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Conundrum and Therapeutic Potential of Curcumin in Drug Delivery

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ABSTRACT: Turmeric, the source of the polyphenolic active compound curcumin (diferuloylmethane), has been used extensively in traditional medicine since ancient times as a household remedy against various diseases, including hepatic disorders, cough, sinusitis, rheumatism, and biliary disorders. In the past few decades, a number of studies have been done on curcumin showing its potential role in treating inflammatory disorders, cardiovascular disease, cancer, AIDS, and neurological disorders. However, the main drawback associated with curcumin is its poor aqueous solubility and stability in gastrointestinal fluids, which leads to poor bioavailability. Multifarious novel drug-delivery approaches, including microemulsions, nanoemulsions, liposomes, solid lipid nanoparticles, microspheres, solid dispersion, polymeric nanoparticles, and self-microemulsifying drug-delivery systems have been used to enhance the bioavailability and tissue-targeting ability of curcumin. These attempts have revealed promising results for enhanced bioavailability and targeting to disease such as cancer, but more extensive research on tissue-targeting and stability-related issues is needed. Tissue targeting and enhanced bioavailability of curcumin using novel drug-delivery methods with minimum side effects will in the near future bring this promising natural product to the forefront of therapy for the treatment of human diseases such as cancer and cardiovascular ailments. We provide a detailed analysis of prominent research in the field of curcumin drug delivery with special emphasis on bioavailability-enhancement approaches and novel drug-delivery system approaches.

KEY WORDS: bioavailability enhancement, cancer targeting, curcumin, irritable bowel syndrome, novel drug delivery, restenosis, solubility enhancement.

ABBREVIATIONS

COX-2	cyclooxygenase-2	DMPC	1,2-dimyristoyl-sn-glycero-3-phosphocholine
DMPG	dimyristoyl phosphatidyl-glycerol	DPPC	dipalmitoylphosphatidylcholine
DMSO	dimethylsulfoxide	EPR	enhanced permeation and retention
HPαCD	hydroxypropyl-α-cyclodextrin	HPβCD	hydroxypropyl-β-cyclodextrin
HPγCD	hydroxypropyl-γ-cyclodextrin	HSA	human serum albumin
HTAβCD	hydroxytrimethylamonium-propyl-β-cyclodextrin	IBD	irritable bowel disease

IL	interleukin
MβCD	2-O-methyl β-CD
NFκB	nuclear factor kappa B
PEG	polyethylene glycol
PLGA	poly-D,L-lactide-co-glycolic acid
RMβCD	randomly methylated β-cyclodextrin
SFCS	silk fibroin chitosan
SLN	solid lipid nanoparticle
TNF	tumor necrosis factor
LEC	liposome-encapsulated curcumin
mPEG	methoxy poly(ethylene glycol)
PBS	phosphate-buffered saline
PEO-PCL	poly(ethylene oxide)-b-poly(e-caprolactone)
PVP	polyvinylpyrrolidone
SBβCD	sulfobutylether-β-cyclodextrin
SLM	solid lipid microparticle
SMEDDS	self-microemulsifying drug-delivery systems

I. INTRODUCTION

From time immemorial, herbal plants have been used as a source of therapeutic compounds or medicine for the treatment of various disorders from head to toe. Turmeric (*Curcuma longa*) is one such herbal remedy. Its dried rhizome is the source of curcumin, which has also been called "Indian solid gold." The characteristic yellow color of turmeric is due to the curcuminoids present in it. The rhizome or root of turmeric is processed into turmeric powder, which contains 2% to 5% curcuminoid and has been used in traditional medicine for centuries as a household remedy for various diseases, including biliary disorders, diabetic wounds, cough, hepatic disorders, AIDS, and sinusitis, and has also been used as a blood purifier.[1–5] Curcumin is also used in perfumes, as a natural yellow coloring agent, and as an approved food additive to flavor various types of curries and mustards. When curcumin is mixed with natural compounds such as slaked lime, it can be used topically for the treatment of inflammation, wounds, tumors, and various skin disorders.

Curcumin was first isolated from turmeric in 1815 by Vogel. It was obtained in crystalline form in 1870, and its structure was delineated in 1910 as diferulolylmethane (Fig. 1) or 1,6-heptadiene-3,5-dione-1,7-bis(4-hydroxy-3-methoxyphe-nyl)-(1E,6E).[6] The orange-yellow color of crystalline curcumin is due to a polyphenolic compound known as diferulolylmethane, which is hydrophobic in nature and practically insoluble in water and ether. Diferulolylmethane is soluble in organic solvents such as ethanol, dimethylsulfoxide (DMSO), acetone, and dimethyl formamide. The solubility of curcumin in acetone is approximately 20 mg/mL, but in the other solvents it is approximately 1 mg/mL. Commercial preparations of curcumin (curcuminoids) are comprised of 77% curcumin I (diferulolylmethane), 17% curcumin II (demethoxycurcumin), and 6% curcumin III (bisdemethoxycurcumin). The maximum absorption of curcumin in methanol occurs at 430 nm and in acetone at 415 to 420 nm. Curcumin exists in enolic and β-diketonic forms due to tautomerism between enol- and keto-structures.[7–9] In solution, curcumin exists primarily in its enolic form characterized by strong intramolecular hydrogen bonds, and this has an important bearing on its radical-scavenging ability. The physicochemical properties of curcumin are due to the

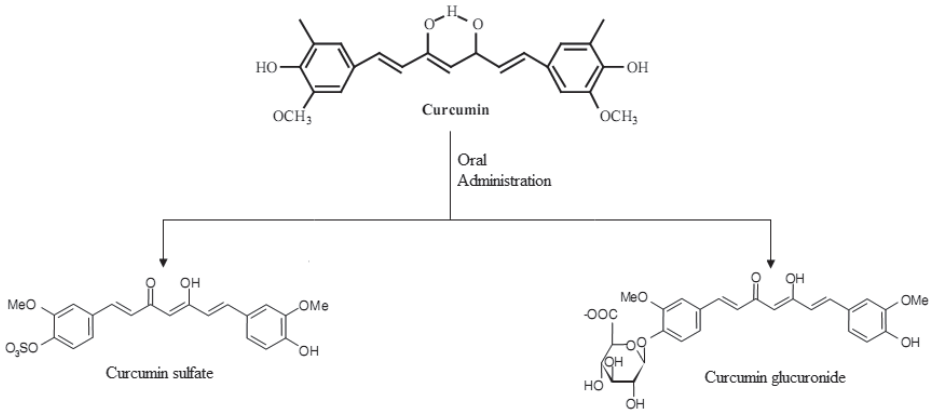

FIGURE 1. Structure of curcumin and its metabolites after oral administration.

formation/disruption of both intra- and intermolecular H-bonds, along with charge delocalizations that are responsible for its therapeutic potential.[10,11]

Curcumin is stable at acidic pH (extremely low degradation), but unstable at neutral and basic pH, and under these conditions it is degraded to trans-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexanal, ferulic acid and feruloylmethane.[12–14] Most of the curcumin (>90%) is rapidly degraded within 30 min of placement in phosphate-buffered saline (PBS) at pH 7.2. This degradation is inhibited by the addition of fetal calf serum, human blood, or antioxidants such as N-acetylcysteine or glutathione and ascorbic acid in culture media or PBS (above pH 7.0).[15,16] When curcumin is orally administered, it undergoes phase II metabolism, predominately glucuronidation and sulfation. Most of the ingested curcumin is excreted in the feces, and only trace amounts of curcumin (or its metabolites) appear in the blood. The absorbed curcumin is rapidly metabolized in the intestinal mucosa and liver to several reduction products (di-, tetra-, and hexahydrocurcumin and hexahydrocurcuminol) and their glucuronide or sulfate conjugates (Fig. 1).[17]

Over the last few years, a number of studies have revealed that curcumin has a surprisingly wide range of beneficial properties, including anti-inflammatory, antioxidant, chemopreventive, radiosensitizing, wound-healing, antimicrobial, antiviral, antifungal, and chemotherapeutic activities. These effects have been demonstrated both in cultured cells and in animal models, and have paved the way for ongoing human clinical trials.[18–24] Numerous preclinical and clinical studies (Table 1) have suggested that serum and tissue levels of curcumin vary depending on the route of administration. An early study in 1978 by Wahlstrom and Blennow[25] reported that 75% of curcumin was excreted in the feces, and only a negligible amount reached the systemic circulation after oral administration to Sprague-Dawley rats. When curcumin was given intravenously to the rats at a dose of 10 mg/kg, a maximum serum curcumin level of 0.36 ± 0.05 μg/mL was observed, compared with 0.06 ± 0.01 μg/mL obtained for a 50-fold dose of

TABLE 1. Preclinical/Clinical Pharmacokinetics of Curcumin After Oral Administration

Human/Animal	Dose	Conclusion	Reference
Sprague-Dawley rats	1 g/kg	Negligible amounts of curcumin in blood plasma	25
Rats	2 g/kg	Serum concentration was observed 1.35 ± 0.23 μg/mL at time 0.83 h	27
Humans	2 g/kg	Either undetectable or extremely low (0.006 ± 0.005 μg/mL at 1 h) serum levels.	27
Rats	340 mg/kg	Serum level 6.5 ± 4.5 nM at 0.5 h	31
Rats	400 mg	Trace amount (less than 5 μg/mL) was found in the portal blood from 15 min to 24 h	32
Humans	4–8 g	Peak plasma levels of 0.41–1.75 μM after 1 h	33
Humans	36–180 mg/kg	Mostly in feces and almost none in urine or blood	34

curcumin administered orally.[26] When 2 g of curcumin was administered orally with piperine to humans, a 2000% increase in bioavailability was observed, suggesting a role of piperine in bioavailability enhancement.[27] Other adjuvants such as bromelain, quercetin, and genistein are also used for to enhance the bioavailability and therapeutic efficacy of curcumin.[28–30]

A plethora of turmeric or curcumin products are currently available on the market in different dosage forms (Fig. 2 and Table 2) either alone or in combination with natural products including piperine, bromelain, and others as an adjuvant for enhancement of therapeutic efficacy. These products are widely used in every corner of the world for the treatment of various ailments such as cough, psoriasis, wound healing, and skin infections, and as a dietary supplement. In spite of its efficacy and safety, curcumin has not yet been approved as a therapeutic agent because of its rapid metabolism, rapid elimination, and poor absorption, which leads to poor bioavailability. The use of curcumin is also limited due to its low water solubility under acidic or neutral conditions, high decomposition rate in alkaline media, and photo-degradation in organic solvents. Most of the published review articles on curcumin have emphasized the mechanism of action of curcumin in different diseases, but to our knowledge, no review has been published on drug delivery. In a review article by Anand et al.,[35] there was a small section dealing with drug-delivery techniques for enhancing the bioavailability of curcumin. Given the explosive growth of interest in curcumin, the purpose of the current review is to present an appraisal of the current level of knowledge regarding the potential of curcumin in drug delivery and bioavailability enhancement. The current review also focuses on the cell line study used in cancer targeting by different drug-delivery techniques for curcumin.

FIGURE 2. Different dosage forms of curcumin alone or in combination available on the market.

II. SOLUBILITY AND BIOAVAILABILITY ENHANCEMENT OF CURCUMIN

Curcumin, which is a polyphenol, has low solubility, and curcumin crystals are not well dispersed in the intestine following oral administration. Restricted bioavailability of dietary polyphenols is not only due to the physiochemical properties of the bioactive compound, but also because of enzyme- and microbial-mediated biotransformation and active efflux. Common approaches used to tackle the challenge of increasing bioavailability are particle size reduction (which includes micro-sizing and nano-sizing), salt formation, solid dispersion, solubilization, and complexation with β-cyclodextrins.[36,37]

II.A. Cyclodextrin Complex

Cyclodextrins, sometimes called cycloamylases or cellulosines, are cyclic oligosaccharides with a somewhat truncated, cone-like structure that are divided into three types, α, β, and γ, depending on whether they contain six-, seven-, or eight-membered sugar ring molecules, respectively. The exterior side of the cone consists of a hydroxy group, making the exterior surface hydrophilic, while the central cavity is somewhat hydrophobic, being lined with the carbons and the ethereal oxygen of the carbohydrate skeleton. Cyclodextrins are popular for their ability to form inclusion complexes by taking up lipophilic drug molecules into the lipophilic central cavity, which increases the bioavailability of poorly soluble drugs by enhancing their aqueous solubility and acts

TABLE 2. Marketed Dosage Form of Curcumin

Active Ingredient	Dosage Form	Brand Name or Formulation	Marketed By
Curcuminoids and Bioperine	Tablet	Curcumin C_3 complex	Sabinsa Corporation , USA
Curcumin, bromelain, and Bioperine	Tablet	Turmeric extract (95% curcumin)	Source Naturals , USA
Curcumin	Capsule	Turmeric	Swanson Health Products, USA
Curcumin	Capsules	Curcumin 95	Jarrow Formulas, USA
95% Curcuminoids	Capsule	Turmeric/curcumin	Nature's Bounty, USA
95% Curcumin	Capsule	Turmeric extract	Planetary Herbals, USA
Curcumin and Bioperine	Capsule	Super curcumin	Life Extension, USA
95% Curcumin	Capsule	Turmeric extract	Vitamin Shoppe, USA
Curcumin	Capsule	Curcumin	Pure Encapsulation, USA
Curcumin	Capsule	Turmeric curcumin	Thompson, USA
Curcumin	Capsules	Turmeric curcumin	Good 'N Natural, Canada
Curcumin and black pepper	Capsule	Curcumin and black pepper	BioActive Nutrients, USA
Curcumin, dl phenylalanine, Boswellia, and nattokinase	Capsule	Phenocane with curcumin and DLPA	OxyLife, USA
Curcumin, fermented soy, and Bioperene	Capsules	Curcumin and fermented soy	Jiva, USA
Curcuminoid complex	Softgels	Curcu-Gel Ultra™	Tishcon Corp., USA
Curcumin	Softgels	Curcu-Gel™	Tishcon Corp., USA
Curcumin	Softgels	Curcumin	Solaray Nutritional Supplement, UK
Curcumin extract , aloe vera	Gel	Psoria-Gold Curcumin Gel	Albi Naturals, Canada
Aloe vera, curcumin	Gel	Psoria Gold Curcumin Gel	Omnicure, USA
Curcumin	Softgel	Curcu-Gel Rx-95	Phyto Therapy, USA
Curcumin	Cream	Vicco Turmeric	Vicco Laboratories , India
Curcumin	Gel	Curcuma Herbal Bath Gel	Bynature, Thailand

as a driving force for diffusion across the biological membrane. Cyclodextrins have both stabilizing and destabilizing effects on drugs from photolytic and hydrolytic degradation.[12,13]

Curcumin is encapsulated in different substituents of cyclodextrin, such as hydroxypropyl-α-cyclodextrin (HPαCD), hydroxypropyl-β-cyclodextrin (HPβCD), hydroxypropyl-γ-cyclodextrin (HPγCD), randomly methylated β-cyclodextrin (RMβCD), 2-O-methyl β-CD (MβCD), sulfobutylether-β-cyclodextrin (SBβCD), and hydroxytrimethylamoniumpropyl-β-cyclodextrin (HTAβCD), for increasing the solubility and stability of the drug. Drug solubility is increased 10^4-fold in an inclusion complex at pH 5.0, having the highest affinity for the relatively hydrophobic cavity of RMβCD and the large cavity of HPγCD, and the least affinity for HTAβCD. The β-cyclodextrin derivative has a stabilizing effect against alkaline hydrolytic (pH 8.0 and 10.0) decomposition in the concentration range of 0.1% to 10%, while it has a destabilizing effect against photolytic degradation for curcumin. At the lowest cyclodextrin concentration (0.1%), the stability increases up to 50-fold, suggesting that 98% of the curcumin molecule is entrapped within the cyclodextrin cavity; on increasing the cyclodextrin concentration, the stability increases up to 500-fold. The 5% and 10 % HPβCD concentration provides the maximum alkaline stability to curcumin, with a half-life of more than 100 h at pH 5.0 and 4.8 h at pH 10.0 for 10%, while lowest for HPγCD, with a half-life of 2.2 h. The formation of an inclusion complex has a destabilizing effect on curcumin with respect to photodecomposition compared with free curcumin solution in organic solvents (methanolic and ethanolic solution of PBS). The α- and γ-derivatives of hydroxypropyl-cyclodextrin have a lesser destabilizing effect than the β-derivative, indicating an effect of cavity size on the stability of the drug.[14,38]

In another study, Kristin et al. determined the effect on solubility of curcumin by complexation with α-, β-, and γ-cyclodextrin and their hydroxyl propylated derivatives. These six cyclodextrins significantly increased the solubility of curcumin, with the greatest solubility observed in HPγCD and then HPβCD. The complex was formed in the ratio of 2:1 host to guest, and the cyclodextrin host encapsulated each of two phenyl group at the ends of the curcumin molecule. In the case of parent cyclodextrins, maximum solubility was obtained with β-cyclodextrin, suggesting that the cavity size of β-cyclodextrin is best matched with curcumin. The cavity of γ-cyclodextrin is too large, but it encapsulated curcumin in HPγCD because the hydroxylpropyl side chain involved in the inclusion process held curcumin in the large cavity.[39] Among the cyclodextrins, the β form is the most versatile and is commonly used due to its cavity size, which is favorable for drug molecules of molecular weights between 200 and 800 g/mol; it is also easily available and economical compared with the other cyclodextrins.[40]

Complexation of curcumin with HPγCD has also been reported for follicular targeting. The complex was applied on the epidermis and observed under fluorescence microscopy for the distribution of the drug complex. It was found that the complex was located in the subcutis of the porcine skin and hair bulbs,

and the authors concluded that they were successful in targeting curcumin to the hair follicle.[41] When curcumin (100 mg) and its β-cyclodextrin complex (at a 1:2 ratio) were orally administered for 10 d, curcumin inhibited the growth of mammary tumors at the beginning of the treatment, while its complex revealed significantly greater activity up to 5 weeks afterward. The effect of curcumin against tumors of the mammary gland was increased due to its enhanced oral absorption and oral bioavailability and its anti-angiogenic properties.[39]

Studies have concluded that among all of the cyclodextrin-curcumin complexes, the hydroxyl propyl derivatives of γ- and β-cyclodextrins are more effective than others due to their similarity in cavity size to curcumin. HPγCD showed more solubility than HPβCD. The solubility and stability of curcumin in alkaline media in these complexes also depend on the concentration of cyclodextrins. During complex formation, there is no covalent bond formed or broken, and the curcumin molecules in complex are in rapid equilibrium with free molecules in the solution. However, the major drawback associated with cyclodextrins is that they have a destabilizing effect on curcumin due to photodecomposition. The cyclodextrin complexes of curcumin are also not suitable for topical delivery because they do not readily permeate biological membranes due to their chemical structures, molecular weights, and low octanol/water partition coefficients.

II.B. PHYTOSOMES/Phospholipid Complex

PHYTOSOMES is an amphiphilic molecular complex in which bioactive molecules (mainly polyphenolics) are complexed with phospholipids, in particular phosphatidylcholine, resulting in the formation of supramolecular adducts having a definite stoichiometry. The PHYTOSOMES formulation improves gastrointestinal absorption upon oral administration by enhancing the rate and the extent of solubilization of bioactive into aqueous intestinal fluids, thus enhancing the ability to cross the lipid-rich biomembranes, yielding higher plasma levels and reducing kinetic elimination and resulting in enhanced systemic bioavailability.[42,43] The pharmacokinetic parameter of bioavailability for curcumin and its phospholipid complex after oral administration were determined in male albino Wistar rats. The complex was developed for curcumin and phospholipid-hydrogenated soy phosphatidyl choline in a molar ratio of 1:1. The aqueous solubility of curcumin alone, mixed with phospholipid, and mixed with the phospholipid complex was found to be 8.33, 12.40, and 26.67 μg/mL, respectively, indicating that the complex was 1.5 times more soluble than curcumin alone. The curcumin-phospholipid complex showed the maximum serum concentration of curcumin (1.20 μg/mL) at 1.5 h after oral administration compared with pure curcumin (0.5 μg/mL) at 0.75 h. The phospholipid complexes also had an impact on elimination half-life and clearance, showing increased elimination half-life (1.96 h) and reduced clearance (22.33 h^{-1}) compared with pure curcumin (1.45 h and 92.26 h^{-1}, respectively). The antioxidant activity and hepatoprotective effect of the complex was significantly higher than pure curcumin, and also restored the normal condition of rat liver enzymes.[44]

In another study, curcumin was complexed with soy lecithin, a major constituent of cell membranes, in order to improve its bioavailability. The aqueous solubility of the physical mixture of soy lecithin curcumin and its soy lecithin complex was found to be 20.2 and 29.4 μg/mL, respectively. The study reported that the curcumin-soy lecithin complex provided a 3-fold increase in the solubility of curcumin compared with pure curcumin. When the curcumin-soy lecithin complex was subjected to in vitro release studies using the everted gut sac method, about 100 μg of the curcumin permeated from the complex, whereas the permeation of curcumin from uncomplexed curcumin was negligible and could not be detected spectrophotometrically. The hepatoprotective effect of pure curcumin at 100 mg/kg was similar to the effect produced by the complex at 50 mg/kg.[45] Commercially available Meriva® (Indena S.p.A, Milan, Italy), is available in the ratio of 1:4 curcumin to phosphatidylcholine in EpiKuron™ 130P (Cargill, Minneapolis, MN, USA). Merczylo et al. conducted an in vivo study in male Wistar rats using unformulated curcumin and formulated curcumin with phosphatidylcholine (Meriva) to explore whether Meriva increased the oral bioavailability or had any effect on the metabolite profile of curcumin. A dose of curcumin equivalent to 340 mg/kg of either unformulated curcumin or Meriva was given by oral gavage, and the plasma and tissue levels of curcumin were compared. Meriva dramatically and significantly increased curcumin levels in the plasma (5-fold) and liver compared with concentrations measured in animals that received unformulated curcumin. In contrast, the curcumin level was moderately lower in the intestinal mucosa after ingestion of Meriva compared with unformulated curcumin. Plasma levels of curcumin glucuronide, tetrahydrocurcumin, curcumin sulfate, and hexahydrocurcumin observed after administration of Meriva were 3- to 20-fold higher than those seen after the administration of unformulated curcumin. Oral administration of curcumin as Meriva is superior to that of unformulated curcumin if tissues other than the gastrointestinal tract are targeted, while maximal levels in the gastrointestinal tract can be achieved with unformulated curcumin.[31]

In a pharmacokinetic study by Liu et al., curcumin and a curcumin-phospholipid complex at a dose of 100 and 300 mg, respectively, were administered orally to male Sprague-Dawley rats, and a significant improvement in curcumin bioavailability, high clearance, and long half-life was observed in rats given the phospholipid complex. The maximum plasma concentration of curcumin in the curcumin-phospholipid complex was 600.93 ng/mL at 2.33 h compared with 266.70 ng/mL at 1.62 h for free curcumin after oral administration. An almost 2-fold increase in bioavailability and 1.5-fold increase in half-life for the curcumin-phospholipid complex over free curcumin was observed in this study.[46] Thus, the phospholipid complex increases bioavailability by increasing the solubility of curcumin in aqueous intestinal fluid, reducing clearance and leading to increased elimination half-life.

II.C. Solid Dispersion

Formulation of solid dispersion in water-soluble carriers has been widely researched over the past four decades for solubility and related bioavailability enhancement. Solid dispersion, also referred to as molecular dispersion, is when a drug is incorporated into a carrier at the molecular level, resulting in a change of the crystalline form of the drug to the amorphous form, which is more soluble.[36] In order to enhance the solubility and bioavailability of curcumin, solid dispersion methods have been developed using different polymers. A solid dispersion of curcumin with polyvinylpyrrolidone (PVP) K-30 was created in different ratios from 1:2 to 1:8 using the solvent evaporation method, and 1:8 was determined to be the best on the basis of an in vitro dissolution study. Pure curcumin, a curcumin-PVP K-30 solid dispersion, and curcumin physical mixture were administered to rats at a dose of 100, 200, and 400 mg/kg, respectively. The plasma level of curcumin was below the detection limit after oral administration of pure drug and the physical mixture, even at a high dose (400 mg/kg), while for the solid dispersion it was detectable at all time points. The peak plasma levels in blood for the three dosages were 74.558, 110.174, and 193.665 ng/mL at about 45 min, respectively, and the bioavailability was 514.646, 609.111, and 1028.627 ng/mL/h, respectively. These results indicate that solid dispersion improved the oral absorption and bioavailability of curcumin.[47]

Paradker et al. formulated a solid dispersion with PVP K-30 using the spray-drying method to improve dissolution of curcumin in acidic medium. Solid dispersions prepared with lower proportions of PVP (1:1–1:3) had rough spherical surfaces with pinholes, whereas in higher proportions (1:5–1:10), a smooth surface with concave depressions was observed, indicating an effect of PVP on the particle shape of solid dispersions. During dissolution in 0.1N HCl, pure curcumin and its physical mixture revealed a negligible release even after 90 min, while the solid dispersion showed a drastic increase in the dissolution rate with increasing concentrations of PVP. This was attributed to changes in the solid state (amorphous) during the formation of dispersion.[48] Onoue et al. formulated a nanocrystal solid dispersion, an amorphous solid dispersion, and a nanoemulsion for curcumin to overcome drawbacks such as poor solubility, bioavailability, and photostability, and to improve physicochemical and pharmacokinetic properties. The dissolution profiles of all three formulations and crystalline curcumin were examined up to 180 min, and it was found that the curcumin nanoemulsion showed the fastest dispersion , while dissolution of crystalline curcumin into water was found to be much slower than the tested curcumin formulations. Both solid dispersions of curcumin exhibited an improved dissolution/dispersion, with 95% and 80% release from amorphous solid dispersion and nanocrystal solid dispersion, respectively, at 180 min. In an attempt to determine the pharmacokinetic parameters, the developed curcumin formulations (20 mg curcumin/kg) and pure curcumin (100 mg/kg) were administered orally to the rats. Oral administration of the nanoemulsion (20 mg/kg curcumin) resulted in a rapid elevation of curcumin blood levels up to a C_{max} of 451 ng/

mL within 10 min, and decreased rapidly with an elimination half-life of 39 min, while for pure curcumin the C_{max} was 35 ng/mL at 80 min with an elimination half-life of 207 min. In contrast, both nanocrystal solid dispersion and amorphous solid dispersion showed maximum plasma concentrations of 194 and 147 ng/mL, which was 3- to 5-fold higher than that for crystalline curcumin, with similar T_{max} and elimination half-lives. These findings indicated that both solid dispersion formulations of curcumin can provide wider therapeutic safety margins than the nanoemulsion. High photochemical stability was seen in the crystal solid dispersion of curcumin and crystalline curcumin, while curcumin in the solution state was found to be photoreactive and photodegradable.[49]

Curcumin exists in the crystalline form, and solid dispersion is a good technique with which to convert the crystalline nature of a drug to the amorphous form, which leads to enhanced solubility. Different methods for the solid dispersion of curcumin were developed, and resulted in good solubility and significant plasma levels of curcumin compared with free curcumin. This was due to the reduction of particles to the absolute minimum during entrapment of the drug, which causes increased surface area and effective dissolution rates. Curcumin is also photostable when in the form of a solid dispersion. However, the scale-up and stability of curcumin in the form of a solid dispersion has been the greatest limitation to its development as a formulation tool because of the need for the maintenance of its physicochemical properties during processing and manufacturing.

II.D. Nanoparticulate System

For drugs that are poorly soluble, particle size is important because it can be a major hurdle in achieving adequate oral bioavailability for a large number of herbal and synthetic drug molecules. For several decades, particle size reduction has been an active area of research in the pharmaceutical industry. Recently, nanoparticle-based drug-delivery systems have emerged as a solution for increasing the bioavailability of potential therapeutic agents. Nanoparticulate drug-delivery systems such as nanoemulsion, liposomes, self-emulsifying drug-delivery systems (SMEDDS), nanoparticles, and dendrimers of submicron size are stabilized with surfactants or polymers in nanosuspensions, which can be further processed into standard dosage forms for oral, parental, or topical delivery to improve the bioavailability of the active ingredients and introduce controlled/target release. Takahashi et al.[50] encapsulated curcumin in liposomes (LEC) for enhancing its delivery. They used two kinds of lecithins, SLP-PC70 and SLP-WHITE, for the preparation of liposomes by the mechanochemical method using a microfluidizer. They observed that the SLP-PC70 LEC solution was stable with encapsulation efficiency for curcumin (68.0 wt%) compared with SLP-WHITE LEC (<10.0 wt%), which became unstable after 1 d of storage. The formulation SLP-PC70 LEC, curcumin, and a curcumin-lecithin mixture at a dose of 100 mg of curcumin/kg body weight were orally administered to male Sprague-Dawley rats. With the LEC formulation, a peak plasma level of 319.2 ±

70.4 μg/L was achieved in 30 min, while for curcumin and its mixture, the peak plasma level was 64.6 ± 10.7 and 78.3 ± 17.9 μg/L in 120 min, respectively. The $AUC_{0\text{-}120}$ value of curcumin after oral administration of LEC was 26502.8 μg min/L, which was 4.96-fold greater than that seen after free curcumin administration. The study demonstrated that encapsulation of curcumin in lecithin led to a substantial improvement in curcumin absorption and systemic bioavailability; however, little absorption was observed with co-administration of curcumin and the lecithin mixture. Plasma antioxidant activity was found to be significantly higher for LEC, while similar for curcumin and its mixture, as measured by the trolox equivalent antioxidant capacity assay.[50] Tiyaboonchai et al.[51] improved the stability of curcuminoides in a cream preparation by developing curcuminoid-loaded solid lipid nanoparticles (SLNs) using the microemulsion technique. The group used stearic acid and glyceryl monostearate as the oil phase, poloxamer as the emulsifier, dioctyl sodium sulfosuccinate as the co-emulsifier, and water for the SLN preparation. After increasing the concentration of poloxamer 188 and dioctyl sodium sulfosuccinate, the mean particle size and polydispersity index increased, while the entrapment efficacy decreased as the amount of lipid increased. The in vitro release studies in 50% v/v ethanol from both a cream containing curcuminoid SLNs and a cream containing free curcuminoids demonstrated more rapid release of curcuminoids from the one containing free curcuminoids (~90%) within 8 h, while 70% of the curcuminoids were released from the SLNs within 12 h, with a 25% burst release within the first 10 min. The lyophilized curcuminoid SLNs demonstrated physical and chemical stabilities for at least 6 months of storage in the absence of sunlight under room temperature, with the percentage of curcumin (99%), bisdemethoxycurcumin (97%), and demethoxycurcumin (95%) remaining stable. Curcuminoid SLNs in a model cream base demonstrated chemical stability in the absence of sunlight, with the percentages of the curcumin (91%), bisdemethoxycurcumin (96%), and demethoxycurcumin (88%) remaining stable, while in presence of sunlight the percentages decreased to 71%, 83%, and 62%, respectively. Furthermore, the stability of non-encapsulated curcuminoids in the cream base in the absence and presence of sunlight for 3 and 6 months showed 50% and 80% curcuminoid loss, respectively, confirming that SLNs significantly improved curcuminoid stability against light and oxidation reaction during storage when incorporated in a cream base.[51]

Shaikh et al. improved the oral bioavailability of curcumin molecules by encapsulating them in nanoparticles. The poly-D,L-lactide-co-glycolic acid (PLGA) nanoparticle of curcumin was developed by the emulsion-diffusion evaporation method using polyvinyl alcohol, Pluronic® F68 (BASF, Ludwigshafen, Germany), vitamin E d-alpha tocopheryl polyethyleneglycol succinate, and cetyl trimethylammoniumbromide as stabilizers. The maximum encapsulation of curcumin was found with polyvinyl alcohol (84.6 ± 1.1%), which had the largest particle size (242 ± 2 nm), while the lowest encapsulation was with cetyl trimethylammoniumbromide (7.5 ± 0.2%), which had the smallest particle size (121 ± 3 nm). On increasing drug loading from 5% to 15% using polyvinyl alcohol as a

stabilizer, particle size and the polydispersity index increased from 242 ± 2 nm and 0.17 ± 0.02 to 264 ± 2 and 0.31 ± 0.05, respectively. In vitro drug release from PLGA nanoparticles was biphasic, with a rapid release of about 24% in 24 h, followed by sustained drug release of about 43% over 20 d. No characteristic peak of curcumin was observed using X-ray diffraction when entrapped into nanoparticles, indicating the formation of an amorphous complex with the intermolecular interaction occurring within the matrix. After 3 months of storage at accelerated conditions, freeze-dried nanoparticles with 5% sucrose (used as a cryoprotectant) were stable without any collapse or shrinkage of the dried cake. Blood levels after oral administration of curcumin nanoparticles (100 mg/kg body weight) to male Sprague-Dawley rats were compared with oral curcumin suspension (250 mg/kg body weight) and suspension of curcumin with piperine as an absorption enhancer (250 mg/kg and 10 mg/kg, respectively). A sustained release of curcumin over 48 h was observed from nanoparticles ($T_{max,}$ 2 h), whereas in case of the simple suspension (T_{max}, 0.5 h) and suspension with piperine (T_{max}, 0.75 h), the levels were not detectable beyond 6 h. However, the plasma concentration of curcumin decreased rapidly, indicating rapid metabolism. Compared with a simple curcumin suspension, curcumin with piperine had a relative bioavailability of 2.8-fold with a high C_{max}, while the nanoparticulate formulation of curcumin had a relative bioavailability of 26-fold compared with the former and 9.2-fold compared with the latter.[52] In another study, curcuminoid-loaded poly(butyl)cyanoacrylate nanoparticles coated with poloxamer 188 were developed by anionic polymerization using the solvent evaporation method. The particle size and zeta potential of liposomes were 178 nm and –28.33, respectively, with 77.99% encapsulation efficiency. The curcuminoids were entrapped inside the nanoparticles in the molecular dispersion form and were released in the form of controlled drug release (following the matrix model) for extended periods of time, with higher release in an acidic environment (85.22%) compared with PBS 7.4 (76.98%). However, curcuminoid-loaded poly(butyl)cyanoacrylate nanoparticles showed an initial burst release for the first 4 to 5 h, and at all time intervals release was faster in 0.1N HCl compared with PBS at pH 7.4. These nanoparticles were stable for 6 months at 40°C and 75% relative humidity in the absence of sunlight, and showed release in the range of 87% to 93%, whereas in the presence of sunlight, the release was slightly reduced and was in the range of 79% to 89%. Further, the amount of curcuminoids remaining after 6 months of storage in the absence and presence of light was also documented and found to be in the range of 55% to 62% and 50% to 57%, respectively.[53]

SMEDDS has recently come into existence as one of the most interesting approaches to improve the solubility, dissolution, and bioavailability for poorly water-soluble drugs. An example of this technology is the Neoral® formulation of cyclosporine A, which is commercially marketed by Novartis Pharmaceuticals (Basel, Switzerland). Curcumin is also formulated in the form of SMEDDS using ethyl oleate as the oil phase, a mixture of emulsifier OP and Cremophor EL-40® (BASF) in a 1:1 w/w ratio as a surfactant, and polyethylene glycol (PEG) as a

co-surfactant based on the miscibility and solubility of curcumin. The in vitro release of curcumin at pH 1.2 and 6.8 from SMEDDS showed fast dissolution, with 85% release in 10 min, and more than 96% after 20 min, while for pure curcumin it was less than 2% even after 60 min. An in situ absorption study showed concentration-independent absorption of curcumin in rat intestine from SMEDDS even at variable doses (25, 50, and 100 mg/kg). The percentage of absorption of curcumin-loaded SMEDDS was higher than that of the curcumin suspension at each time point, which was 93.8% for SMEDDS at 24 h after administration and 3.86 times that of the curcumin suspension (24.3%).[54]

Nanoparticulate systems are exciting delivery approaches with the potential for multiple applications in the pharmaceutical and food industries. The solubility of curcumin in lipid is a important factor for better encapsulation efficiency in developing these systems for the delivery of curcumin. Lipid-based systems that promote the absorption of curcumin through the lymphatic system and avoid hepatic first-pass metabolism are a good approach for enhancing the bioavailability of curcumin. Furthermore, the stability of curcumin due to oxygen and light sensitivity is strongly reduced by incorporating curcumin into these unique types of systems. Compared with cyclodextrin complexes, nanoparticulate systems have a stabilizing effect against the photoreactivity of curcumin, while cyclodextrins have destabilizing effects. Apart from enhancing stability, these approaches also show promising results for enhancing bioavailability due to enormous increases in solubility.

III. POTENTIAL THERAPEUTIC USES OF CURCUMIN

Extensive research within the last half decade has created an awareness that curcumin has potential against a wide variety of diseases, including pancreatitis, arthritis, irritable bowel disease (IBD), psoriasis, diabetes, multiple sclerosis, Alzheimer's disease, AIDS, and different types of cancer.[24,30,55–59] Recent studies have shown that telomerase, a reverse transcriptase enzyme, also acts as a key modulator in the development of different types of cancer cells, and may be a good target for different drugs such as curcumin for the prevention of cancer. Mishra et al. highlighted in their review the role of curcumin-mediated nanoparticles in the down-regulation of telomerase activity.[60] Modern research has provided considerable evidence about the mechanism through which curcumin interacts physically with its diverse range of molecular targets, including transcription factors, enzymes, growth factors and their receptors, gene-regulating cell proliferation, cytokines, and apoptosis. Despite extensive research and development, the poor solubility of curcumin in aqueous solution remains a major barrier to its bioavailability and clinical efficacy.[61–63] Nanoscale drug-delivery systems including nanoemulsions, liposomes, nanoparticles, and dendrimers formulated from biocompatible biodegradable polymers or excipients constitute an evolving approach to drug delivery and tumour targeting that appear to provide longer circulation, resistance to metabolic processes, and better permeability. The potential of curcumin in drug-delivery methods, however, has not

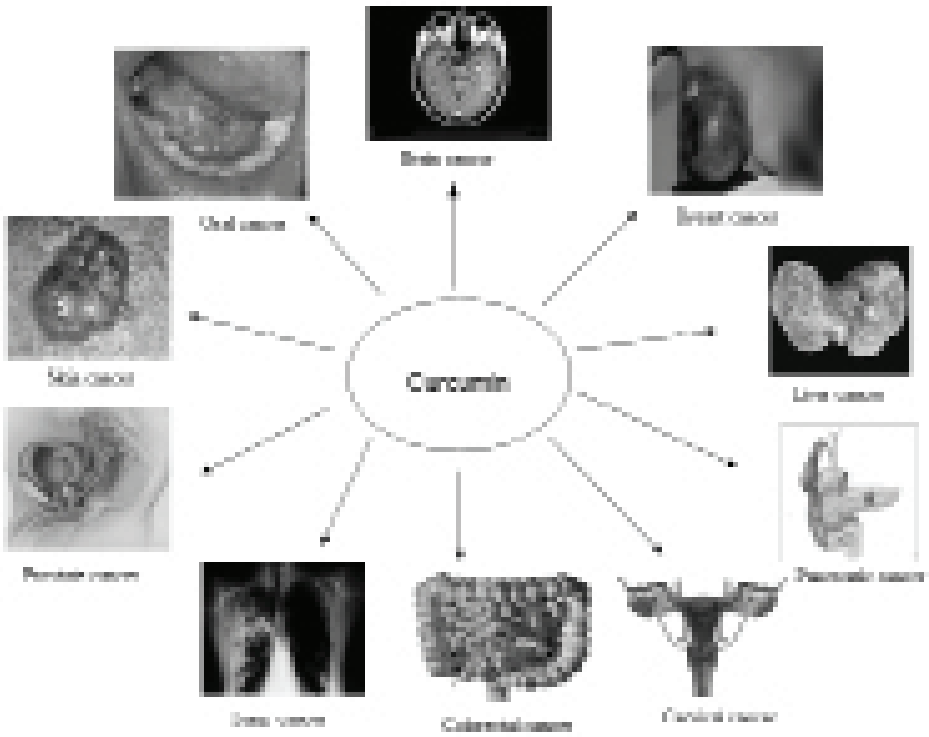

FIGURE 3. Role of curcumin against various cancers.

yet been systematically examined through modern multi-center, randomized, double-blind, placebo-controlled clinical trials.[64–68]

III.A. Anticancer

Over the last few years, a number of studies on curcumin have provided evidence that it is effective against various types of cancer (Fig. 3), including oral, cervical, breast, colorectal, lung, pancreatic, brain, osteosarcoma, head and neck, and kidney cancers, as well as leukemia and melanoma. Curcumin suppresses the activation of nuclear factor kappa B (NFκB) and activator protein 1, and modulates the expression of early growth response protein 1, peroxisome proliferator associated receptor-gamma, β-catenin, and NF-E2-related factor-2. Cancer is a hyperproliferative disorder marked by metastasis into the vital organs of the body through invasion and angiogenesis. Curcumin arrests the growth of cancer cells in the G2/S phases of the cell cycle.[9,69,70] Nanocarriers offer greater promise in improving the therapeutic effectiveness and safety profile of bioactive molecules than conventional cancer therapy by allowing for increased accumulation of drugs at tumors via the enhanced permeation and retention (EPR) effect. Such approaches made it possible to develop US Food and Drug Administration- and

European Medicine Agency-approved liposomal doxorubicin (DOXIL®, Centocor Ortho Biotech Products L.P., Horsham, PA, USA), PEGylated liposomal daunorubicin (DaunoXome®, Gilead Sciences, San Dimas, CA, USA), and, recently, albumin-bound paclitaxel-loaded nanoparticles (Abraxane®, Abraxis Bioscience, LLC, Bridgewater, NJ, USA) for clinical oncology use.[71–73] Extensive research on curcumin encapsulated in nanoparticulate systems is ongoing and may be helpful in the future in developing particulate carrier systems of curcumin for approval by different regulatory bodies.

1. SLN/Polymeric Nanoparticles

Polymeric, curcumin-loaded N-isopropylacrylamide-N-vinyl-2-pyrrolidone-poly(ethyleneglycol) monoacrylate nanoparticles demonstrated comparable efficacy over free curcumin against pancreatic cancer cell lines in vitro by inhibiting cell viability and colony formation in soft agar. The average particle size of the nanoparticles was less than 50 nm, with narrow size distribution, and the entrapment efficiency was more than 90%. The in vitro release profile of the loaded curcumin from the nanoparticles at physiological pH (in PBS) occurred in a sustained manner: only 40% of the total drug was released from the nanoparticles after 24 h. The void polymeric nanoparticles were safe, as no toxicity was observed in either in vitro (pancreatic cell line) or in vivo (athymic mice) toxicity studies. The polymeric nanoparticles encapsulating curcumin were robustly taken up by pancreatic cancer cells, as indicated by the fluorescence emitted from the accumulated intra-cytoplasmic drug, and were effective in their ability to block clonogenicity of the MiaPaca pancreatic cancer cell line in soft agar assays. Nanocurcumin robustly inhibited NFκB function by inhibiting its DNA-binding ability in the pancreatic cancer cell lines BxPC3 (1–2 h) and MiaPaca, as assessed by a shift in migration of radiolabeled p65-binding oligonucleotide (electrophoretic mobility shift or "gel shift" assays). Drastic results were obtained with the MiaPaca cell line, in which there was persistent activation of NFκB in cells exposed to free curcumin after overnight incubation, while a perceptible gel shift was observed in the nanocurcumin-treated cells. Incubation of stimulated peripheral blood mononuclear cells with both free and nanocurcumin decreased steady-state mRNA levels of interleukin-6 (IL-6), IL-8, and tumor necrosis factor-alpha (TNF-α) compared with DMSO and void nanoparticle-treated cells, with evidence of a dose-dependent reduction of IL-6 by both agents.[64]

In another study, the composite nanoparticles were developed by using three biocompatible polymers, alginate, chitosan, and Pluronic® F127 (BASF), referred to as ALG-CS-PF127 nanoparticles, by ionotropic pre-gelation followed by polycationic crosslinking at a mass ratio of 6:1 and a final pH of 4.7 for encapsulation of the anticancer drug curcumin with a mean size of 100 ± 20 nm. However, with ALG-CS-PF127 nanoparticles, an increase in the initial curcumin concentration caused an enhancement of percentage entrapment efficiency, which started decreasing with further increases in the curcumin concentration

(1 mg). The in vitro release of curcumin in PBS (pH 7.4) from ALG-CS-PF127 nanoparticles occurred in a controlled manner, 36% release in 12 h, which increased up to 51% in 24 h, followed by gradual and sustained release up to 72 h. Thereafter there was a drop in the rate of release, and finally by the end of 96 h, about 75% of curcumin was released from the nanoparticulate matrix. The Pluronic® F127 increased curcumin loading or encapsulation efficiency in the formulation at 0.1% w/v, but at a high concentration increased the nanoparticle size and reduced the drug release from ALG-CS-PF127 nanoparticles compared with ALG-CS alone, which may be beneficial for effective passive targeting of cancerous tissues by retaining curcumin for a prolonged time in circulation. A nanoparticle concentration of 500 μg/mL was safe in the HeLa (a human cervical cancer cell line) study. The in vitro cytotoxicity of free curcumin and curcumin-loaded nanoparticles on HeLa cell proliferation was studied, and the estimated half-maximal inhibitory concentration values against cell viability for free and encapsulated curcumin were 13.28 and 14.34 μM, respectively. Cellular uptake studies of curcumin-loaded nanoparticles were done by visualizing the intrinsic fluorescence of curcumin using fluorescence microscopy, and confirmed by cellular internalization of the drug-loaded nanoparticles.[74]

Gupta et al. focused on harnessing the properties of the silk fibroin polymer as a drug-delivery agent to increase the retention, efficacy, and bioavailability of curcumin by encapsulating it as a nanoparticle. The nanoparticles were prepared at 0.1 and 10% w/v with silk fibroin and a blend of silk fibroin chitosan (SFCS) in different ratios as a polymer by the capillary microdot technique. The particle size of all the silk fibroin- or SFCS-encapsulated curcumin nanoparticles formulation was less than 100 nm, except for 0.1% w/v 50:50 SFCS (130 ± 4.2 nm). The entrapment efficiency of curcumin was more than 96% for silk fibroin-coated nanoparticles for both the 0.1% and 10% concentrations, and this decreased to 64% to 73% for SFCS-coated nanoparticles depending on concentration of silk fibroin and chitosan. The curcumin showed an initial burst release from nanoparticles up to 2 d from both silk fibroin and SFCS blends, and in large amounts over 8 d from silk fibroin alone. However, curcumin release from SFCS blends did not increase any further over 8 d. The intracellular uptake of curcumin in MCF-7 and MDA-MB-453 breast cancer cells was highest from silk fibroin-coated nanoparticles compared with the respective 0.1% w/v and 10% w/v SFCS blend groups, which also increased efficacy against breast cancer cells and reduced the viability of Her2/neu high-expressing breast cancer cells.[75] The curcumin-loaded PLGA nanoparticles were designed using PLGA-PEG and Pluronic® F68 and the nanoprecipitation technique with the goal of enhancing their bioavailability without the loss of biological activity. The PLGA nanoparticle contained 4 μg of curcumin per milligram of nanoparticles, with an 80.9 mean diameter and 97.5% encapsulation efficiency. The curcumin nanoparticle was easily taken up by human chronic myeloid leukemia KBM-5 cells as early as 5 min after exposure, reached a maximum at 30 min, and showed a dose-related apoptotic response. In contrast, the earliest uptake of free curcumin occurred at 30 min and did not reach maximum even at 180 min; curcumin nanopar-

ticles quickly disappeared, whereas curcumin disappeared slowly from these cells. Neither curcumin nor curcumin nanoparticles alone activated NFκB but did inhibit the TNF-induced activation of NFκB in a dose-dependent manner, and showed that suppression of NFκB activation was not due to loss of cell viability. The curcumin nanoparticles suppressed the TNF-induced expression of NFκB -regulated gene products more potently than curcumin alone. The curcumin nanoparticles had almost twice the serum levels of curcumin and a substantially longer half-life compared with curcumin alone.[76]

2. Liposomes

Among different drug-delivery vehicles, liposomes have been explored for decades due to their biodegradability and potential to load large concentrations of drug. Liposomes are composed of lipid layers and are either unilamellar, which encapsulate water-soluble drugs, or multilamellar, which are hosts for lipid-soluble drugs, and have the capacity to alter the biodistribution of drugs through delayed clearance and longer intravascular circulation times. Li et al. addressed the in vitro and in vivo antitumor activity of liposomal curcumin against human pancreatic carcinoma cells. The liposomal curcumin was prepared with 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), and the proliferation/survival of pancreatic cells was assessed with the (3-4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide assay. The liposomal curcumin (IC_{50} 2.0–37.8 μM) inhibited pancreatic cell growth for 72 h in a time-dependent manner, and its antiproliferative and apoptotic effects were equivalent to or better than those of free curcumin (IC_{50} 5.4–46 μM) at equimolar concentrations in all cell lines. Liposomal curcumin decreased NFB binding, and its effects were as potent as those of free curcumin. NFκB inhibition led to down-regulation of expression of the gene products cyclooxygenase-2 (COX-2) and IL-8, and was accompanied by marked in vitro growth suppression and apoptosis. In vivo, liposomal curcumin inhibited pancreatic cell growth in murine xenograft models, and these effects were accompanied by a potent anti-angiogenic response.[77] In another approach, liposomal curcumin was used alone and in combination with oxaliplatin against colorectal cancer. The complete internalization of PEGylated liposomal curcumin into the colorectal cells after 2 h of treatment with a 10 μmol/L concentration was observed and was comparable to that of free curcumin. The in vitro growth-inhibitory effect of liposomal curcumin was less than that of oxaliplatin for LoVo cells and equivalent for Colo205 cells. PEGylated liposomal curcumin and oxaliplatin both induced PARP (an enzyme involved in DNA damage and repair mechanisms) cleavage, but the combination of liposomal curcumin and oxaliplatin produced an increase in PARP cleavage in LoVo cells. The suppressive effects of liposomal curcumin in the growth of Colo205 and LoVo tumors in a murine xenograft model were equal to or greater than those of oxaliplatin. The combination of liposomal curcumin and oxaliplatin at a 4:1 molar ratio resulted in a synergistic, enhanced growth-inhibitory effect.[78]

Kunwar et al. synthesized curcumin-loaded liposomes with phosphatidylcholine, cholesterol, and human serum albumin (HSA), and made quantitative estimations on the loading of curcumin from phosphatidylcholine liposomes and HSA to cellular systems. The curcumin was entrapped in the gel phase of liposome formulation and was located in the vicinity of tryptophan (hydrophobic environment) in HSA, which was confirmed by steady-state fluorescence anisotropy measurements. The average binding affinity of curcumin to HSA was higher compared with liposomes. Cell uptake studies for liposomal and HSA formulation in normal mouse lymphocytes and the mouse T-lymphoma cell line EL4 revealed that liposomal formulation was efficiently taken by cells compared with HSA and free curcumin. The study also found that the uptake of curcumin was significantly higher in EL4 cells (nearly 1.5 times, $P < 0.05$) with all of the vehicles compared with splenic lymphocytes, indicating that tumor cells showed preferential uptake of curcumin.[79]

Wang et al. used the liposomal curcumin protocol for the delivery of the drug to head and neck squamous cell carcinoma cell lines (CAL27 and UM-SCC1), and showed growth inhibition in vitro and suppression of tumor growth in nude mice. The lipid polymers used for the preparation of liposomes were 1,2-dimyristoyl-sn-glycero-3-phosphocholine and 1,2-dimyristoyl-sn-glycero-3-phospho-rac-(1-glycerol). The liposomal curcumin inhibited cell growth significantly ($P < 0.0001$) compared with the empty liposome treatment. Growth suppression by liposomal curcumin was not as dramatic as that by curcumin dissolved in DMSO, although this may have been secondary to the toxicity of DMSO alone to the cells. The liposomal curcumin showed dose-dependent cytotoxicity to CAL27 and UM-SCC1, with optimal growth suppression at 200 μmol/L and 50 μmol/L, respectively. The liposomal curcumin treatment of CAL27 and UM-SCC1 in the concentration range of 50 to 600 μmol/L suppressed the activation of NFκB in both cell lines, with a maximal reduction of 40% observed at 200 μmol/L in CAL27 and 25% at 400 μmol/L in UM-SCC1 cells. There was a 55% and 33% reduction in pI_kB_α levels in the CAL27 and UM-SCC1 cells, respectively, obtained with a 50 μmol/L concentration of liposomal curcumin, while there was no effect on expression levels of pAKT. The curcumin level in 5-week-old female athymic nude mice (nu/nu; Harlan Laboratories, Indianapolis, IN, USA) after intravenous injection of 1 mg/100 μL liposomal curcumin was detected to be over 5 μg/mL in the serum and 0.07 pmol in 0.1 g of liver at 2 h, which decreased with time, was not detectable at 48 h in serum, and decreased by 8-fold in liver after 4 h. In contrast, in DMSO curcumin-injected mice, there was an absence of detectable curcumin with lysis of red blood cells in the plasma, whereas curcumin was detected in the liver. Xenograft tumors in nude mice treated with liposomal curcumin (mean tumor weight, 33.09 mg) showed tumor growth suppression compared with control mice (mean tumor weight, 117.52 mg) and mice treated with liposomes alone (mean tumor weight, 89.36 mg).[80]

Chen et al. formulated curcumin-loaded liposomes in a molar ratio with three different lipid and curcumin compositions: i) lipo-curcumin-DMPC:dimyristoyl

phosphatidylglycerol (DMPG):cholesterol:curcumin (7:1:8:0.5); ii) high-lipo-curcumin- DMPC:DMPG:cholesterol:curcumin (70:10:80:0.5); and iii) high-lipo-high-curcumin-DMPC:DMPG:cholesterol:curcumin (70:10:80:1.5). The stability of all three complexes was compared with free curcumin, and it was found that only 16% of the free curcumin remained in PBS (pH 7.2), indicating that it gradually degraded. Liposomal curcumin was quite stable, with maximum stability in the high-lipo-curcumin formulation (100% remain) in 180 min. Only 25% degradation of free curcumin occurred in whole blood (pH 7.4, 37°C), and there was no degradation in the high-lipo-curcumin formulation after 180 min of incubation. The drug-free liposomes DMPC and DMPC/DMPG (7:1) were toxic to human lymphocytes, splenocytes, and Epstein-Barr virus-transformed B-cells, which was attributed to more cellular uptake by liposomes. The toxicity of these liposomes was nearly eliminated by adding cholesterol in a 1:1 molar ratio of lipid to cholesterol,[81] The reduced toxicity of cholesterol to the cells might have been due to the stabilizing and protective effect caused by the decreasing lipid bilayer hydration, improving the quality of liposome preparations, or decreasing either the direct effects of liposomes at the cell surface or the secondary effects after the liposomes had undergone endocytosis.[82–84] Liposomal curcumin inhibited Epstein-Barr virus-transformed human B-cell proliferation and concanavalin A-stimulated human lymphocyte and splenocyte proliferation similar to or stronger than free curcumin.[81] Encapsulation efficiency in liposomes depends on the type of lipid used for the preparation. Liposomal formulations were made at a 1:10 curcumin:lipid (DMPC, dipalmitoylphosphatidylcholine [DPPC], egg phosphatidylcholine) ratio (w/w basis) of about 100 to 150 nm. DMPC-based liposomes allowed the greatest amount of curcumin to be intercalated into the lipid membrane followed by DPPC, whereas egg phosphatidylcholine-based liposomes had the lowest amount of curcumin intercalation. DPPC and DMPC liposomal curcumin had more efficacy compared with free curcumin in inhibiting the proliferation of the prostate cancer cells LNCaP and C4-2B. However, DMPC liposomal curcumin was found to be the most effective, and control liposomes showed toxic effects (10%–15%) at higher doses.[83,85] Narayanan et al. tested the hypothesis that liposome-encapsulated forms of resveratrol and curcumin in combination may inhibit prostate cancer by increasing their bioavailability synergistically and enhancing the anticancer effects. The liposomal formulation of curcumin (lipo-curcumin) and resveratrol (lipo-resveratrol) were prepared with lipid DMPC in 1:5 ratios. For combination, the lipo-curcumin and lipo-resveratrol (2.5 mg/kg/body weight each) were mixed using a 3-way adjuvant mixer. The serum level after oral administration in male B6C3F1/J mice was determined to be 100 ng/mL after 1.5 h with liposomal curcumin. However, the mice that received lipo-curcumin co-administered with resveratrol showed a higher serum concentration of curcumin 252 ng/mL, which was stable for up to 4 h in the range between 245 and 238 ng/mL and 151 ng/mL in prostate tissue after 3 h, and was stable for up to 6 h in the range between 151 and 148 ng/ml compared with curcumin alone. These findings revealed that the mean genitourinary tract and prostate weights were remarkably reduced in mice that received lipo-curcumin

co-administered with resveratrol, indicating a decrease in the tumor growth and a 5-fold increase (55%–62%) in the rate of apoptosis in PTEN (phosphatase and tensin homolog)-CaP8 cells. Furthermore, these agents in combination had an inhibitory effect on the pAkt (Ser473), AR (androgen receptor), cyclin D1, and mTOR (mammalian target of rapamycin) proteins in PTEN Cap8 cells with a loss of PTEN, suggesting that these agents can target multiple mechanisms of prostate carcinogenesis, and indicating a synergistic interaction between these two phytochemicals in promoting anticancer effects.[86]

3. Nanoemulsions

Nanoemulsions are isotropic, highly kinetically stable, transparent (or translucent) systems of oil, water, and surfactant, frequently in combination with a co-surfactant having a droplet size usually in the nanometer range (typically less than 200 nm).[87] Although nanoemulsions are chiefly seen as vehicles for administering water-insoluble drugs, they have more recently received increasing attention as colloidal carriers for targeted delivery of various anticancer drugs due to their sub-micron size, which makes it possible to target the tumor area for improved efficacy and/or reduced toxicity.[88] Ganta et al. examined augmentation of therapeutic efficacy upon co-administration of curcumin and paclitaxel, an inhibitor of NFκB and a potent down-regulator of ABC (ATP-binding cassette) transporters, in wild-type SKOV3 and drug-resistant $SKOV3_{TR}$ human ovarian adenocarcinoma cells. Paclitaxel and curcumin-containing nanoemulsion formulations were prepared using the high-energy ultrasonication method. The optimized nanoemulsion formulations consisted of paclitaxel or curcumin (0.2% w/v), flaxseed oil (20% w/v), egg phosphotidylcholine (lecithin) (1.2%, w/v), and deionized water. Additionally, PEG-modified nanoemulsions were prepared by incorporating 0.3% w/v of distearoylphosphatidylethanolamine-PEG2000. The average particle size of the blank nanoemulsion (without any drug) was 133 ± 1.5 nm, which did not change with the incorporation of paclitaxel or curcumin, and the average surface charge of the oil droplets was in the range of −35.37 to −44.53 mV. The encapsulation efficiency of both plain and PEG-modified nanoemulsions was 100% for paclitaxel and 97% for curcumin, and these nanoemulsions were stable for 3 months. Qualitative cellular uptake analysis demonstrated that the nanoemulsion formulations were efficiently internalized in SKOV3 and $SKOV3_{TR}$ cells. The curcumin nanoemulsion was much more efficient in down-regulating P-glycoprotein and inhibiting NFκB in tumor cells compared with curcumin and curcumin-PEG-modified nanoemulsions. In the presence of curcumin solution, the IC_{50} of the paclitaxel solution was reduced to approximately 1.8-fold in $SKOV3_{TR}$ cells and 2.3-fold in SKOV3 cells. This combination therapy was significantly effective ($P < 0.05$) when the paclitaxel and curcumin were delivered in nanoemulsion formulations compared with when they were delivered in solution or in PEG-modified nanoemulsion. It was concluded that nanoemulsions were effective in delivering the paclitaxel and curcumin to the cells, and combination therapy with paclitaxel and curcumin

delivered in nanoemulsions indeed showed higher therapeutic efficacy in SKOV3 and SKOV3TR cells.[89]

4. Polymeric Micelles

Polymeric micelles are nano-sized assemblies of spherical or globular structures created from amphiphilic polymers that spontaneously form nano-sized aggregates when the individual polymer chains are directly dissolved in aqueous solution (dissolution method) above the critical micelle concentration and the critical micelle temperature. In an aqueous medium, micelles consist of a hydrophilic shell that minimizes clearance by the mononuclear phagocytic system and a hydrophobic core that acts as a host for hydrophobic drugs. Polymeric micelles have several advantages over conventional delivery, such as low toxicity, high stability, and small size (less than 200 nm), which makes them ideal for passive targeting of solid tumor tissue sites by the EPR effect. The alkaline stability of curcumin can be increased by forming curcumin-encapsulated cationic micelles with the small molecular weight surfactant dodecyl trimethylammoniumbromide and cetyl trimethylammoniumbromide.[90]

Ma et al. investigated amphiphilic block copolymer micelles of poly(ethylene oxide)-b-poly(e-caprolactone) (PEO-PCL) as vehicles for the solubilization, stabilization, and controlled delivery of curcumin for cancer. Curcumin-loaded PEO-PCL micelles were prepared by a co-solvent evaporation technique using curcumin and PEO-PCL copolymer (PEO_{5000}, PCL_{5000}, PEO_{5000}-PCL_{13000}, PEO_{5000}-PCL_{24500}) in different ratios (1:20, 2:20, 4:20). The level of curcumin encapsulation increased with increase in molecular weight of the PCL block (5000–24500 g/mol) at all ratios, with an average diameter of micelles in the range of 64.6 to 198 nm. It was also observed that with an increase in applied curcumin, the molar ratio of loaded curcumin to polymeric micelles with 24,500 g/mol of PCL was also increased from 2.36 to 3.47 and 4.73 in the ratio 1:20, 2:20, and 4:20, respectively, and maximum curcumin solubility of 628 μg/mL with a 4:20 ratio of drug and polymer was achieved. The curcumin encapsulated in PEO_{5000}-PCL_{13000} micelles was stable up to 8 h in PBS (pH 7.4), while in presence of HSA in PBS, 90% of the micelles remained similar to those with free curcumin under similar conditions. The in vitro release of curcumin from free curcumin was 98.8% (6 h) and 89.5%, 36.0%, and 56.1% from polymeric micelles with 5000, 13,000, and 24,500 g/mol of PCL, respectively, after 24 h in distilled water. The release of curcumin increased from 36% to 70.6% from PEO_{5000}-PCL_{13000} micelles in the presence of HSA in distilled water at 24 h and 98.5% after 4 d, revealing sustained release of the drug, while it decreased from 98.8% to 68% at 6 h from free curcumin. The cytotoxicity of free and micelle-encapsulated curcumin was dose dependent in B16-F10 (mouse melanoma cells) cells and MCL (mantle cell lymphoma) cells. Micelle-encapsulated curcumin was significantly less toxic than free curcumin at a higher dose of 80 μM after 24 h of incubation, but became equally cytotoxic as the free drug after 96 h of incubation. This might have been because micelles prevent the direct interaction between

encapsulated curcumin and melanoma cells at early time points of incubation. However, at 96 h, the level of drug release or micellar internalization provides enough drug concentration within the cell for a similar effect to that of free drug. Interestingly, encapsulated curcumin was found to be significantly more toxic than free curcumin at lower curcumin concentrations (10 μM).

In Mino and JeKo-1 cells (MCL cells), micelle-encapsulated curcumin was found to be less toxic than free drug at high doses, but more toxic than free drug at low doses.[91] Sahu et al. synthesized a novel biodegradable and self-assembling methoxy poly(ethylene glycol)-palmitate nanocarrier for curcumin delivery to cancer cells with methoxy poly(ethylene glycol) (mPEG) as the hydrophilic and palmitic acid as the hydrophobic segment. The conjugate (mPEG-PA) prepared in a single-step reaction had ester linkage, which is biodegradable and showed minimal toxicity on HeLa cells. The mPEG-PA conjugate formed micelles in the aqueous solution with critical micelle concentration of 0.12 g l^{-1}. The mean diameters of the blank and drug-loaded micelles were 41.43 and 47.36 nm, respectively. The encapsulation efficiency of curcumin in mPEG-PA micelles was 31% when the drug-to-conjugate ratio (w/w) was 0.05, and close to 100% at a 0.01 ratio, indicating that encapsulation efficiency increased with increasing amounts of mPEG-PA. Drug retention inside the mPEG-PA micelles after 48 h of incubation was over 92%, 80%, and 84% in physiological conditions (pH 7.4), simulated gastric fluid (pH 1.2), and simulated intestinal fluid (pH 6.8), respectively. In vitro lipase-catalyzed release of curcumin from the core of the micelle was 92% after 48 h, and 26% release from the HeLa cell lysate after 24 h, which indicated cleavage of the ester bond of the conjugate and enzyme-triggered drug release.[92]

In another study, hydrophobically modified starch was used to form polymer micelles and to encapsulate curcumin for increased anticancer activity in vitro. The polymer micelles were formed at 0.36% critical aggregation concentration of hydrophobically modified starch, and the solubility of curcumin in these micelles was 1670-fold more than that in distilled water, which may have been due to the combination of hydrophobic microenvironment of curcumin and hydrogen bonding between the modified starch and curcumin. The in vitro anticancer activity of curcumin encapsulated in hydrophobically modified starch against HepG2 cells was significantly higher than that of DMSO-dissolved curcumin,[93] which was similar to that of the curcumin-casein micelle complex.[94]

The use of nanoparticulate drug-delivery methods for the encapsulation of curcumin has revolutionized cancer treatment with herbal bioactives. The encapsulation of curcumin in the core of a lipid or polymer increases the aqueous solubility of curcumin and shows a promising stability over a wide range of pH values. Furthermore, the curcumin released from these systems over a period of 2 to 5 d showed longer duration of action, which may have also been due to the increased half-life of the drug in the body. The small size of curcumin-loaded nanoparticles also provided for passive targeting of tumors via the EPR effect. The in vitro cell line study showed that curcumin-loaded nanoparticle were efficiently taken up by the cells and were more cytotoxic to the cells than free curcumin. These nanoparticles are suitable for oral as well as intravenous administration

depending on the route for which they are developed. Since the solubility of curcumin in lipids is less, this is the main limitation for curcumin drug delivery because it leads to less drug loading in formulation. This could be overcome by using a lipid or polymer in which curcumin is more soluble.

III.B. Irritable Bowel Disease

IBD is one of the most common functional disorders of the gastrointestinal tract, and is characterized by Crohn's disease and ulcerative colitis. Symptoms include abdominal pain, altered bowel habits, changes in stool frequency, and frequent complications of and risk for colorectal cancer in humans.[95–97] Several cytokines, including TNF-α and IL-1β, have been shown to be up-regulated in IBD, and they amplify and perpetuate tissue damage.[98] Current therapy for IBD involves the use of immunosuppressives such as corticosteroids, azathioprine, mercaptopurines, ciclosporin, and monoclonal antibodies against TNF-α.[99,100] However, these agents are expensive and sometimes limited by drug-induced toxicity. Curcumin is effective against IBD due to its anti-inflammatory effects, which involve reductions in myeloperoxidase activity, in the number of infiltrating neutrophils, and in the expression of IL-1β. Some recent studies have also shown the effect of curcumin in reducing the clinical symptoms of IBD in human patients.[101,102] However, the therapeutic efficacy of curcumin is reduced due to a lack of proper delivery. Such problems can be addressed by the incorporation of curcumin into a colloidal carrier system. Yadav et al. prepared and evaluated the anti-inflammatory activity of solid lipid microparticles (SLMs) of curcumin for the treatment of IBD in a colitis-induced rat model using a colon-specific delivery approach. Curcumin-loaded SLMs were prepared by the microemulsion technique using soy lecithin, stearic acid, and palmitic acid as a lipid phase (1%–10% w/w), Poloxamer-188 (0.5% w/w) as an emulsifier, and water at a temperature range of 35°C to 40°C. The particle size and entrapment efficiency of SLMs were found to be in the range of 108 to 342 nm and 58% to 79%, respectively; the minimum particle size (108 ± 0.25 nm) and maximum entrapment efficiency (79.24 ± 0.21%) was with stearic acid in a 1:1 ratio of curcumin and lipid. The entrapment efficacy decreased as the amount of lipid increased due to the insolubility of the curcumin in lipid. The stearic acid formulation had enhanced release in the in vitro dissolution media compared with pure curcumin and other formulations, indicating the amorphous nature of curcumin in formulation. The chorioallantoic membrane study showed vascular regression and zones that were devoid of a capillary network, which indicated that the angiostatic activity of SLMs of curcumin was more than that for pure curcumin. Dextran sulfate sodium-induced colitis in rats treated with SLMs of curcumin showed minimal changes in the surface epithelium and no infiltration of inflammatory cells to the mucosa (reduced level of disease activity index) compared with those receiving pure curcumin. That study demonstrated that the degree of colitis caused by administration of dextran sulfate sodium is significantly attenuated by the SLM formulation of curcumin.[103]

III.C. Restenosis

Restenosis means the recurrence of stenosis, a narrowing of blood vessels that leads to restricted blood flow. Restenosis after percutaneous coronary intervention continues to be a serious problem, limiting the long-term clinical outcome in approximately 20% to 40% of patients.[104,105] Studies have revealed that curcumin is effective in suppressing the proliferation of a wide variety of cells by down-regulating transcription factors and by inhibiting the expression of COX-2 and other mediators.[57] However, there have been no reports investigating curcumin as an antirestenotic agent in vivo. Jang et al. developed a curcumin-coated stent using a dip-coating method and investigated the antirestenotic effect in an animal model and in vitro. Curcumin inhibited platelet-derived growth factor-induced proliferation of vascular smooth cells in a dose-dependent manner at concentrations of 1 to 10 μM, and this was maintained over a period of 3 d. The drug release from high-dose (30 mg/mL) and low-dose (10 mg/mL) curcumin-coated stents was characterized by an exponential function, starting with a burst release of about 30 μg in both. However, there was a significant difference in the duration of drug maintenance between the two: about 3 d for the low-dose and 21 d for the high-dose stents. In an in vivo test (rabbit iliac artery stent model), the curcumin-eluting stent was shown to decrease neointimal formation and to increase vessel area in a dose-dependent manner. The high-dose curcumin-eluting stent was more effective in reducing restenosis than the low-dose one.[106]

In another study, a drug-eluting stent of curcumin was developed by electrophoretic deposition technology using PLGA nanoparticles embedded with curcumin. Curcumin-loaded PLGA nanoparticles prepared using polyacrylic acid as a surfactant were 276.0 ± 3.4 nm in size and had a zeta potential of –53.5 ± 5.8 mV. The amount of curcumin deposited onto the stent in 50% ethanol medium was about 0.71 μg, compared with 491 μg in 100% deionized water. The higher the concentration of ethanol, the lower the amount of curcumin; in particular, curcumin dramatically decreased at ethanol concentrations above 30%. However, no studies have been performed on the antirestenotic effect.[107] Pan et al. developed curcumin-eluting stents with PLGA as the drug carrier using the ultrasonic spray method. After loading curcumin in the PLGA coating, the roughness of the stent (0.55 nm) was greater than that for the PLGA-only coated stent (0.14 nm). However, no drug particles were seen on the stent surfaces (smooth and uniform), indicating that curcumin was mixed with PLGA at the molecular level using an ultrasonic atomization spray method.[108] A smooth surface coating can significantly decrease injury to blood vessels and reduce platelet activation and aggregation, consequently leading to less thrombus formation and neointimal proliferation.[109]

The sustained release of antirestenotic drugs for at least 3 weeks is required to prevent smooth muscle cell migration and proliferation.[110] The in vitro release of curcumin from curcumin-loaded stents exhibited a nearly linear sustained-release profile, with no significant burst release over a period of 18 d. The

sustained-release duration of curcumin was approximately 27 d for low-dose (140 μg/stent) stents, 38 d for medium-dose (280 μg/stent) stents, and 59 d for high-dose (490 μg/stent) stents in PBS (pH 7.4). The in vitro anticoagulation studies of drug-eluting films with different curcumin doses and control films were done by platelet adhesion measurements and activated partial thromplastin time tests. Drug-eluting films with different drug doses showed significantly less platelet adhesion and aggregation than the PLGA-only film and the stainless steel plate. Adhered platelets on the surface of curcumin-loaded film decreased significantly with increasing drug dose. The curcumin released from drug-loaded films might inhibit the cyclooxygenase pathway by blocking the GPllb/llla receptor, inhibiting platelet aggregation and leading to the formation of blood clots. The activation of the intrinsic blood coagulation system was suppressed by curcumin release from the drug-eluting stent, and the extent of suppression was related to the curcumin content.[108]

III.D. Anti-inflammatory and Wound-Healing Effects

Curcumin, with its impressive antioxidant and anti-inflammatory properties, has been used in treating a wide array of diseases, including psoriasis, diabetic wound healing, Alzheimer's disease, and rheumatoid arthritis. The anti-inflammatory effect of curcumin is mediated through its ability to inhibit COX-2, lipoxygenase, and inducible nitric oxide synthase, important enzymes that mediate inflammatory processes. Since most inflammatory diseases occur locally and near the surface of the body, topical application of curcumin can offer the advantage of delivering a drug directly to the disease site and producing its local effect. However, the barrier properties of intact skin (stratum corneum) limit the permeability of wide variety of substances, including pharmaceutical active agents. Patel et al. addressed the anti-inflammatory effects of curcumin in a topical gel formulation. Topical gel was formulated with hydroxyl propyl cellulose and Carbopol® 934 polymer (Lubrizol Corp., Wickliffe, OH, USA) using menthol as a penetration enhancer. The permeability of curcumin in formulation was enhanced in the presence of menthol and was concentration dependent. A constant flux up to 5% menthol was observed, which gradually increased with increased concentrations of menthol (7.5% or more) in both types of gel formulations. There was an 8-fold and 7-fold increase in the permeability of the drug observed from the Carbopol® 934 and hydroxyl propyl cellulose gel containing 12.5% w/w of menthol, respectively. Carbopol® 934, hydroxyl propyl cellulose, and standard gel (gel containing 2 mg of diclofenac) formulations showed better reduction in paw volume (edema inhibition) in Wistar albino rats compared with control formulations. Carbopol® 934 and hydroxyl propyl cellulose gel formulations (containing 4 mg of curcumin) showed a similar effect as that of the standard gel formulation.[111]

In another study, in vitro and in vivo skin absorption of curcumin was investigated after the application of enhancers using Wistar rats as an animal model. Cyclic monoterpenes (terpineol, carveol, and eugenol) generally showed higher activity in promoting curcumin permeation than the other enhancers (hespere-

tin, farnesol). Terpineol provided the best enhancing activity on curcumin flux, followed by carveol and nerolidol. Hydrogels of curcumin using carboxymethyl cellulose with different counter ions of Na^+ and NH_4^+ were formulated. There was no significance difference between the flux and skin reservoir of curcumin from pH 7.4 buffered solutions and 3% carboxymethyl cellulose-Na hydrogel, indicating comparable flux and skin deposition to those of solution vehicle. However, the hydrogel composed of 3% carboxymethyl cellulose-NH_4 completely retarded curcumin flux without affecting the partitioning of curcumin into the skin. The skin deposition of curcumin after in vivo topical application of curcumin hydrogels showed that eugenol had the highest enhancement of curcumin partitioning to the skin at 2 h, but was comparable to that of terpineol at 8 h. Terpineol produced the highest transepidermal water loss values (a parameter used for assessment of the skin irritant potential), whereas ketocholestanol produced a negligible increase in water loss as compared with control.[112]

Wang et al. encapsulated curcumin in an oil-in-water (o/w) nanoemulsion in order to improve its anti-inflammation activity, which was evaluated by using a mouse ear inflammation model. A nanoemulsion was prepared with a high-pressure homogenizer and high-speed homogenization using medium-chain triacylglycerols as oil and Tween 20 as an emulsifier, with mean droplet sizes ranging from 618.6 to 79.5 nm. The nanoemulsion viscosity increased slightly as the average droplet diameter decreased because the number and the interfacial area of droplets became larger as the droplet size decreased. The emulsion showed no change in physical properties (droplet size and viscosity) during 7 d of storage, and there was a nearly unchanged absorption peak of curcumin at pH between 5.0 and 5.5. The oral administration of 1% curcumin in Tween 20 water solution showed little or no inhibition effect of 12-O-tetradecanoylphorbol-13-acetate-induced edema of mouse ear. Nevertheless, 1% curcumin encapsulated in o/w emulsions of 618.6 and 79.5 nm showed 43% to 85% inhibition, respectively. This revealed synergistic effects of both emulsion droplet size and the presence of lipid in the emulsion that provide the optimum anti-inflammatory activity of curcumin.[113]

Lin et al. formulated a curcumin-encapsulated o/w microemulsion system using food-acceptable components, lecithin, and Tween 80 as the surfactants and ethyl oleate as the oil phase. The solubilities of curcumin in soybean oil, isopropyl myristate, peppermint oil, and ethyl oleate were 0.02, 0.03, 0.03, and 0.04 wt%, respectively. The curcumin-encapsulated microemulsion formulated with isopropyl myristate became turbid after 1 week, while the microemulsion with ethyl oleate remained transparent even after 2 weeks. The mean diameters of microemulsion droplets were gradually increased at d 14 in the microemulsion formulation containing ethyl oleate, with more than 0.5% wt% or less of the surfactant ratio due to the aggregation of oil droplets. A microemulsion containing wt% 0.4% ethyl oleate was stable with no change in mean droplet diameter (72.8 ± 2.76 nm) for up to 14 d at room temperature (25°C). Moreover, it was also stable in a long-term study (60 d) at 4°C with almost similar mean droplet diameter (71.8 ± 2.45 nm). Three microemulsion formulations containing 10 mg (MEC1), 20 mg (MEC2), and 50 mg (MEC3) of curcumin per 10 mL of microemulsion solution

were tested for in vitro skin permeation. Time-dependent increases in permeated curcumin were observed with MEC1 and MEC2. On the contrary, MEC3 exhibited lower permeation activity in a non-time-dependent manner compared with the results of MEC1 and MEC2 due to an abrupt increase in droplet diameter (1 μm), which led to the breaking of thermodynamic stability.[114]

IV. CONCLUSION AND FUTURE PROSPECTS

A plethora of research studies in the last half-century have revealed that curcumin has wide therapeutic actions such as antiinflammatory, antidiabetic, anti-spasmodic, antimicrobial, anticancer, hepatoprotection, and neuroprotection effects. However, as we have discussed, these effects are negligible when curcumin is given to patients because of its poor bioavailability. Multifarious approaches have been developed for the formulation of curcumin to improve its bioavailability and tissue targeting. Among them, nanocurcumin opens up avenues for systemic therapy of human cancer, as well as other human diseases such as Alzheimer's, diabetes, IBD, and cystic fibrosis, for which the beneficial effects of curcumin have been propounded, but extensive research is still needed. Overall, nanoparticle-based systems for curcumin delivery are still in their infancy, and much progress is warranted in the area of formulation development for effective delivery. Future studies using relevant experimental models will address these scenarios in an in vivo setting, and should facilitate the eventual clinical translation of nanocurcumin.

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Inhalational Therapy for Pulmonary Arterial Hypertension: Current Status and Future Prospects

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ABSTRACT: This review summarizes the pathophysiology and current therapeutic and drug delivery strategies for pulmonary arterial hypertension (PAH), a rare but devastating disorder of the pulmonary circulation affecting 50,000 to 100,000 persons in the United States. Chief clinical features of PAH include increased mean pulmonary arterial pressure (>25 mm Hg) and right ventricular and smooth muscle hypertrophy. A wide variety of agents have been studied for use as anti-PAH drugs, including prostacyclin analogues, endothelin receptor antagonists, and phosphodiesterase-5 inhibitors, to name a few. However, a major shortcoming of anti-PAH medications is their short half-lives, requiring them to be administered via parenteral routes, which lead to undesirable side effects, including systemic vasodilation. Inhalational delivery of anti-PAH drugs provides an attractive alternative to conventional routes, with ease of administration and minimal systemic vasodilation. Recently, the U.S. Food and Drug Administration approved inhalable iloprost (Ventavis®), a prostacyclin analogue, for PAH treatment. Other drugs being studied for their potential in inhalable PAH therapy include PGE1, treprostinil, vasoactive intestinal peptide, and fasudil. Controlled-release inhalable delivery systems for anti-PAH medications have also been proposed to facilitate long-term and selective vasodilation of pulmonary arteries. Extensive studies are warranted to develop safe and effective drug delivery systems that will provide a better quality of life to patients.

KEY WORDS: pulmonary arterial hypertension, mean pulmonary arterial pressure, prostacyclin analogues, rho-kinase inhibitors, inhalational therapy

I. PULMONARY ARTERIAL HYPERTENSION—THE DISEASE

Pulmonary arterial hypertension (PAH) is a disease of the pulmonary circulation that belongs to a major subdivision of a complex disease called pulmonary hypertension (PH). Left-sided heart disease is reported to be one of the most common factors contributing to PH. However, PH patients often die from right ventricular failure, the most prominent outcome of PAH.[1] For this reason, both terms, PH and PAH, are used interchangeably. PAH severely impairs the ability of affected individuals to perform daily activities and many routine chores.

This invariably fatal disorder is associated with complex pathophysiological and molecular mechanisms that include vasoconstriction, increased mean pulmonary arterial pressure (MPAP), vascular wall remodeling, *in situ* thrombosis, and right ventricular failure.[2] In recent years there has been tremendous advancement toward understanding PAH pathogenesis and its management. Although there are a number of drugs available for the treatment of PAH, there is still no cure; death is inevitable from cardiopulmonary and drug-delivery-related complications. Because the disease is localized in the pulmonary arteries, the pulmonary route of drug administration has emerged as one of the most attractive avenues for treatment of PAH. In this review, we have summarized recent developments in aerosolized drug delivery for PAH treatment and progress toward understanding PAH pathogenesis, diagnosis, and its molecular mechanism.

PAH affects approximately 50,000 to 100,000 persons in the United States,[3] with approximately 300 new cases diagnosed each year. The estimated mean survival time among patients with untreated PAH is 2.8 years in adults and less than 1 year in children. Females of child-bearing age are two to three times more prone to PAH than males. Typical PAH patients are usually the young and childbearing women in their 30s.[4] The pathophysiology of PAH is poorly understood, but the disease is considered to be a multi-factorial disorder, with multiple mechanisms responsible for its development and progression; it is often described as a cascade of diseases rather than a single disease.

PAH was first identified by Dr. Ernst Von Romberg in 1891, although it was not until 1951 that it was first reported in the literature. However, the development of drug therapy for PAH and progress toward long-term management of the disease have been relatively slow, perhaps because the disease affects only a small number of persons compared to other cardiovascular disorders. The National Institutes of Health Registry defines PAH as an elevated mean pulmonary arterial pressure (MPAP) of more than 25 mm Hg at rest, or 30 mm Hg after exercise (compared to normal MPAP of 12–16 mm Hg).[5] Increased MPAP, the principal manifestation of PAH, is associated with several other complications, including vascular wall remodeling, increased pulmonary vascular resistance, smooth muscle hypertrophy, intimal hyperplasia, *in situ* thrombus formation, and right ventricular hypertrophy (RVH).

The symptoms of PAH are rather nonspecific—dyspnea, fatigue, nonproductive cough, syncope, angina pectoris, peripheral edema, and hemoptysis—which is one of the main reasons that the disease can remain undiagnosed for many years. Furthermore, progression of PAH is usually slow; the symptoms take 2 to 3 years to develop. Thus, patients may delay seeking medical attention, which further complicates management of the disease. Moreover, such delay also contributes to the high mortality of PAH patients, due to right ventricular failure.

II. CLASSIFICATION OF PULMONARY HYPERTENSION

The World Health Organization (WHO) was the first to classify PH. In 1973, the WHO categorized PH into primary and secondary PH.[6] In the WHO clas-

sification, the term "PAH" includes all types of PH caused by diseases proximal to pulmonary capillaries. Later, in 1998, at the Second World Conference on Pulmonary Hypertension held in Evian, France, the classification was modified based on pathophysiology, clinical presentation, and therapeutic management. The classification was then further modified at the Third World Conference on Pulmonary Hypertension held in Venice, Italy, in 2003.

The Venice conference divided PH into five classes: Group 1 PH, known as PAH, is the largest of all the groups and comprises several subgroups: (i) idiopathic PAH (IPAH), which includes primary PAH without any known etiology, sporadic and with no familial history; (ii) familial PAH (FPAH), which clusters within a family; (iii) associated with PAH (APAH), which is associated with many other disease conditions; (iv) PAH associated with significant venous or capillary involvement; and (v) persistent PAH of the newborn. Group 2 PH is known as pulmonary venous hypertension, with disorders in the left side of the heart (atrial or ventricular, and/or valvular heart disease). Group 3 PH comprises PH associated with various lung diseases and/or hypoxemia. Group 4 PH comprises pulmonary hypertensive disorders due to chronic thrombotic or embolic disease such as thromboembolic obstruction of proximal or distal pulmonary arteries. Finally, Group 5 includes disorders that are very rarely associated with PH such as histiocytosis X, fibrosing mediastinitis, and sarcoidosis. At the Fourth World Congress on Pulmonary Hypertension in 2008, in Dana Point, California, the Evian-Venice classification was again modified to include information published over the previous 5 years and also to clarify ambiguity in the Venice classification.[7] Table 1 shows the Dana Point classification and the recent changes.[7] In this review we will focus on the pathogenesis, long-term management, and inhalational therapy for Group 1 PH.

Based on a wide array of pathophysiological findings, a histopathological classification system for PAH was proposed in 2004.[8] This classification helps in diagnosis of the disease based on the predominant pathological and co-existing changes.[9] In addition, the New York Heart Association (NYHA)/WHO divided PAH into four classes according to the severity of the symptoms (Table 2).[10]

III. PATHOPHYSIOLOGICAL MECHANISMS INVOLVED IN PAH

The pulmonary circulation is a low-pressure and high-flow vascular bed. Pulmonary vasculature receives the entire cardiac output. However, the pulmonary arterial pressure (PAP) does not necessarily change with the changes in cardiac output.[11] The blood vessels of the pulmonary vasculature are thinner than those of the systemic circulation. The smooth muscle cells in the pulmonary vessels are normally in a state of relaxation because of the presence of an opposing cascade of pulmonary vasodilators and vasoconstrictors. Although PAH is characterized by increased PAP and pulmonary vascular resistance (PVR), as well as decreased cardiac output, the left-sided pressures are reported to be normal, as represented by pulmonary capillary wedge pressure (PCWP) of $\leq$15 mm Hg.[2] As the disease progresses, there is an increased degree of vasculopa-

TABLE 1. Updated Clinical Classification of Pulmonary Hypertension (Dana Point, California, 2008)

1.	**Pulmonary Arterial Hypertension (PAH)**
1.1.	Idiopathic PAH
1.2.	Heritable
1.2.1.	BMPR2
1.2.2.	ALK1, endoglin (with or without heritable hemorrhagic telangiectasia)
1.2.3.	Unknown
1.3.	Drug and toxin induced
1.4.	Associated with
1.4.1.	Connective tissue diseases
1.4.2.	HIV infection
1.4.3.	Portal hypertension
1.4.4.	Congenital heart diseases
1.4.5.	Schistosomiasis
1.4.6.	Chronic hemolytic anemia
1.5.	Persistent pulmonary hypertension of the newborn
1.' Pulmonary veno-occlusive disease (PVOD) and/or pulmonary capillary hemangiomatosis (PCH)	
2.	**Pulmonary hypertension owing to left heart disease**
2.1.	Systolic dysfunction
2.2.	Diastolic dysfunction
2.3.	Valvular disease
3.	**Pulmonary hypertension owing to lung diseases and/or hypoxia**
3.1.	Chronic obstructive pulmonary disease (COPD)
3.2.	Interstitial lung disease
3.3.	Other pulmonary diseases with mixed restrictive and obstructive pattern
3.4.	Sleep-disordered breathing
3.5.	Alveolar hypoventilation disorders
3.6.	Chronic exposure to high altitude
3.7.	Developmental abnormalities
4.	**Chronic thromboembolic pulmonary hypertension (CTEPH)**
5.	**Pulmonary hypertension with unclear multifactorial mechanisms**
5.1.	Hematologic disorders: myeloproliferative disorders, splenectomy
5.2.	Systemic disorders: sarcoidosis, pulmonary Langerhans cell histolysis: lymphangioleiomyomatosis, neurofibromatosis, vasculitis
5.3.	Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders
5.4.	Others: tumoral obstruction, fibrosing mediastinitis, chronic renal failure on dialysis

Modified from Simmonneau et al.[7] with permission from Elsevier.
ALK1, activin receptor-like kinase type 1; BMPR2, bone morphogenetic protein receptor type 2; HIV, human immunodeficiency virus

TABLE 2. Functional Classification for Pulmonary Arterial Hypertension as Described by New York Heart Association (NYHA)/World Health Organization (WHO)

Class I	No limitation of usual physical activity. Ordinary physical activity does not cause undue dyspnea and pain, chest pain, or near syncope. Asymptomatic.
Class II	Slight limitation of physical activity; no discomfort at rest, ordinary activity causes undue dyspnea, fatigue, chest pain, or near syncope.
Class III	Marked limitation of physical activity; no discomfort at rest, but less than ordinary physical activity causes undue dyspnea, fatigue, chest pain, or near syncope.
Class IV	Inability to perform any physical activity without symptoms. Signs of right ventricular failure or syncope. Dyspnea and/or fatigue may be present at rest and discomfort is increased by any physical activity.

Modified from Rich.[10]

thy, vascular remodeling, smooth muscle hypertrophy, hyperplasia, and *in situ* thrombus formation. All of these pathological changes can cause narrowing of the pulmonary arteries and increased right ventricular afterload that eventually leads to RVH, dilatation, and subsequent RV failure and death.

The pathogenesis of PAH can be attributed to several factors, including exposure to toxins, inflammatory mediators, and genetic predisposition. The mediators that regulate vasodilation and proliferation in the pulmonary circulation include prostacyclins, nitric oxide, adenosine, and endothelium-derived hyperpolarizing factors, whereas the vasoconstricting factors are thromboxane A2, endothelin-1, hypoxia, serotonin, and interleukin. Imbalance among these mediators of vascular tone causes constriction of pulmonary vascular smooth muscle (Fig. 1). Pulmonary endothelial cell dysfunction also promotes the triad of vasoconstriction, thrombosis, and cellular proliferation. For this reason, it is suggested that pulmonary vasoconstriction can both be a cause or a secondary response to PAH development.

One of the characteristic changes that occurs in the pulmonary vasculature of patients with PAH is remodeling of blood vessels that are <500 μm in diameter. The remodeling occurs in all layers of cells of the vascular wall and includes hypertrophy, hyperplasia, and reduction in the number of small vessels. The roles of different cell types—endothelial cells, smooth muscle cells, and fibroblasts—involved in these processes will be discussed later in detail. In addition to the above changes, remodeling also stimulates deposition of connective tissue matrix substances such as fibronectin, collagen, and elastin and thus causes narrowing of the arteries. The possible causes of remodeling include higher MPAP, hypoxia, viral infection, lack of apoptosis, endothelial cell injury, and lack of anti-proliferative factors.

The development of *in situ* thrombi in small pulmonary arteries due to thrombin deposition in the lumen of arteries is another important characteristic of PAH pathogenesis. Thrombi develop because of the pro-coagulative

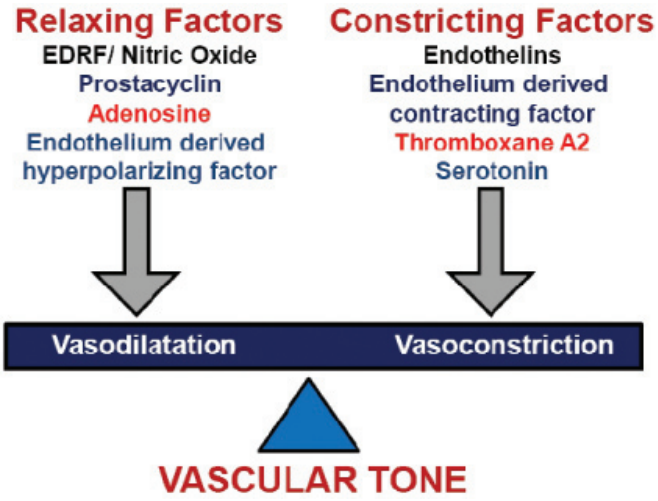

FIGURE 1. Factors responsible for maintaining vascular tone in the pulmonary circulation. EDRF, endothelium-derived relaxing factor.

environment created by interactions between platelets and growth factors. *In situ* thromboses may also contribute to the narrowing of the lumen of the pulmonary vessel.

In more advanced stages of the disease, occlusive changes in the pulmonary arteries and development of plexiform lesions can also occur because of progressive intimal hyperplasia and enhanced distal arterial muscularization. These events lead to development of aberrant channels in the lumen and adventitia of the vascular wall due to proliferation of apoptosis-resistant endothelial cells. Additional features of PAH include venous hypertrophy, thickening of pulmonary adventitia, macrophage infiltration, and increased expression of matrix proteins and transforming growth factor (TGF)-β. Overall, the common features of PAH include intimal fibrosis, increased medial thickness, vascular remodeling, pulmonary arteriolar occlusion, and plexiform lesions that are summarized in Figure 2.[12]

IV. GENETICS IN THE PATHOGENESIS OF PAH

Familial PAH (FPAH) has been reported to be an autosomal dominant disease. A gene associated with PAH was localized to a 3-cM region of chromosome 2q31-33.[13] Furthermore, mutations associated with the development of FPAH were determined to affect the gene encoding bone morphogenetic protein receptor II (BMPR II).[14] To date, more than 46 mutations have been reported in the BMPR II gene, 60% of which may be responsible for the development of PAH. Bone morphogenetic protein (BMP) is a member of the TGF-β superfamily and is synthesized by both endothelial and smooth muscle cells. It is responsible for inhibiting smooth muscle proliferation and inducing apoptosis. Mutation of

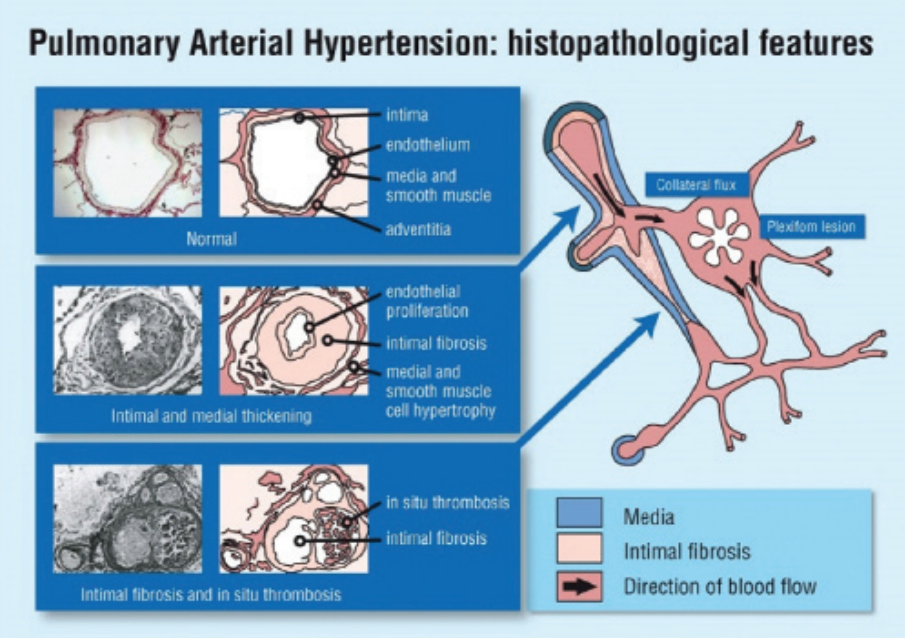

FIGURE 2. Schematic representation of different vascular abnormalities and histopathological features associated with pulmonary hypertension and compared with normal circulation throughout the pulmonary circulation. EC, endothelial cell; SMC, smooth muscle cell. (Reproduced from http://www.pah-info.com/What_is_PAH.[12])

BMPR II may disrupt the receptor's kinase activity, thus blocking the signaling pathway and resulting in excessive cell proliferation and vascular structural remodeling. Approximately 10% to 25% cases of IPAH and >50% of cases of FPAH have been identified to have mutations in BMPR II.[15]

V. CELLULAR MECHANISMS RESPONSIBLE FOR PAH DEVELOPMENT

As discussed above, remodeling of the small arteries of the pulmonary vasculature is the main pathological feature of PAH. In patients with PAH, all three layers of the pulmonary vascular wall—inner, media, and outer—undergo profound structural changes. Below is a brief discussion of the changes that may occur in cells of the pulmonary vascular wall upon development of PAH.

V.A. Endothelial Cells

Under normal physiological conditions, the vascular endothelium is responsible for maintaining the structure of blood vessels and the integrity of smooth muscle cells by secreting various vasoactive substances. However, when endothelial cells are damaged by hypoxia, inflammation, shear stress, or toxins, the link between smooth muscle cells and the barrier function of vascular endothelial

cells becomes disrupted, which can lead to vascular injury, vasoconstriction, and *in situ* thrombosis. As a result, smooth muscle cells proliferate and cause remodeling of the pulmonary vasculature. It has been reported that 90% of endothelial cells in PAH-associated lesions do not express TGF-β2 receptors, suggesting involvement of tumor-inhibiting genes in PAH pathogenesis.[16]

V.B. Smooth Muscle Cells

In patients with PAH, smooth muscle cells proliferate and enlarge; thus, the normally static tunica media layer of the pulmonary vasculature becomes hypertrophic. The intermediate cells and pericytes also begin to differentiate into new smooth muscle cells. Nonmuscular and partially muscular arteries start to muscularize and new muscular arteries develop. Increased proliferative index (PI), apoptotic index (AI), and PI:AI ratio are indicators of imbalance between hyperplasia and apoptosis contributing to pulmonary vascular remodeling. Also, various vasoactive substances secreted by smooth muscle cells may participate in pulmonary vascular remodeling and PAH progression.

V.C. Fibroblasts

Fibroblasts are present in the outer layer of blood vessels. Fibroblast proliferation, connective tissue deposition, and changes in extracellular matrix (ECM) can also contribute to pulmonary vascular remodeling. Up-regulation of collagen I and II and procollagen I and III mRNAs has been reported in the pulmonary arteries of PAH-induced rats. Furthermore, elevated levels of other ECM components, including elastin, fibronectin, collagen degradation regulatory enzymes, and metalloproteinase-I, have also been observed in the pulmonary arteries of these same rats.[17] Furthermore, many published reports suggest that adventitial fibroblasts play a major role in pulmonary vascular remodeling. Readers interested in this aspect of PAH pathogenesis are directed to an excellent review article by Stenmark et al. that elucidates the role of adventitial fibroblasts in pulmonary vascular remodeling.[18]

V.D. Inflammatory Cells

The levels of inflammatory mediators such as interleukin (IL)-1 and IL-6 increase with PAH progression.[19] Moreover, the histology of pulmonary hypertensive lungs shows infiltration of macrophages and lymphocytes, thus indicating the involvement of inflammatory cells in PAH.

The potential involvement of bone-marrow (BM)-derived progenitor cells in the pathophysiology of pulmonary hypertension has been studied extensively. In fact, BM progenitor cells are now suggested to play a role in endogenous maintenance and repair of the damaged vascular endothelium.[20–22] Treatment with progenitor cells has been demonstrated to prevent the development of PH in monocrotaline (MCT)-induced PAH rats and restore capillary continuity and

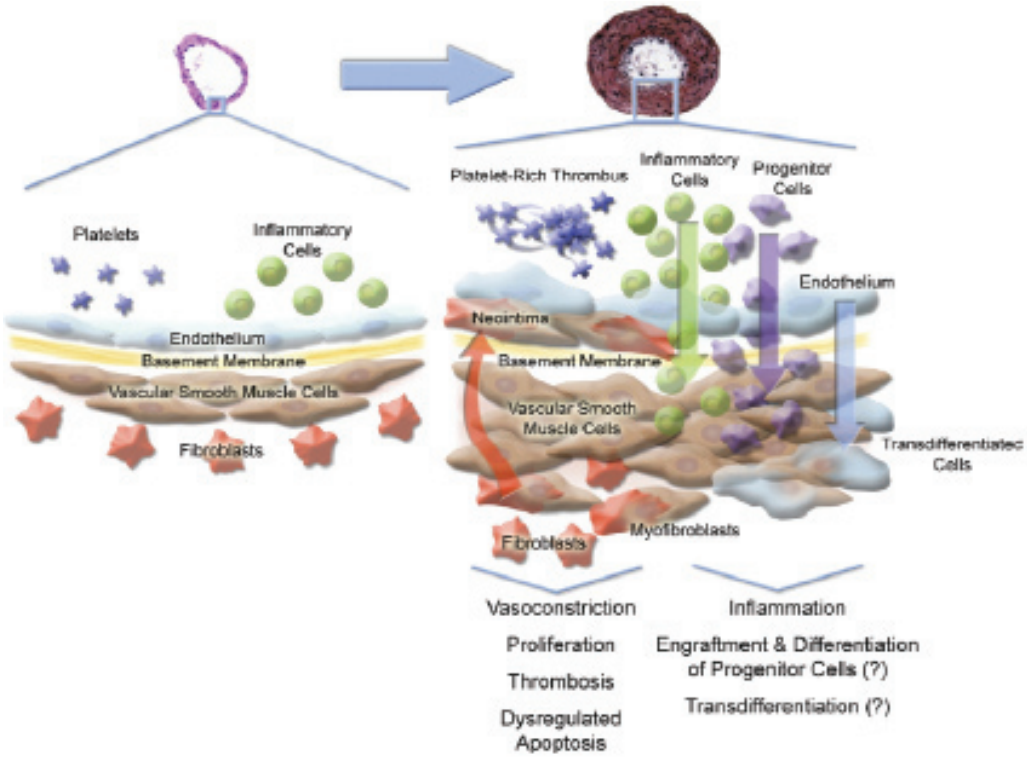

FIGURE 3. Diagrammatic illustration of different cell layers involved in the pathophysiology of pulmonary hypertension. The figure depicts different factors and phenotypic responses involved in histological progression of the pulmonary circulation from normal to pathogenically activated in pulmonary arterial hypertension. (Reproduced from Chan and Loscalzo[24] with permission from Elsevier.)

microvasculature architecture.[23] Bone marrow- and endothelial-derived progenitor cells are discussed in more detail below.

V.E. Platelets

As discussed above, *in situ* thrombosis is one of the main features of PAH. Development of thrombi in pulmonary arteries may be attributed to endothelium damage and thus induction and agglutination of platelets. These activated platelets not only create the thrombus, but they also promote vasoconstriction and remodeling of pulmonary blood vessels. Figure 3 describes the roles of the various cell layers of the pulmonary vasculature in PAH pathogenesis.[24]

VI. PAH—CLINICAL ASSESSMENT

Because the symptoms of PAH are vague and nonspecific, it is often difficult to diagnose the disease or it remains undiagnosed for many years. A series of

diagnostic tests is performed to determine the class of PAH and to evaluate the extent of hemodynamic and functional impairments. Because patients with PAH are more likely to experience dyspnea, a reduction in exercise tolerance, exertional chest pain, syncope, and fatigue, it is important to rule out other more common cardiopulmonary disorders before proceeding to the diagnosis of PAH.[25]

VI.A. Physical Examination

Individuals with PAH often demonstrate altered heart sounds, including loud P_2 (pulmonary valve closure sound), paradoxical splitting of S2, the second heart sound, a possible S_3 or third heart sound, and murmur from pulmonary valve or tricuspid valve regurgitation, with increased jugular venous pressure, all of which are suggestive of elevated right-heart pressure. Hepatomegaly and lower-extremity edema indicate a more advanced stage of the disease with right ventricular failure.

VI.B. Pulmonary Function Test

Pulmonary function tests can be performed to determine the exact etiology of symptoms, especially of dyspnea. Patients with advanced-stage PAH demonstrate reduced lung volume and, more importantly, exhibit a decline in diffusing lung capacity for carbon monoxide (DLCO).

VI.C. Electrocardiography

Electrocardiographic (ECG) examination can reveal the presence of RVH, right axis deviation, and right-peaked P waves. However, due to a lack of sensitivity, ECG is not used as the gold standard for diagnosis of PAH (specific in approximately 55% of patients with PAH). Moreover, a normal ECG does not necessarily rule out the presence of severe PAH.[26]

VI.D. Radiographic Imaging

Various PAH-related abnormalities such as oligemia with decreased vascularity, prominent pulmonary arteries, and right ventricular enlargement can be observed upon radiography of the lateral chest.

VI.E. Tomographic Scanning

Computed tomographic scanning (CT scan) can assist in identifying enlarged pulmonary arteries and evaluating embolic or thrombotic disease. Magnetic resonance imaging is used to determine the size of the right ventricle and presence of chronic thromboembolic disease. A high-resolution CT scan can identify enlarged pulmonary arteries and assess embolic or thrombotic diseases. Furthermore, ventilation perfusion scans are important tools for identifying the presence of any large central occlusive clots in the pulmonary arteries.

VI.F. Echocardiography

Transthoracic echocardiography is the gold standard among the noninvasive techniques for PAH diagnosis. It gives an estimate of pulmonary arterial systolic pressure and right ventricular systolic pressure by evaluating regurgitation across the tricuspid valve. It can also be used to determine the presence of left ventricular dysfunction and aortic and mitral valve disease.

VI.G. Six-Minute Walk Test

The 6-minute walk test gives an idea of the severity of the disease and is used to assess response to therapy and prognosis. It has been reported that patients who are able to walk less than 300 m in 6 minutes have a 2.4 times higher risk of mortality than healthy individuals.[27] Furthermore, individuals who are able to walk less than 332 m have a lower survival rate than those able to walk more than 332 m. The results of the 6-minute walk test correspond with the NYHA functional classification in terms of disease severity, that is, the greater the distance a patient can walk in 6 minutes, the higher the survival rate.[28]

VI.H. Right-Heart Catheterization

Once PH is diagnosed by means of noninvasive techniques, right-heart catheterization is used to differentiate between PAH and pulmonary venous hypertension by directly measuring PAP. This invasive procedure provides direct hemodynamic measurements of PAP, PCWP, and cardiac output and thus allows calculation of PVR. It also facilitates evaluation of intra-cardiac shunts, establishes the site of hypertension in arterial or venous beds, and helps determine the degree of vasoreactivity of the pulmonary circulation upon exposure to oxygen and nitric oxide.

VI.I. Serological Examination

Currently, there are no specific serological tests or biomarkers for diagnosis of PAH. However, to determine the etiology of the disease, patients with suspected PAH may undergo some serological tests. Common biomarkers that can be tested include autoantibodies such as antinuclear, anticentromere, anti-SCL-70, and anti-U1 RNP. In addition, increased plasma brain natriuretic peptide (BNP) has been shown to be associated with increased PAP and PVR, and thus with the development of PAH.

VII. THERAPEUTIC TARGETS FOR PAH MANAGEMENT

Three major pathways are reported to play important roles in the pathophysiology of PAH: (1) the prostacyclin pathway, (2) the endothelin pathway, and (3) the nitric oxide (NO) pathway. Intervention of these pathways has been the

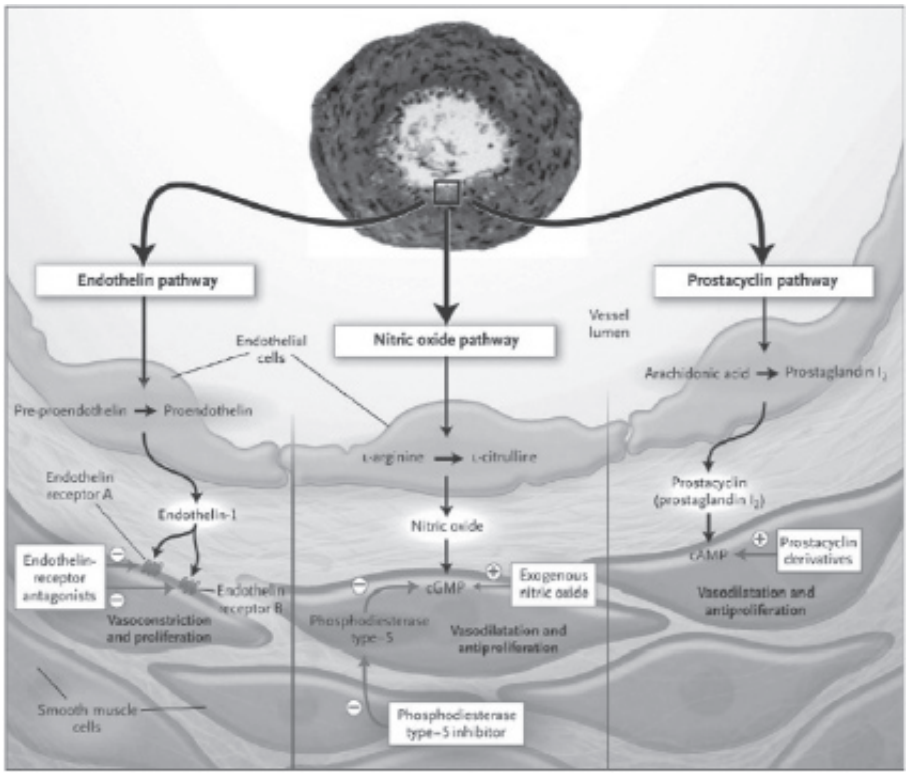

FIGURE 4. Targets for current and emerging therapies in pulmonary arterial hypertension. (Reproduced from Humbert et al.[54] with permission from the Massachusetts Medical Society.)

basis of the four therapeutic categories of drugs used in the treatment of PAH: (i) prostacyclin derivatives, (ii) endothelial receptor antagonists, (iii) NO inhalation, and (iv) phosphodiesterase-5 inhibitors. Recent studies have implicated a number of additional pathways in PAH, which may also serve as potential therapeutic targets for PAH treatment.[29] Figure 4 illustrates current therapeutic targets for PAH treatment.[4]

VII.A. Prostacyclin Pathway

Prostacyclins are members of the eicosanoid family, a group of 20 essential fatty acids derived from arachidonic acid. Other members of this family include the thromboxanes and leukotrienes. Prostacyclins exert a variety of effects on the systemic and pulmonary vasculature, including vasodilatory, anti-proliferative, anti-platelet, anti-aggregation, and anti-inflammatory effects, and they also facilitate marginalization and adherence of leukocytes.

Prostacyclin was first described in 1976 as an "endothelium dependent and aggregation inhibiting factor with vasodilatory properties" and named pros-

taglandin I_2 (PGI_2).[30] To date, prostacyclin is one of the strongest pulmonary vasodilators known. However, chemically it is very unstable and has a biological half-life of only 2 to 3 minutes. In the pulmonary circulation, prostacyclin is synthesized from arachidonic acid via the cyclooxygenase pathway in endothelial cells. Prostacyclin synthesis is mediated by a constitutive enzyme, prostacyclin synthase, present in endothelial cells. In the pulmonary circulation, prostacyclin acts as a local hormone and causes smooth muscle relaxation and exhibits anti-platelet effects. The biological activity of prostacyclins is mediated by a G protein-coupled receptor, prostacyclin receptor IP, via stimulation of adenylate cyclase, which leads to activation of protein kinase A by cAMP.

In patients with PAH, synthesis of PGI_2, prostacyclin synthase activity, and physiological levels of PGI_2 are all severely reduced, resulting in a lack of vasodilatory and anti-proliferative effects.[2,24,31] Furthermore, the production of thromboxane A2, a vasoconstrictor, is increased in the pulmonary circulation. Published reports suggest that overexpression of PGI_2 synthase protects animals from development of PAH after exposure to hypoxia or to monocrotaline.[32–35] This observation agrees with reports of the loss of PGI_2 synthase activity in pulmonary arteries isolated from patients with PAH. A decrease in excretion of urinary metabolites of prostacyclin and an increase in excretion of thromboxane A2 metabolites have also been reported. These findings confirm the fact that, in patients with PAH, the balance between PGI_2 and thromboxane A2 synthesis is disrupted.[36]

It is still unknown if reduction in PGI_2 levels in the blood is one of the multiple factors contributing to PAH progression or if PGI_2 levels decline as a consequence of disease development. However, administration of PGI_2 to patients with PAH has shown promising results in symptomatic improvements and, in fact, is the only treatment that confers a survival advantage. Because PGI_2 is chemically unstable, administration of this drug is very complex and may cause adverse effects, including diarrhea, headache, and nausea. The quest for developing new prostacyclin analogues with enhanced stability but pharmacological activity similar to that of PGI_2 has resulted in the development of a variety of stable analogues that are briefly discussed below.

1. Epoprostenol

Epoprostenol (Flolan®), a prostacyclin analogue, is a very potent vasodilator and anti-proliferative agent. Flolan® was the first drug to be approved by the US Food and Drug Administration (FDA) for PAH. It is the only approved PAH-medication that has shown survival benefits in clinical studies.[37] However, this drug has a half-life of 2 to 3 minutes. It rapidly converts to inactive metabolites because of its enzymatic degradation and hydrolysis. For this reason, epoprostenol has to be administered via continuous intravenous (I.V.) infusion by means of a central catheter. Moreover, because of its degradation at physiological pH, it is required to be administered at high alkaline pH. Because the drug is also unstable at room temperature, epoprostenol must be stored in the cold. These factors negatively impact the acceptability of this medication for PAH treatment.

Both short-term and long-term randomized clinical trials have demonstrated the efficacy of epoprostenol in treating various classes of PAH (NYHA class III and IV). An improvement in hemodynamic parameters and survival rates was observed in a study of 162 NYHA class III and IV patients who were followed for 3 years.[38] The survival rates of the epoprostenol treatment arm were 88%, 76%, and 63% versus 59%, 46%, and 39% of the control arm at years 1, 2, and 3, respectively. Similar results were observed by Sitbon et al. in a clinical study with 178 patients.[39] Side effects of epoprostenol are moderate and generally dose-dependent. Frequently observed side effects were flushing, jaw pain, nausea, and musculoskeletal pain; in >1% of patients, hyperthyroidism was observed. Side effects associated with the use of a central catheter include pain, infection, and thrombosis at the infusion site, and cardiovascular collapse due to infusion pump malfunction. Because of the complications associated with epoprostenol, efforts have been made to develop longer-lived chemical analogues such as treprostinil, iloprost, and beraprost.

2. Treprostinil

Treprostinil, a tricyclic benzene analogue of prostacyclin, was approved by the FDA in 2002 for the treatment of NYHA class II-IV PAH. It has a half-life of approximately 4.6 hours when administered subcutaneously (S.C.) and approximately 4.4 hours for I.V. administration. Moreover, it is chemically stable at room temperature and at neutral or physiological pH. Unlike epoprostenol, it does not require ice packs and daily mixing. Furthermore, treprostinil can be administered using a small infusion pump on alternate days from premixed syringes available at different strengths.

In a 12-week double-blind clinical trial with 470 patients with PAH, Simonneau et al. reported that treprostinil was efficacious at a dose of 1.25 to 22.5 ng/kg/min. The drug improved PAH symptoms and quality of life, and produced significant improvements in hemodynamic parameters.[40] In another clinical trial, Barst et al. reported on the long-term efficacy of treprostinil in 860 patients with PAH. Treprostinil gave a sustained clinical improvement, with 87% survival after 1 year and 68% after 4 years.[41] Recently, the FDA approved I.V. treprostinil infusion for PAH treatment. The advantages of I.V. treprostinil include reduced risk of cardiovascular collapse due to pump malfunction, and less cumbersome administration compared to I.V. epoprostenol. In an open-label clinical trial with untreated patients with PAH, Tapson et al. demonstrated that I.V. treprostinil improves hemodynamics and 6-minute walking distance (6MWD) by 82 m.[42] The main side effect of treprostinil is pain at the site of infusion (80%–85%). There are also a number of studies that have documented the efficacy of treprostinil when administered as an inhalable solution to patients with PAH. Studies on inhaled treprostinil are summarized in Section IX.B.

3. Iloprost

Iloprost is a chemically stable carbacyclin analogue of prostacyclin with a biological half-life of approximately 30 minutes. Iloprost generated tremendous interest in PAH treatment following reports of its efficacy upon administration as an inhalable solution.[43,44] Iloprost has been approved for NYHA class III and IV PAH as both an I.V. infusion and inhaled solution (Ventavis®). Intravenous iloprost infusion has been reported to have the same acute hemodynamic effects as epoprostenol (I.V.) and treprostinil (S.C.) at a dose of ≤3 ng/kg/min.[45,46] The advantages of iloprost infusion are that the drug is stable at room temperature and physiological environment, and there is less risk of accidental disruption of the therapy. Details on the use of inhaled iloprost for PAH treatment are discussed in section IX.A.

4. Beraprost

Beraprost sodium is an orally active prostacyclin analogue that garnered attention in 2002. A 12-week randomized clinical trial of 130 patients in Europe showed that the drug was efficacious in treating PAH symptoms, as demonstrated by an increase in 6MWD of 25 m.[47] However, a study conducted in the United States with NYHA class II and III patients showed that the anti-PAH effects of beraprost were not maintained after 9 and 12 months, despite improvement in 6MWD at 3 and 6 months. These data raised questions regarding the long-term efficacy of beraprost.[48] Therefore, beraprost cannot be used as a stand-alone therapy for PAH. However, these studies propelled the development of new orally active prostanoids and a combination therapy of prostacyclin and beraprost. Currently, beraprost is approved as an anti-PAH medication in Japan; it has yet to receive approval of the US FDA and European regulatory agencies.

5. NS-304/ACT-293987

NS-304/ACT-293987 is a synthetic prostacyclin receptor agonist. Chemically, this drug is different from prostacyclin analogues. It metabolizes to MRE-269, a highly orally bioavailable compound with vasodilator properties. NS-304/ACT-293987 was shown to be efficacious in improving PAH symptoms in monocrotaline-treated rats; the drug produced a reduction in MPAP and pulmonary arterial hypertrophy, and an improvement in survival rate.[49] Based on these preliminary *in vivo* experiments, NS-304/ACT-293987 can be considered a promising candidate for oral PAH therapy.

Overall, PAH therapy with prostacyclin analogues seems very promising, but one of the main problems with this therapy is its high cost. The cost-effectiveness of prostacyclin analogues has been challenged by the United Kingdom's National Institute for Health and Clinical Excellence (NICE). Table 3 summarizes the major shortcomings and limitations of current PAH treatment with prostacyclin analogues.[50]

TABLE 3. Advantages and Limitations of Prostacyclin Analogues for PAH Treatment

Name	Route	Half-Life	Advantages	Disadvantages	FDA Approval
Epoprostenol (Flolan®)	I.V.	6 min	Easy to titrate Longest experience	Requires permanent I.V. catheter Risk of line infection Risk of syncope or cardiovascular collapse Need for ice packs Mixing every 24 hours	Class III Class IV
Treprostinil (Remodulin®)	S.C. I.V.	4.6 h 4.4 h	Smaller pump No mixing Cassette changed every 48 h No need for ice packs Less risk of cardiovascular collapse	Pain at the site of infusion Risk associated with the permanent I.V. catheter	Class II Class III Class IV
Iloprost (Ventavis®)	Inhaled I.V.	6.5–9.5 h 23 min	No need for IV catheter Local delivery limits side effects Same as epoprostenol but less experience	6–9 inhalations per day More risk of syncope Same as epoprostenol	Class III Class IV
Beraprost	Oral	35–40 min	Oral delivery	Gastrointestinal intolerance Unclear efficacy	No

Modified from Gomberg-Maitland and Olschewski.[165]

VII.B. Endothelin Pathway

The role of endothelins in the pathogenesis of PAH has been an intensive area of research. Endothelins are a family of 21-amino-acid peptides secreted mainly by endothelial cells, smooth muscle cells, and fibroblasts. Endothelin-1 (ET-1), the most active isoform of three distinct isoforms (ET-1, 2, and 3), has been documented to play a critical role in the progression of diseases involving the cardiopulmonary vascular system.[51] ET-1 was first reported in 1988 as a potent endothelial vasoconstrictor peptide by Yanagisawa et al.[52] Primarily synthesized by endothelial cells, ET-1 is the most potent vasoconstrictor ever found, and acts as a pulmonary vasoconstrictor and potent co-mitogen, that is, it promotes smooth muscle cell proliferation and alters angiogenesis, inflammation, and fibrosis. In the pulmonary circulation, ET-1 has both autocrine and paracrine effects (primarily the latter) on two different types of G protein-coupled recep-

tors: ET_A and ET_B. ET_A receptors are located on pulmonary vascular smooth muscle cells, and ET_B receptors are located mainly on pulmonary endothelial cells and to some extent on smooth muscle cells.

ET_A receptors of smooth muscle cells mediate ET-1-associated vasoconstriction and smooth muscle cell proliferation by increasing intracellular calcium concentration. ET_B receptors, when present on vascular smooth muscle cells, mediate similar signals as ET_A receptors and thus exert similar functions. However, ET_B receptors present on endothelial cells of the pulmonary vasculature produce vasodilatory effects by promoting the release of prostacyclins and NO. In addition, ET_B receptors have a major role in clearing ET-1 from both the pulmonary and systemic circulations.[53,54]

In normal pulmonary circulation, a physiological balance between ET_A and ET_B receptor activity keeps the pulmonary vasculature in a relaxed state. Upon development of PAH, the balance between ET receptor signaling is disrupted, which results in pathobiological complications.

An increase in ET-1 levels has been observed in different animal models of PAH induced by chronic hypoxia, monocrotaline, or hemodynamic stress.[55–57] Plasma and pulmonary tissue levels of ET-1 and its receptors have been reported to be increased in patients with PAH.[58] Increased ET-1 levels are directly related to the severity of PAH and inversely correlated with survival rate. Many reports indicate that ET_A receptor-mediated vasoconstriction and proliferation play a key role in the pathogenesis of PAH. Overexpression of ET_A receptor is reported to be associated with remodeling of arteries. However, ET_B receptors counteract PAH development by mediating vasodilation and antagonizing ET_A activity. The role of ET-1 in the progression of PAH is summarized in Figure 5.[59] Thus, attenuation of endothelin activity in the pulmonary circulation by blocking either ET_A only or both ET_A and ET_B may be a potential therapeutic approach for PAH treatment.

In fact, published data have shown that prolonged treatment with an ET_A blocker protects the pulmonary circulation from hypertensive vascular remodeling and increased PAP in the ovine fetus.[60] It has also been reported that PH is exacerbated in rats in which ET_B receptors have been down-regulated by chronic hypoxia.[61] In a separate study, Sakai et al. showed that selective inhibition of ET_A receptors is more effective in alleviating PAH symptoms than is nonselective inhibition of ET_A and ET_B.[62] These studies underscore the importance of selective ET_A inhibition in PAH treatment. However, Jasmin et al. proposed that dual-receptor blockade may be more efficacious in treating the symptoms in the monocrotaline-induced PAH rat model.[63] These conflicting reports highlight the complex etiology of ET-1-mediated PAH and the need for further studies. Nonetheless, the protective effects of endothelin receptor antagonists (ERAs) in preventing remodeling and maintaining vascular tone in patients with PAH, and thus increasing the survival rate, are very much evident. Currently, three ERAs have been approved in different countries for treatment of PAH. Of these three drugs, bosentan is a dual antagonist, whereas sitaxsentan and ambrisentan are selective ET_A receptor antagonists.

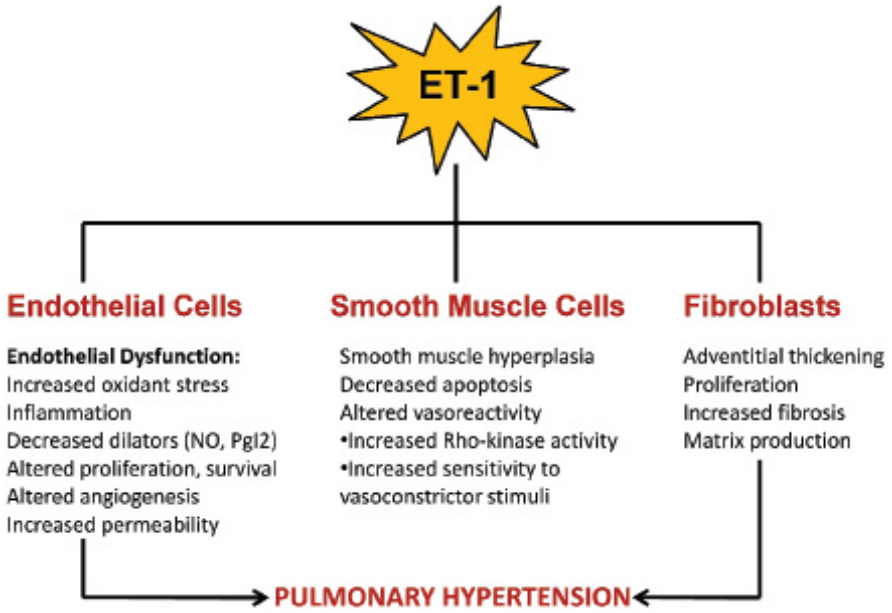

FIGURE 5. Mechanisms of endothelin-1 (ET-1)-induced pulmonary arterial hypertension. (Modified from Abman.[59])

1. Bosentan

Bosentan, with the brand name Tracleer™ (Actelion Inc., San Francisco, California), was the first ERA to be approved by the FDA for PAH treatment. It is a dual ET_A and ET_B receptor antagonist with slightly more selectivity toward ET_A receptors. In preclinical studies, bosentan produced reductions in MPAP, PVR, and vascular remodeling, and showed positive effects on pulmonary fibrosis and vascular inflammation.[64] In clinical trials, bosentan showed a 70-m improvement in the 6MWD, and significant changes in the Borg dyspnea index and hemodynamic parameters. These trials were conducted to test the efficacy of bosentan in the treatment of IPAH and PAH associated with connective tissue diseases.[65]

BREATHE-1 was a clinical study that compared the efficacy of bosentan at two different doses, 125 and 250 mg bid, in NYHA class III and IV patients with PAH. Bosentan produced a 36-m improvement in the 6MWD and significantly reduced the "time to clinical worsening" of the disease. The patients in these trials were followed for 3 years and the 12- and 24-month survival rates were 96% and 89%, respectively.[66] Similar data were obtained in another clinical trial (EARLY) that tested bosentan in NYHA class II patients with PAH.[66]

The main limiting factors for the widespread use of bosentan for PAH treatment are a variety of adverse effects associated with long-term therapy, including elevation of liver transaminase levels (11%), lower-extremity edema,

nasal decongestion, and severe drug interactions with cytochrome P450 (CYP)-inhibiting agents such as cyclosporine and ketoconazole. There are also reports of potential teratogenic effects,[67] which further increase the risk for pregnant women. With long-term use of bosentan, a reduction in serum hemoglobin levels has been reported.[65,68–70]

2. Sitaxsentan

Sitaxsentan is one of two selective ET_A antagonists. This drug has been approved in Europe, Canada, and Australia under the brand name Thelin™ (Encysive Inc., Houston, Texas; now acquired by Pfizer Inc., New York), but it has not yet been approved for use in the United States. Sitaxsentan is 6000 times more selective for ET_A than for ET_B receptors, and has a longer half-life than bosentan, which allows for once-daily dosing.[59] In different clinical trials (STRIDE-1 and STRIDE-2) with NYHA class II, III, and IV patients, the efficacy of sitaxsentan was tested at doses ranging from 50 mg to 300 mg. Patients treated with 100 mg or 300 mg of sitaxsentan showed improvements in 6MWD and functional class, with slight improvement in the primary endpoint "maximum oxygen consumption" (3.1%).[71]

Adverse effects due to long-term use of sitaxsentan include increased levels of liver enzymes in 7% of patients, with inhibition of cytochrome enzymes, thus decreasing the metabolism of some drugs such as warfarin.

3. Ambrisentan

Ambrisentan is a selective ET_A antagonist approved for PAH treatment. The drug is called Letairis™ (Gilead Inc., Foster City, California) in the United States and Volibris™ (GlaxoSmithKline, United Kingdom) in Europe. Several clinical trials were conducted to establish the efficacy of ambrisentan in patients with PAH with connective tissue disease, with HIV, and in patients with PAH taking anorexigen. ARIES-1 studied the effects of 5-mg and 10-mg daily doses of ambrisentan, whereas ARIES-2 studied the effects of 2.5-mg and 5-mg daily doses.[72] Both the 5- and 10-mg doses increased the 6MWD (by 31 m and 51 m, respectively) and significantly improved the Borg dyspnea index with minimal adverse effects. In the ARIES-2 study, the 5-mg dose led to a 59-m improvement in the 6MWD, with reductions in both time to clinical worsening and Borg dyspnea index. Unlike bosentan, only a few patients (2%) demonstrated elevation in liver enzyme levels: >3 times normal. Although data on the safety of ambrisentan are limited, the drug promises fewer side effects and lower daily-dosing compared to bosentan. Although all three currently approved ERAs have common side effects, with liver injury being the most important one, there is no evidence to correlate ERA with permanent liver damage. A novel dual ERA, macitentan, has recently entered a Phase III clinical trial (SERAPHIN) for PAH treatment (Actelion Inc., San Francisco, California).

VII.C. Nitric Oxide Pathway

Nitric oxide is a free radical with a short biological half-life. It elicits a variety of responses upon interacting with various cellular targets. These responses include vasodilation, inhibition of platelet activation, and inhibition of vascular smooth muscle proliferation.[73] In the pulmonary vasculature, NO is synthesized by vascular endothelial cells from L-arginine in the presence of endothelial NO synthase (eNOS), an endothelial isoform of NO synthase. Pulmonary NO is a potent vasodilator and plays a pivotal role in maintaining low vascular tone in the pulmonary circulation. The vasodilatory effects of NO are elicited by its ability to increase the production of cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP) by stimulating soluble guanylate cyclase (sGC). cGMP is a critical second messenger regulating smooth muscle contractility by activating cGMP-dependent protein kinases, phosphodiesterases, and ion channels leading to vascular relaxation.[74] In addition to the sGC-dependent cGMP activation pathway, there are several other pathways through which NO may elicit its cardiopulmonary effects. These alternate pathways include interactions of NO with heme-containing molecules and with proteins containing reactive thiol groups (to form S-nitrothiols); NO may also be oxidized to nitrite.[75]

As noted earlier, cGMP activation is a vital step in promoting the vasodilatory and anti-proliferative effects of NO. Phosphodiesterases (PDEs) are enzymes that convert cGMP to 5′-GMP via hydrolysis, thus abrogating the NO-cGMP signaling pathway. In the pulmonary vasculature there are three main PDE isoforms (PDE1, 5, and 9) that act on cGMP, thus reducing the vasodilatory capacity of vascular smooth muscle cells and promoting cell proliferation. Of the three isoforms, PDE5 is mainly responsible for cGMP breakdown in the pulmonary circulation.[76] Figure 6 depicts various therapeutic targets for PAH treatment in the NO-sGC-cGMP pathway.[77]

Upon development of PAH, a wide array of factors may disrupt the NO-mediated signaling cascade and thus promote vasoconstriction, vascular proliferation, and remodeling. Reduced expression and activity of eNOS has been reported in many adult and infant patients with PAH. The lack of eNOS leads to reduced NO production in the pulmonary circulation, resulting in vasoconstriction and cell proliferation.[78–80] Oxygen is also required for eNOS activity, which may account for the increased vascular tone observed in animals exposed to hypoxia. However, the underlying mechanisms are not well understood. Because of these important roles of NO in PAH pathogenesis, the NO pathway continues to be explored as a therapeutic target for PAH.

1. Inhaled Nitric Oxide

Patients with PAH have been reported to produce smaller amounts of NO,[81] and exhale lesser amounts of NO in their breath than healthy individuals. This suggests a direct correlation between reduced NO production and PAH progression. Thus, administration of exogenous NO can alleviate or reverse PAH

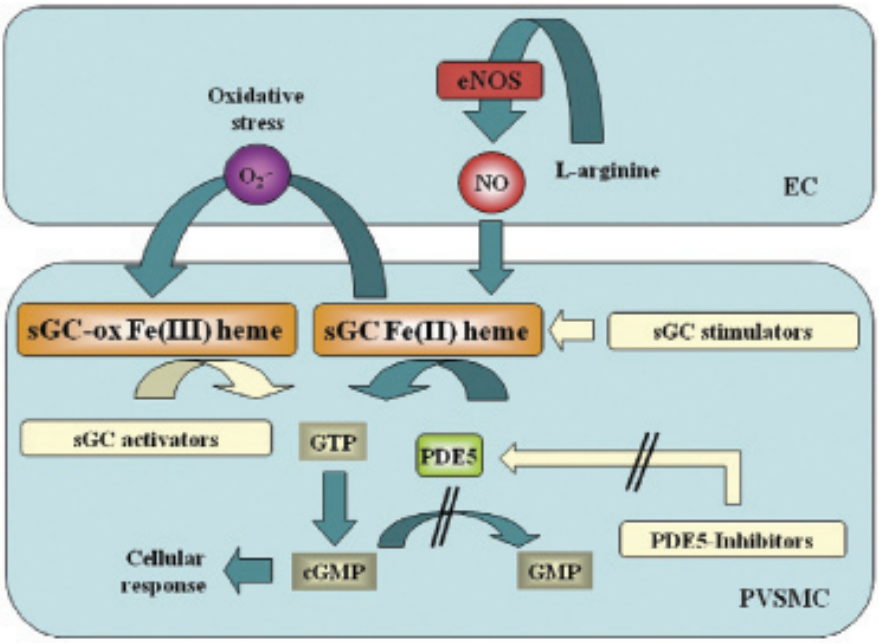

FIGURE 6. Pharmacological targets of the nitric oxide (NO)–soluble guanylate cyclase (sGC)–cyclic GMP (cGMP) system for treatment of pulmonary arterial hypertension. EC, endothelial cells; PVSMC, pulmonary vascular smooth muscle cells; eNOS, endothelial NO synthase; GTP, guanosine triphosphate. (Reproduced from Olsson and Hoeper[77] with permission from Elsevier.)

symptoms. NO donor compounds can be administered systemically to increase NO availability. However, their efficacy to the pulmonary vasculature is limited by their systemic absorption, which results in systemic hypotension. Inhaled NO is reported to produce selective pulmonary vasodilation, thus decreasing MPAP and PVR with minimal systemic side effects.[82] Inhaled NO is approved by the FDA for use in hospitalized pediatric patients with PAH. The efficacy of inhaled NO in PAH therapy has been documented in a number of clinical trials.[83–86] Inhaled NO also reduces pulmonary vascular remodeling and RVH.

The major side effects associated with the use of inhaled NO include sudden reversal of the drug's effects and rebound of symptoms soon after discontinuation of therapy. The rebound may occur within 30 minutes of discontinuation. To prevent the rebound effect, NO is administered by continuous inhalation through a nasal cannula (up to 40 ppm) or via a ventilator (up to 80 ppm). Moreover, methemoglobin levels have to be monitored during the course of NO treatment due to the drug's interaction with oxygenated hemoglobin to form nitrate and methemoglobin. In addition to these side effects, it has been reported that approximately 40% of infants suffering from PAH do not respond to inhaled NO. There is no report showing that NO inhalation reduces mortality or morbidity associated with PAH.

2. Phosphodiesterase Inhibitors

Up-regulation of PDE1 and PDE5 has been observed in remodeled pulmonary arteries of patients with IPAH.[87] PDEs also block NO-cGMP signaling by degrading cGMP to 5′-GMP. These observations were the basis for targeting of PDEs for the treatment of PAH. Sildenafil is an oral PDE5 inhibitor that was approved for PAH treatment in Europe in 2005 (Revatio™) at a dose of 20 mg tid. Sildenafil inhibits PDE5 activity with some efficacy for PDE1 as well, which is involved in proliferation of smooth muscle cells in the pulmonary vasculature. Sildenafil increases the levels of cGMP in the pulmonary circulation, promoting pulmonary vasodilation. When administered in combination, it is also reported to augment the vasodilatory effects of inhaled NO and/or prostacyclin analogues.[88] In a recent clinical trial (SUPER-1), the efficacy of sildenafil for treatment of NYHA class II and III PAH was studied at three different doses (20, 40, and 80 mg daily) for a period of 12 weeks. These studies showed that sildenafil improved the 6MWD by 45 to 50 m at all three doses, with significant hemodynamic and functional class improvement. However, no significant improvement was observed in the time span to clinical worsening of symptoms,[89] which underlines the role of sildenafil as an adjuvant rather than stand-alone therapy.

Side effects associated with the use of sildenafil include headache, dizziness, blue-lavender vision, and a few cases of sustained erections. Sildenafil is contraindicated with nitrates because concurrent use of sildenafil and nitrates may cause severe systemic hypotension. There are no data on the safety of long-term use of sildenafil. Tadalafil, a new PDE5 inhibitor, showed promising results in a Phase III clinical trial in 405 symptomatic (idiopathic/heritable or related to anorexigen use) patients with PAH.[90] It is a selective PDE5 inhibitor with no PDE1 inhibition. Because PDE1 is involved in pulmonary smooth muscle proliferation, it is still not clear if tadalafil will have the same long-term effects as sildenafil on PAH progression.

VII.D. Soluble Guanylate Cyclase Pathway

As discussed above, the NO-cGMP-mediated signaling pathway is mediated by soluble guanylate cyclase (sGC), a heme-iron (II)-containing enzyme. sGC is a heterodimer of α and β subunits. sGC present in vascular smooth muscle cells converts GTP to cGMP, a critical second messenger responsible for maintaining low pulmonary vascular tone. sGC-mediated signaling has been reported to play a critical role in NO-mediated signaling selective to the pulmonary circulation.[91] In patients with PAH, sGC expression is increased in pulmonary vessels, but the enzymes are inactivated by oxidation of the heme-iron due to oxidative stress and generation of reactive oxygen species.[92] This renders inhaled NO and other sGC-dependent vasodilators ineffective as therapeutic agents; indeed, approximately 40% of patients with PAH are nonresponders to inhaled NO therapy for this reason.

As shown in Figure 6, two different approaches have been investigated for targeting sGC-dependent pulmonary vasodilation: (i) sGC activators and (ii) sGC stimulators. These small-molecular-weight drugs are orally active, powerful vasodilators that are not subjected to tolerance. They can target both oxidized and reduced forms of sGC, thus allowing treatment of PAH based on its etiology.

1. sGC Activators

sGC activators can activate the oxidized or heme-free form of sGC. These drugs promote a robust synergistic vasodilatory response in the absence of NO. Several novel sGC activators have been investigated for their potential use as anti-PAH drugs, including HMR-1766 (Sanofi-Aventis, Bridgewater, New Jersey) and BAY 58-2667 (Bayer-AG, Wayne, New Jersey). A recently published study suggests that HMR-1766 produces pulmonary vasodilation in a dose-dependent manner when tested on hypoxia-induced PAH in isolated mouse lungs.[93] Furthermore, BAY 58-2667 has been reported to reverse vascular remodeling and decrease PH. However, it failed to reverse RVH in eNOS knockout PAH mice, suggesting a potential synergism between NO and these compounds.[94]

2. sGC Stimulators

sGC stimulators stimulate sGC directly and thus augment the sensitivity of the enzyme even at very low levels of NO. This class of drugs does not affect the oxidized form of sGC. YC-1 is one of several lead compounds that have shown promise as NO-independent stimulators.[95] YC-1 stimulates sGC in the presence of a reduced heme group on the α subunit of sGC. More recently, YC-1 was used as the lead compound to synthesize several derivatives, including BAY 41-2272 and BAY 63-2521 (Riociguat). In a sheep model of PAH, BAY 41-2272 was reported to cause a prolonged reduction in PVR.[96] A dose-dependent reduction in MPAP and PVR and an increase in cGMP levels were observed in U-46619-induced PAH in lambs.[97] In a very recent study, Schermuly et al. reported that treatment with BAY 63-2521, a chemical analogue of BAY 41-2272, reversed hemodynamic and structural changes in both hypoxic mice and monocrotaline-induced PAH rats.[92] In a recent clinical trial with 10 patients with PAH, BAY 63-2521 produced a marked improvement in hemodynamic parameters,[98] and this product will soon be entering Phase III clinical trials.

VII.E. Carbon Monoxide and Hydrogen Sulfide Pathways

1. Carbon Monoxide

Carbon monoxide (CO) plays an important role in hypoxic PH. CO is endogenously produced by endothelial cells of the pulmonary vasculature upon breakdown of heme in the presence of heme oxygenase (HO). CO is responsible for relaxation of blood vessels and for inhibiting cell proliferation. Yun et al. reported

that exogenously supplied CO decreases PH caused by hypoxia and reverses pulmonary vascular structural remodeling.[99]

2. Hydrogen Sulfide

Hydrogen sulfide (H_2S) has been known as an environmental pollutant for decades. Abe and Kimura were first to suggest the existence of endogenous H_2S.[100] H_2S is endogenously synthesized from cysteine by cystathione β-synthase (CBS) and cystathione γ-lyase (CSE). It has been demonstrated that H_2S plays a major role in the pathophysiology of various cardiopulmonary disorders such as hypertension. A reduction in H_2S levels and CSE enzymatic activity was observed in hypoxia-induced PAH rats.[101] In two separate studies, it was shown that administration of sodium hydrosulfide (NaHS), an H_2S donor, significantly reduced MPAP, attenuated vascular remodeling, and prevented RVH in both hypoxia-induced and high pulmonary blood flow rat models.[101,102] Further studies are required to establish the long-term safety and efficacy of H_2S donors in PAH.

VII.F. Rho Kinase Pathway

Rho kinase (ROCK) is a serine/threonine-specific protein kinase that is activated by the Rho (Ras-homologous) family of GTP-bound proteins. This pathway mediates a plethora of cellular functions, including smooth muscle contraction, proliferation, angiogenesis, and vascular remodeling. RhoA is one of the best-known members of the Rho family, which acts by exchanging GDP for GTP and then translocating the latter to the plasma membrane. GTP-bound RhoA activates two isoforms of ROCK: ROCK 1 and ROCK 2. This activation of the ROCK pathway has been reported to be responsible for vasoconstriction and vascular remodeling during PAH progression, which can be attributed to inhibition of myosin light chain phosphatase (MLCP) by ROCK-mediated phosphorylation of the myosin-binding subunit and Ca^{++} sensitization.[103–105] Figure 7 depicts the pathways influenced by ROCK signaling,[106] and other pathways affecting RhoA activity are described in Figure 8.[107]

There are reports that suggest that ROCK-mediated vasoconstriction plays a major role in PAH pathogenesis in various animal models. The role of ROCK signaling in pulmonary vascular tone management was first proposed by Robertson et al., who showed that ROCK inhibition produces vasodilation in isolated, perfused rat lung.[108] Since then, several specific inhibitors of ROCK have been developed, including fasudil (HA-1077) and Y-27632 as lead molecules. These ROCK inhibitors have been tested in various animal models of PAH at different doses and have been documented to be efficacious in the treatment of PAH.[106,109,110] The mechanisms by which ROCK inhibitors alleviate the symptoms of PAH include down-regulating the expression of growth factors, cell proliferation markers, and matrix proteins, and up-regulating the expression of apoptotic markers.

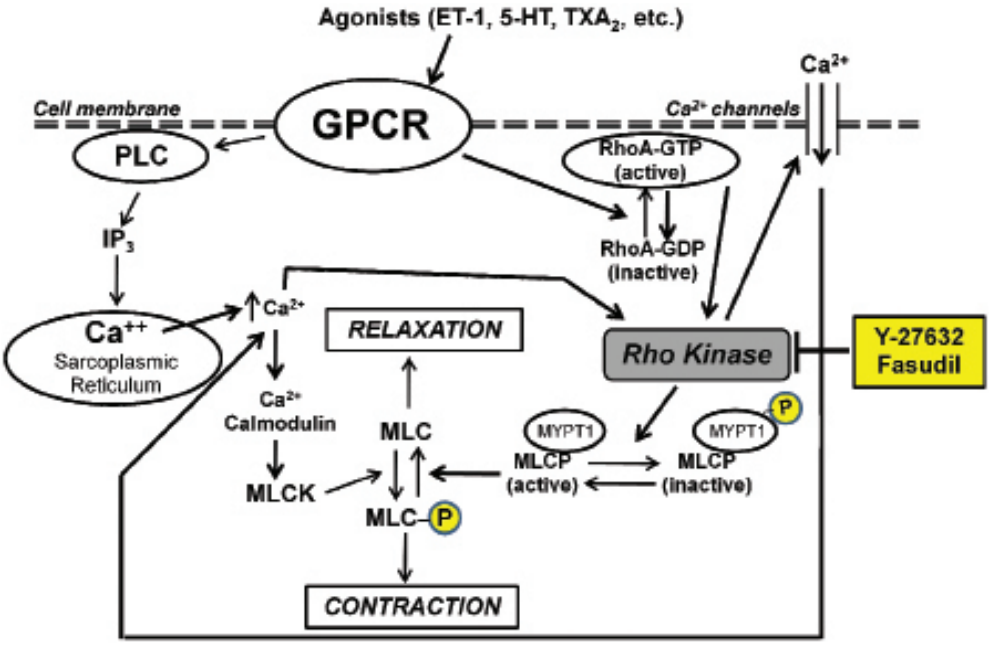

FIGURE 7. Regulation of smooth muscle contraction and proliferation by the RhoA/Rho kinase pathway. ET-1, endothelin-1; GPCR, G protein-coupled receptor; 5-HT, 5-hydoxytryptamine (serotonin); IP3, inositol triphosphate; MLC, myosin light chain; MLCK, myosin light chain kinase; MLCP, myosin light chain phosphatase; PLC, phospholipase C; TXA2, thromboxane A2. (Modified from Nagaoka et al.[106])

The first clinical trial on fasudil, conducted in 2005, showed a 17% reduction in pulmonary vascular resistance in 9 severely ill patients with PAH.[111] In a separate trial, the efficacy of intravenous fasudil was studied in 8 severely ill patients. Fasudil improved MPAP and vascular resistance but caused systemic vasodilation. These observations prompted the development of inhalational fasudil, which has been tested in PAH-induced rats.[111] In addition to ROCK inhibitors, there are several other treatment regimens that are believed to act, in part, through ROCK inhibition. These include statins, which act by blocking post-translational isoprenylation of RhoA, and sildenafil, which acts by promoting phosphorylation and blocking the translocation of RhoA.

Recently, Do e et al. reported up-regulation of Rho kinase activity in the small pulmonary arteries of patients with IPAH. Increased expression of ROCK 1 and 2 was observed in whole lung tissues, specifically in the intima and media of small pulmonary arteries, isolated from patients with IPAH. Do e et al. also demonstrated a direct correlation between Rho kinase activation and the severity and duration of PAH.[112] Recent data also suggest that RhoA/Rho kinase becomes activated in human patients with PAH.[113,114] Fasudil indeed presents a very attractive treatment regimen for PAH. However, more studies are warranted on the long-term safety and efficacy of this drug.

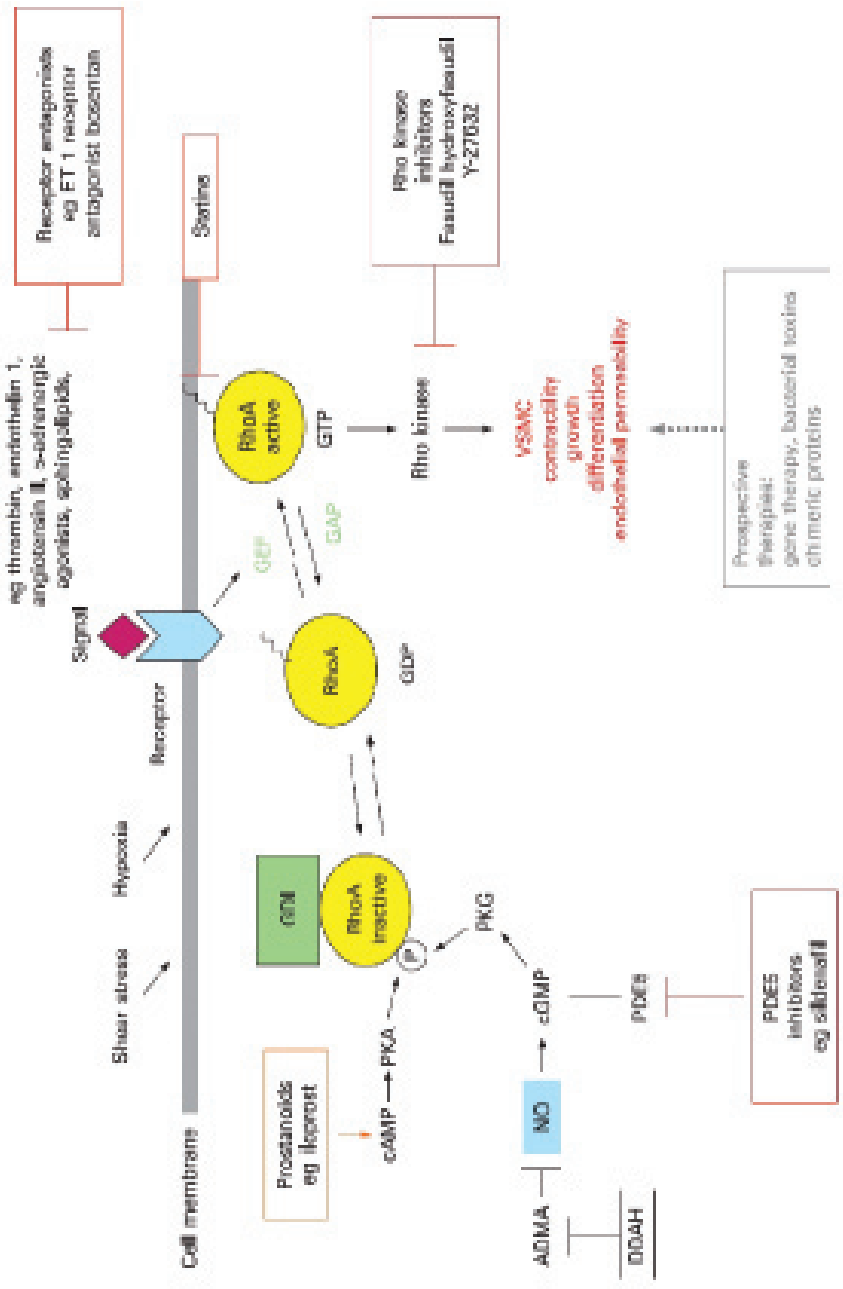

FIGURE 8. Schematic representation of pathways affecting RhoA activity in the pulmonary vasculature (see text for details). ADMA, asymmetric methylarginine; DDAH, dimethylarginine dimethylaminohydrolase; GDP dissociation inhibitor, GDI; GEF, guanosine nucleotide exchange factor; NO, nitric oxide; PDE5, phosphodiesterase-5; VSMC, vascular smooth muscle cell. (Reproduced from Wojciak-Stothard[107] with permission from BMJ Publishing Group.)

VII.G. Statins

Several studies suggest that statins may potentially play a role in PAH treatment. Statins are HMG-CoA reductase inhibitors that work by blocking the rate-limiting step in cholesterol synthesis. Statins are also reported to block the Rho and Ras family of GTPases by inhibiting synthesis of farnesyl and geranylgeranyl pyrophosphate that are required for isoprenylation of the GTPases. Additional means by which statins produce their effects in PAH include enhancing eNOS expression, reducing expression of the adhesion molecules vascular cell adhesion molecule-1 (VCAM1) and intercellular adhesion molecule-1 (ICAM1), increasing tetrahydrobiopterin (BH_4) levels, and inhibiting leukocyte-endothelium interactions.[115–117] Simvastatin has been reported to reduce MPAP by 50%, decrease pulmonary arterial muscularization and RVH, and reduce neointimal occlusion in both chronic hypoxia- and monocrotaline-induced rat models of PAH.[118] An open-label trial conducted in 16 patients with PAH showed dose-dependent anti-PAH effects of simvastatin (20–80 mg/day).[119] Over a 3-month period, simvastatin significantly improved 6MWD and right ventricular systolic pressure (RVSP). The dose of prostacyclin analogues remained unchanged or was reduced during the course of treatment. Although statins sound promising, randomized clinical trials should be performed to establish the efficacy of these drugs in PAH treatment. In a recent double-blind, randomized, and placebo-controlled study, the efficacy of simvastatin was demonstrated as an add-on therapy to conventional treatment of PAH.[120] The drug produced a small and transient early reduction in RV mass and N-terminal (NT)-proBNP levels in patients with PAH. However, the drug did not maintain the beneficial effects after 12 months of therapy.

VII.H. Tyrosine Kinases

Various growth factors acting via receptor tyrosine kinases have been reported to play a role in smooth muscle cell remodeling and endothelial cell proliferation and dysfunction. Expression of platelet-derived growth factor (PDGF) is reported to be up-regulated in lung-tissue isolates from patients with PAH and in PAH-induced lambs.[121,122] A PDGF receptor antagonist, imatinib mesylate (Gleevac®), has been shown to have beneficial effects when administered to hypoxia- and monocrotaline-induced animal models of PAH.[123] Imatinib administration resulted in complete regression of vessel remodeling, reduced RVH, and increased cardiac output. In clinical studies, imatinib showed beneficial effects in patients with PAH. Patients showed improvement in hemodynamics, functional class, and in the 6MWD test during the 6-month follow-up period.[124–126] Due to reported cardiotoxic effects of imatinib,[127] the long-term safety of this drug remains a concern. Sorafenib, a new tyrosine kinase inhibitor that inhibits multiple kinases, showed encouraging data in both hypoxia- and SU-5416- induced PAH[128]; the latter compound is an inhibitor of vascular endothelial growth factor receptor (VEGFR) and potentiates the effects of hypoxia in the pulmonary circulation.

VII.I. Vasoactive Intestinal Peptide and Other Vasoactive Substances

Vasoactive intestinal peptide (VIP) is a neuropeptide that mediates a wide array of physiological activities in the body. It is a 28-amino-acid peptide that was discovered by Said and Mutt in 1970.[129] In the cardiopulmonary system, VIP inhibits platelet activation, maintains cardiac output, and works as a potent vasodilator. VIP signaling is mediated via two G protein-coupled receptors, VPAC1 and 2, and results in activation of the adenylate and guanylate cyclase pathways.[130] Depletion of serum VIP has been reported in the pulmonary circulation of patients with PAH.[131] Moreover, VIP knockout mice develop moderate PAH with pulmonary hypertrophy.[132] Intravenous administration of VIP produced selective vasodilatory effects in the pulmonary circulation in a piglet model of PAH.[133] In a clinical study, VIP administered as an inhalable solution at a daily dose of 200 μg for 3 months to 8 WHO class III or IV patients with PAH produced improvements in many symptoms: 6MWD was increased by 113 m, MPAP was decreased by 13 mm Hg, and PVR was decreased by 50%. There were little or no vasodilatory effects on the systemic circulation. Although no major side effects were reported,[131] more studies are required before the clinical efficacy of VIP can be established.

Adrenomedullin (ADM), another vasoactive peptide, has drawn attention as a drug for PAH. ADM has been implicated as a key player in cardiovascular events and has vasodilatory and antiproliferative properties. It acts via different receptors in the pulmonary vasculature, including the calcitonin receptor-like receptor and receptor-activity-modifying proteins-2 and -3.[134] Beneficial effects of ADM in PAH treatment are summarized in Table 4.[135] In hypoxic rat lung tissues, the levels of ADM and its receptors are elevated, with increased circulating levels of ADM.[136] In hypoxic PAH rats, I.V. infusion of ADM decreased MPAP and muscularization and attenuated vascular remodeling, indicating a therapeutic role of ADM in PAH.[137] In an initial clinical case study, I.V. ADM markedly reduced symptoms of PAH. In a small clinical trial, 11 patients with PAH who were treated with aerosolized ADM showed significant improvement in PAH symptoms with minimal or no systemic side effects.[135]

VII.J. Voltage-Dependent Potassium Channels

Voltage-gated potassium channels (Kv's) play an important role in PAH pathogenesis. Expression of Kv1.5, a gene encoding a channel subunit, is reduced in patients with PAH, leading to Kv impairment.[138] Kv channels are responsible for smooth muscle cell contraction. Kv down-regulation causes depolarization of the cell membrane due to reduced potassium outflow that results in increased levels of cytosolic Ca^{++} via opening of calcium channels. Dexfenfluraine, a Kv2.1 inhibitor, has been used in PAH treatment,[139] which demonstrates the importance of Kv channels as a therapeutic target in PAH.

VII.K. Serotonin

Serotonin (5-hydroxytryptamine, 5-HT) is a potent pulmonary vasoconstrictor and causes mitogen-mediated smooth muscle cell proliferation. Serotonin signaling in the pulmonary circulation is mediated by 5-HT_{1B}, 5-HT_{2A}, and 5-HT_{2B} receptors present in pulmonary vascular endothelial and smooth muscle cells.[140] The 5-HT transporter (5-HTT or SERT) is also present in the pulmonary vasculature, and 5-HTT blocks the activity of serotonin by transporting the neurotransmitter from the circulation to platelets for storage. In patients with PAH, high levels of serotonin are reported to be present in the plasma, with corresponding low levels in platelets. The first evidence of a role for serotonin in PAH was provided by Schweizer in 1969, who linked its association with anorectic agents.[141] Anorectic agents (e.g., aminorex) block 5-HTT activity, thus blocking serotonin reuptake and increasing the levels of free 5-HT in the circulation, which is responsible for the vasoconstrictive and proliferative effects of 5-HT. In patients with IPAH, a genetic polymorphism in the 5-HTT gene promoter specifies a long (L) variant that confers on smooth muscle cells increased sensitivity to serotonin. In one study, 65% of the patients with IPAH were reported to have an LL homozygous genotype for the 5-HTT promoter compared with 27% of individuals in the control group.[142]

Evidence of 5-HT involvement in PAH pathogenesis has been provided by various groups. Keegan et al. showed reduced vascular remodeling in hypoxic mice lacking 5-HT_{1B} or 5-HT_{2B} receptors.[143] Eddahibi et al. showed protective effects of 5-HTT deficiency against PAH in hypoxic mice.[144] Additional reports have suggested that the vasoconstrictor and proliferative effects of 5-HT are mediated mainly by 5-HT_{1B} receptors and not by 5-HT_{2A} and 5-HT_{2B} receptors.[145]

Therapeutic agents that have been used to target the 5-HT-mediated vasoconstrictive pathway for PAH treatment include selective serotonin reuptake inhibitors (SSRIs) and 5-HT receptor antagonists. Fluoxetine and citalopram are two 5-HTT inhibitors that have been reported to decrease both RVH and muscularization in male hypoxic mice.[146] Recently, an investigational 5-HT_{2B} receptor antagonist, PRX-08066, produced a significant dose-dependent improvement in post-exercise systolic PAP in 58 patients with chronic obstructive pulmonary disease (COPD)-associated PAH. Very recently, Guignabert et al. suggested that serotonin may play an important role in PAH progression by decreasing Kv1.5 expression via 5-HTT, and by inhibiting nuclear translocation of nuclear factor of activated T-cells (NFATc2).[147] In summary, 5-HT may have a critical role to play in PAH pathogenesis either through receptor signaling or 5-HTT-mediated transport. However, its mechanism of action remains to be elucidated in PAH.

VII.L. Dichloroacetate—A Metabolic Modulator

Dichloroacetate (DCA) is a small molecule inhibitor of mitochondrial pyruvate dehydrogenase kinase (PDK), a gate-keeping enzyme of the mitochondrial tri-

carboxylic acid (TCA) cycle. In addition to inhibiting PDK, DCA also inhibits pyruvate dehydrogenase (PDH), thus interrupting the entry of pyruvate into the TCA cycle. In addition, DCA has also been reported to restore the activity of Kv channels by activating the NFATc2 transcription factor. As was discussed in the preceding sections, expression of the Kv1.5 gene is reduced in patients with PAH. Reduced Kv1.5 expression is associated with depolarization of the cell membrane, increased cytosolic Ca^{++}, and subsequent vasoconstriction.[138] Many studies have reported that DCA improves PAH symptoms in different rodent models of PAH, including in monocrotaline-induced, chronic hypoxia-induced, and fawn-hooded rats, a strain of rats with an inherited platelet storage disorder that develop PH on exposure to mild hypoxia.[148–150] In addition to Kv reactivation, DCA-mediated effects can be attributed to increased mitochondrial oxidative phosphorylation, increased mitochondrial depolarization, and restoration of a proper balance between apoptosis and proliferation of smooth muscle cells. Guignabert et al. suggested that DCA slows the progression of established PAH in SM22-5-HTT^{+} mice.[147] The main characteristic of DCA as a potential anti-PAH drug is that it targets a unique feature of hypertensive cells and thus does not affect normal rats or normal vascular cells. Moreover, there are minimal toxicities related to DCA use, and its effect is reversible and dose-dependent.

VII.M. Endothelial Progenitor Cells

Endothelial progenitor cells (EPCs) are a hypothetical population of heterogeneous mononuclear cells thought to be circulating in the blood in low numbers and which have the ability to differentiate into endothelial cells. These cells arise from mesodermal stem cells in the bone marrow and express CD34, CD133, and vascular endothelial growth factor-2 (VEGF-2).[151] In addition to their roles in replacing damaged endothelial cells, EPCs can also be used as carriers for genes for various neurotransmitters, including ADM, eNOS, VEGF, and angiopoietin-1. In 2007, Raoul et al. reported that EPCs (bone marrow-derived cells) showed some beneficial effects in vascular injury promoted by pulmonary vascular remodeling, but had no effects in vascular remodeling associated with hypoxia-induced PAH.[152] The above information suggests that, despite initial failures of EPCs as a progenitor-cell-based gene therapy in PAH treatment, the approach can facilitate reversal of vascular remodeling, the characteristic pathological feature of PAH.

VII.N. TGF-β/BMPR II-Associated Pathway

As discussed above (section IV), mutations in bone morphogenetic protein receptor-2 (BMPR2) have been reported to predispose to PAH. BMPR2 is predominantly present in the pulmonary vascular endothelium, in medial smooth muscle cells, and in macrophages, where it mediates a variety of functions such as cell proliferation, differentiation, and apoptosis in conjunction with BMPs.

TABLE 4. Biological Roles of Adrenomedullin in PAH Treatment

Biological Activity	Second Messenger or Signal
Potent pulmonary vasodilation	cAMP, NO/cGMP, PI3K/Akt
Inhibition of endothelial cell apoptosis	PI3K/Akt
Inhibition of smooth cell proliferation and migration	cAMP, Ca^{++}
Positive inotropic effect	cAMP, Protein kinase C, Ca^{++} release or efflux
Diuresis and natriuresis	NO/cGMP, cAMP
Suppression of aldosterone production	Ca^{++}
Induction of angiogenesis	PI3K/Akt, MEK/ERK
Anti-inflammation	cAMP

Modified from Nagaya and Kangawa[135] with permission from Elsevier.

In PAH, BMP signaling is disrupted, as evidenced by decreased expression of BMPR2 mRNA and proteins.[153] These observations establish BMP as a potential therapeutic target for PAH treatment. Recently, Reynolds et al. reported the successful reduction of PAH pathology through adenoviral transfer of BMPR2 to the pulmonary vascular endothelium in chronic hypoxic rats.[154]

Two other members of the TGF-β superfamily, endoglin and activin receptor-like kinase 1 (ALK-1), also play some roles in PAH pathogenesis. TGF-β has been reported to elicit a pro-mitogenic response in pulmonary vascular smooth muscle cells isolated from patients with PAH.[155] In a recent study, ALK-5 inhibition was shown to attenuate PAH signs in monocrotaline-induced PAH rats.[156] Although the exact role of TGF-β signaling in PAH prognosis is largely unknown, TGF-β inhibition can still be regarded as a potential target for PAH treatment.

In summary, there are a number of different pathways that may form the basis for development of an effective therapy for PAH. However, to date, only a few drugs have been approved for PAH therapy and these are listed in Table 5.[157]

VIII. INHALATIONAL THERAPY AS AN APPROACH FOR LOCALIZED DELIVERY OF ANTI-PAH DRUGS

One of the major limitations of many investigational and commercially available drugs is that they are required to be administered via I.V. and S.C. injection. Although anti-PAH drugs are intended to produce vasodilation in the pulmonary vasculature, parenteral formulations produce both pulmonary and systemic vasodilation. Systemic vasodilators such as calcium channel blockers

TABLE 5. Current Therapeutic Approaches for PAH

Class of Medication	Route of Administration	Agent	Classification
Anticoagulant therapy	Oral	Coumadin	Conventional therapies
Diuretic	Oral	Mainly loop diuretics/ spironolactones	
Oxygen	Inhaled		Vasodilator therapies
Calcium channel blocker	Oral		
Endothelin receptor antagonist	Oral	Bosentan, sitaxsentan, ambrisentan	Current specific therapies
Prostacyclin analogue	Intravenous	Epoprostenol	
	Subcutaneous/I.V.	Treprostinil	
	Inhaled/I.V.	Iloprost	
	Oral	Beraprost	
Phosphodiesterase inhibitor	Oral	Sildenafil	

Modified from Yildiz[157] with permission from Elsevier.

have been investigated for pulmonary vasodilation and PAH treatment,[158,159] but all of them have systemic side effects. With the advent of FDA-approved prostacyclin analogues—epoprostenol, treprostinil, and iloprost—concerns have been raised regarding their short biological half-life and side effects related to their systemic administration. Because all prostacyclin analogues have a very short half-life—2 to 3 minutes for epoprostenol, approximately 80 minutes for treprostinil, and approximately 30 minutes for iloprost—they were initially approved for administration via systemic infusions: I.V. for epoprostenol and S.C/I.V. for treprostinil. However, long-term safety and efficacy studies revealed that systemic administration of prostacyclin analogues is associated with a wide range of complications, including systemic hypotension, reduction in right coronary blood flow, deterioration of right ventricular performance, and increase in shunt function with worsening oxygenation. Moreover, there were concerns regarding continuous infusions, including pain at the site of infusion, infection and thrombosis due to repeated administration of the drug at the same site, use of catheters, and risk of cardiovascular collapse due to infusion pump malfunction. Furthermore, both the method of catheterization and need to carry the cumbersome pump everywhere eventually lead to noncompliance with the therapy.

Because of the above limitations of injectable prostacyclin analogues, efforts have been made to develop anti-PAH drugs that can be administered via noninvasive routes. An orally active prostacyclin analogue, beraprost sodium, is currently available. However, this drug fails to provide hemodynamic effects past 6

months. This propelled the development of drug-delivery systems that can provide selective pulmonary vasodilation and overcome side effects associated with the use of infusion pumps. One of the approaches that would address many of the complications associated with systemically administered anti-PAH drugs is to deliver inhalable formulations directly to the lungs.

For many years, macromolecular and small-molecular-weight drugs have been administered as aerosolized formulations to the lungs to achieve both local and systemic effects. The lungs offer several advantages over other routes of administration, including: (i) a large surface area (approximately 140 m^2) available for drug absorption; (ii) high blood flow that bypasses the clearance mechanisms present in the liver; (iii) thin epithelial surface (0.5 to 1.0 μm) for better absorption than any other mucosal route of administration; and, importantly, (iv) accessibility for self-administration of the therapeutic agents.[160–162] Moreover, lungs have a lower drug metabolizing and efflux transporter activity than the gut or liver, thus keeping the drug intact for a longer period of time.[163,164] These are the main factors that contribute to the enhanced bioavailability of drugs administered via the pulmonary route.

In the case of PAH, an ideal delivery system should provide selective pulmonary vasodilation and be tolerable over a long period. Because there are presently no medications that provide pulmonary-selective vasodilation, attempts have been made to deliver currently available agents via the pulmonary route so as to target only the pulmonary circulation. The advantages that inhaled PAH treatment can offer include: (i) targeted delivery of the medications to the diseased pulmonary circulation; (ii) avoidance of cumbersome I.V./S.C. infusion; (iii) minimal systemic side effects; and (iv) avoidance of right-to-left shunt blood flow.[165] The pulmonary route promotes drug deposition and activity in well-ventilated areas, thus minimizing ventilation-perfusion mismatch. Because of the close proximity of the airways to the small pulmonary arteries, anti-PAH medications administered via the pulmonary route produce localized vasodilation in the pulmonary arteries. In fact, it has been shown that prostacyclin analogues act directly on the pulmonary arterial wall from the adventitial side, and not upon recirculation of the drugs from pulmonary or bronchial arteries.[166] The inhalable route of administration for prostacyclin analogues is diagrammed in Figure 9.[165]

Two of the currently FDA-approved prostacyclin analogues have been studied for direct delivery to the lungs. One of them, inhaled iloprost, has already been approved by the FDA for treatment of NYHA class III and IV patients under the brand name Ventavis®. Inhalable treprostinil is now in a Phase III trial (TRIUMPH-1; see Section IX.B.) for its long-term safety and efficacy in humans. In addition to prostacyclin analogues, other anti-PAH molecules have also been studied to establish a treatment regimen for PAH using an inhalable drug formulation. In the following section, we have summarized studies that have evaluated the safety and efficacy of various inhalable formulations of anti-PAH drugs.

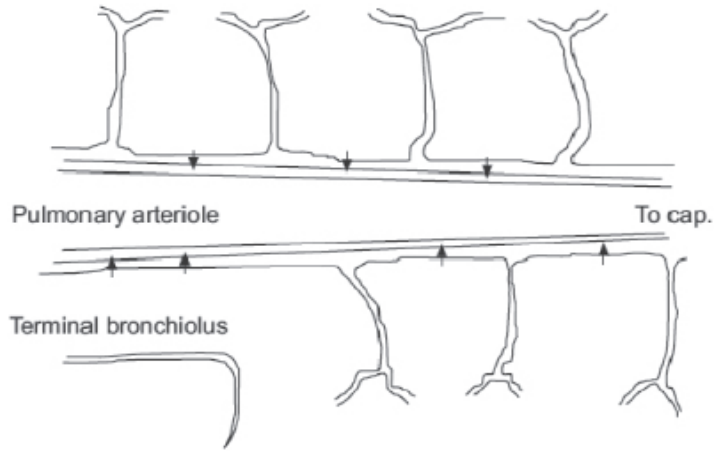

FIGURE 9. Inhaled route of administration of prostacyclin analogues. Black arrows indicate the areas where locally deposited drug can penetrate the airway wall and directly diffuse into the pulmonary arterial wall. Terminal arterioles, carrying most of the resistance, are completely surrounded by alveolar surfaces. Cap, pulmonary capillaries. (Reproduced from Gomberg-Maitland and Olschewski[165] with permission from the European Respiratory Society.)

IX. AEROSOLIZED DELIVERY OF THERAPEUTIC AGENTS FOR PAH

IX.A. Iloprost

Iloprost is a synthetic analogue of prostacyclin and the first FDA-approved inhalable therapy (Ventavis®) for NYHA class III and IV PAH. The efficacy of inhaled iloprost in PAH treatment has been studied in patients with PAH and various animal models of PAH. Inhaled iloprost reverses vascular remodeling in a chronic monocrotaline-induced rodent model of PAH. Iloprost, when administered in nebulized form for 15 minutes, 12 times a day, at a dose of 6 μg/kg/day to monocrotaline-induced PAH rats, caused a reduction in right ventricular systolic pressure, an increase in cardiac output, and a decrease in the pulmonary vascular resistance index. Inhaled iloprost also significantly decreased the degree of muscularization. The percentage of fully vascularized vessels and median wall thickness were decreased significantly in inhaled iloprost-treated rats: 21.8% ± 2.8% compared to 32.0% ± 5.0% for monocrotaline-treated rats. Inhaled iloprost caused a reduction in the expression of matrix metalloproteinases (MMPs), especially MMP-2, in monocrotaline-treated rats.[123]

In an isolated rabbit lung model, the pharmacokinetics and vasodilatory effects of inhaled iloprost were compared with an infused formulation of iloprost. PAH (MPAP ≈ 32 mm Hg) was induced by infusing U46619, a thromboxane A2 agonist, for 210 minutes. Nebulization of 75 ng iloprost over a period of 10 minutes resulted in a significant reduction in PAP. The anti-hypertensive effect was maintained for 50 minutes and then leveled off at the end of 210 minutes.

When the drug was administered as an I.V. infusion at a dose of 200 ng bolus + 33 ng/h infusion, the PAP decreased by a mean of 9.5 mm Hg, but the effects began to diminish within 40 to 60 minutes of the treatment and completely disappeared after 210 minutes.[167]

A number of clinical trials were conducted before the approval of iloprost for treatment of NYHA class III and IV patients with PAH. Olschewski et al. published the first clinical study with aerosolized iloprost in 6 patients with PAH at a dose of 100 μg in 6 to 9 divided doses. The study demonstrated acute and long-term effects on the hemodynamics of the patients.[168] A randomized and placebo controlled multicenter study was performed with 203 patients with IPAH, NYHA class III or IV. In that study, the effects of 6 to 9 inhalations of 5 μg iloprost per day on 6MWD and NYHA class improvement were investigated. An improvement in 6MWD (>10%) was observed in 17% of patients and the hemodynamic parameters were also reported to be improved at the end of the 12-week study.[169] This study, called AIR (Aerosolized Iloprost Randomized study), eventually led to the approval of inhalable iloprost by the FDA in 2006. Hoeper et al. also conducted a clinical trial to determine the long-term efficacy of inhaled iloprost in 24 patients with IPAH. The trial showed a significant improvement in hemodynamics and exercise capacity after 12 months of therapy, with a mean 6MWD increase of 85 m.[170]

The effect of different inhaler devices on the nebulization of iloprost has also been studied by Olschewski et al. No differences in hemodynamic improvements or half-lives were observed when three different nebulizers (Iloneb/Aerotrap®, Ventstream®, and Halolite®) were used to administer the drug.[166] A clinical trial was also performed to compare the effect of aerosolized and I.V. iloprost in patients with severe PAH. Although both I.V. and inhaled iloprost produced similar hemodynamic changes, the inhaled drug demonstrated more selectivity toward the pulmonary circulation.[171] In another study, McLaughlin et al. reported successful transition from oral bosentan to inhaled iloprost in 67 patients with IPAH and observed significant improvement in 6MWD and hemodynamic parameters.[172]

Most recently, Ivy et al. reported short- and long-term effects of inhaled iloprost in 22 pediatric patients with PAH who received 2.5 to 7.5 μg/dose at five to nine inhalations per day. It was shown that the acute vasodilator response to inhaled iloprost was similar to that induced by inhaled NO in children, and the addition of inhaled iloprost reduced the need for I.V. prostacyclin therapy.[173] In another study, improved gas exchange and exercise tolerance were observed in PAH and COPD patients following two doses of inhaled iloprost (2.5 μg).[174] As is evident from the above studies, iloprost has to be inhaled six to nine times a day to maintain the drug levels within the therapeutic range. To overcome the problems of multiple dosing, there is an urgent need to develop a controlled-release formulation of iloprost.

IX.B. Treprostinil

Treprostinil is one of the chemically stable tricyclic benzidine prostacyclin analogues currently approved by the FDA for the treatment of PAH. The efficacy of the inhaled treprostinil formulation in PAH therapy has been studied both in animals and human subjects. Sandifer et al. compared the efficacy of aerosolized and I.V.-administered treprostinil on the pulmonary circulation in a PAH-induced sheep model. PAH was induced by infusing a prostaglandin H_2 (PGH_2) analogue, U-44069, at a rate of 1000 ng/kg/min for 180 minutes. Aerosolized treprostinil was more effective than I.V. treprostinil as a pulmonary vasodilator even at a dose of 250 ng/kg/min. At the highest dose used in the experiment (1000 ng/kg/min), treprostinil decreased both PAP and PVR to baseline levels, even with continuous infusion of the vasoconstricting agent. Pulmonary delivery also resulted in a more localized delivery of treprostinil into the alveolar regions.[175]

In a placebo-controlled, randomized clinical trial, Voswinckel et al. showed that treprostinil, when inhaled, produced sustained pulmonary vasodilatory effects with excellent tolerability at relatively low doses when compared with inhaled iloprost.[176] Inhalation of both iloprost and treprostinil decreased MPAP and PVR, but no significant differences were observed between the AUCs (area under curves) of PVR for iloprost and that for treprostinil (12.6% ± 7.0% vs. 13.3% ± 3.2%). Treprostinil did not show any significant changes in cardiac output and systemic arterial pressure (SAP). In addition, the maximum effects of iloprost and treprostinil on PVR were comparable. The maximal effect of treprostinil was observed 18 ± 2 minutes after inhalation, whereas for iloprost it took 8 ± 1 minutes for the maximal effect to occur. Importantly, the effect of treprostinil lasted for approximately 60 to 180 minutes (Fig. 10).[176] In another study, Channick et al. reported the efficacy of inhaled treprostinil administered at 30 to 45 μg/dose four times daily using an ultrasonic nebulizer as an add-on therapy to bosentan in 12 NYHA class III or IV patients. At 12 weeks, patients showed an improvement in 6MWD (by 86 m), significant improvements in MPAP and PVR, and improvement from NYHA class III to class II.[177] Voswinckel et al. reported the efficacy of treprostinil after delivery via a metered dose inhaler (MDI) at a dose of 30, 45, and 60 μg at one time. Various efficacy parameters were recorded for 180 minutes. The authors demonstrated that the AUCs of PVR and PAP decreased significantly with all three doses of treprostinil delivered by MDI, whereas changes in systemic hemodynamics—SAP, cardiac output, and heart rate—were minimal or unaltered.[178]

Inhaled treprostinil has recently entered Phase III clinical trials [Treprostinil Sodium Inhalation Used in the Management of Pulmonary Arterial Hypertension (TRIUMPH-1)]. In this 12-week, double-blind, randomized, placebo-controlled study—data from which are yet to be published—comprising 235 NYHA class III and IV patients, inhaled treprostinil administered at doses up to 45 μg four times daily showed improved 6MWD by 20 m, with no change in clinical class or disease worsening. Furthermore, a biomarker of right ventricular enlargement,

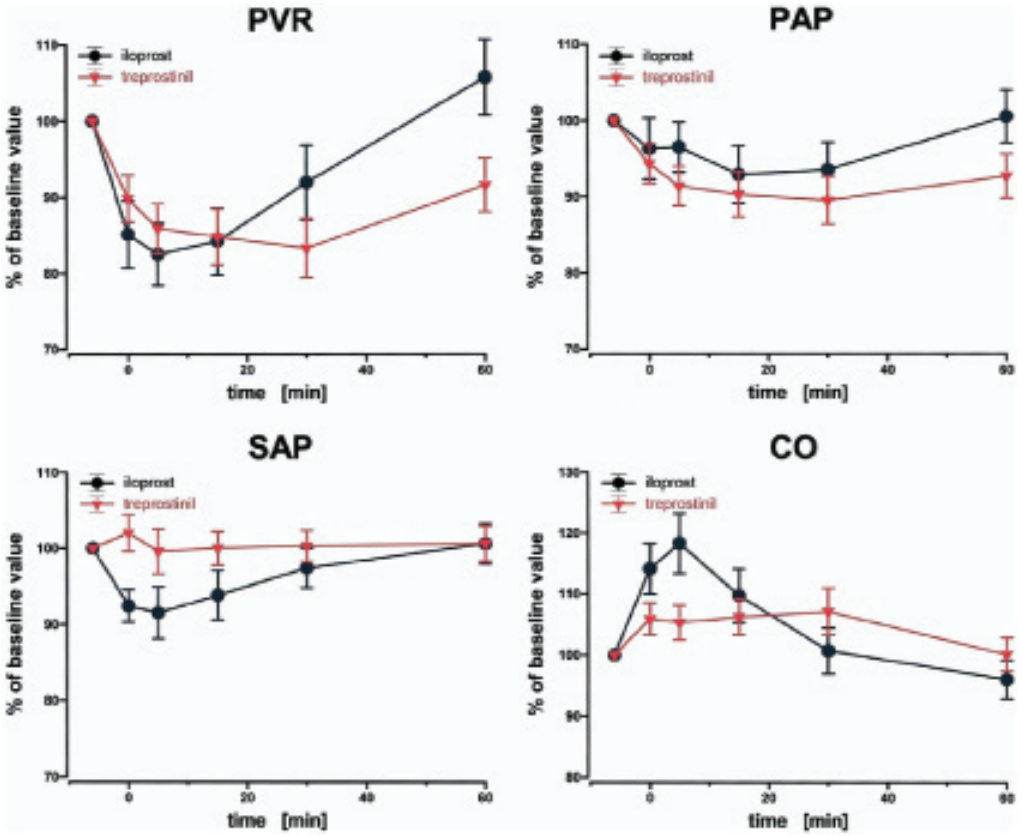

FIGURE 10. Hemodynamic response to inhalation of treprostinil versus iloprost. Data from 44 patients who inhaled both drugs in randomized order. CO, cardiac output; PAP, pulmonary arterial pressure; PVR, pulmonary vascular resistance; SAP, systemic arterial pressure. (Reproduced from Voswinckel et al.[176] with permission from Elsevier.)

BNP (brain natriuretic peptide), decreased significantly, suggesting improvement in the disease. A controlled-release oral formulation of treprostinil is now in a Phase III trial as a combination therapy with sildenafil or bosentan (FREEDOM-C). Preliminary results do not show any significant improvement in 6MWD, the primary endpoint of the study. The same formulation has also been studied as a stand-alone therapy for PAH (FREEDOM-M), and the results are yet to be published.

IX.C. Prostaglandin E_1

Prostaglandin E_1 (alprostadil, PGE_1) is a potent vasodilator that has been approved by the FDA for the treatment of erectile dysfunction. Because it is a pulmonary-selective vasodilator, PGE_1 has also been investigated as a potential treatment option for PAH by I.V. administration.[179,180] Several studies have shown that

PGE_1 is efficacious in the treatment of several respiratory conditions, including acute respiratory distress syndrome, hypoxemic respiratory failure, and lung transplantation.[181] Like other prostacyclins, PGE_1 is a very short-acting, potent vasodilator, with a half-life of 5 to 10 minutes. Intravenous administration of PGE_1 results in side effects similar to those caused by other short-acting prostacyclin analogues, such as systemic hypotension and low cardiac output.[181]

Nakazawa et al. used aerosolized PGE_1 for the treatment of PH and hypoxia in oleic acid-induced lung injury in Japanese white rabbits. The authors reported that aerosolized PGE_1 along with partial liquid ventilation improved gas exchange, relaxed the pulmonary circulation, and reduced PVR without causing systemic hypotension or reduction in cardiac output.[182]

Sood et al. reported the results of a Phase I/II clinical trial of inhaled PGE_1 for the treatment of hypoxemic respiratory failure and neonatal PAH associated with respiratory failure. In this study, 20 infants with hypoxemic respiratory failure were enrolled and received aerosolized PGE_1 at a dose ranging from 100 to 300 ng/kg/min. A significant improvement in oxygenation was observed following 3 hours of inhalation, thus establishing the short-term safety and efficacy of inhaled PGE_1 in neonates with hypoxemic respiratory failure.[183] A recent clinical trial also documented the efficacy of aerosolized PGE_1 in improving pulmonary hemodynamics and oxygenation in 18 patients undergoing lung transplantation. The authors reported that PGE_1 administered at a low dose produced a reduction in PAP and improvement in oxygenation without impairing systemic hemodynamics.[184] Although there is compelling evidence that PGE_1 is effective as a pulmonary vasodilator, more studies are needed to develop PGE_1 as a therapeutic agent for PAH treatment.

IX.D. Vasoactive Intestinal Peptide

VIP, a 28-amino-acid peptide, plays an important role in maintaining pulmonary vascular tone. VIP has been investigated for treatment of asthma-related bronchoconstriction.[185–188] The levels of VIP are reduced in patients with PAH, and inhaled VIP has been used in the treatment of PAH, with some beneficial effects. In a preliminary clinical study, Petkov et al. reported that inhaled VIP (100 μg) improves pulmonary hemodynamics. Daily administration of VIP (200 μg) for 3 months produced a significant improvement in MPAP, cardiac output, PVR, and 6MWD, without altering systemic parameters.[131] An open-label clinical study by Leuchte et al. showed the efficacy of VIP or aviptadil (a generic name given to VIP by the WHO in 1997) in 20 patients with PAH who were undergoing right-heart catheterization. The study showed that aviptadil, when administered as an aerosol using the Optineb®-ir nebulizer at a dose of 100 μg, caused a reduction in PAP and PVR and improved oxygenation without affecting systemic hemodynamic parameters. In addition, the study by Leuchte et al. showed beneficial and statistically significant hemodynamic effects, although the beneficial effects of aviptadil were modest and short-lived.[189] These short-lived effects can be attributed to rapid degradation of the peptides by endogenous

proteases,[190] and thus call for the development of a sustained-release formulation of VIP (aviptadil).

IX.E. Adrenomedullin

The circulating levels of ADM, a potent and long-lasting vasodilator peptide, are reported to be increased in patients with PAH. ADM is reported to play a role in maintaining pulmonary vascular tone in both animal models and clinical studies.[135] The efficacy of both I.V. and inhaled ADM has been investigated. ADM, administered by an ultrasonic nebulizer, was reported to decrease MPAP and PVR, with minimal effects on systemic vascular tone, following 3 weeks of treatment in monocrotaline-induced PAH rats. ADM inhalation also inhibited an increase in medial wall thickness in PAH-induced rats.[191] Similar results were obtained in a surfactant-depleted piglet model.[192] Nagaya et al. reported beneficial effects of ADM inhalation in 11 patients with IPAH. When inhaled at a dose of 10 μg/kg, ADM produced a significant increase in MPAP and PVR in patients with IPAH, without altering SAP or heart rate. ADM inhalation also improved oxygenation and exercise tolerance in patients with PAH.[193] Although the results on inhaled ADM are promising, more studies are required to establish the long-term efficacy of this new drug.

IX.F. Soluble Guanylate Cyclase

The activators and stimulators of sGC, an important mediator in NO-cGMP signaling, produce their effects in PAH treatment by increasing cGMP production in the pulmonary circulation. The efficacy of sGC activators and stimulators has been studied in various animal models of PAH and clinical settings.[94] In a recent study, Evgenov et al. reported the efficacy of different sGC stimulators (BAY 41-2272 and BAY 41-8543) and sGC activators (BAY 58-2667) in providing selective pulmonary vasodilation in lambs following aerosolized delivery of the drug in microparticulate formulations. All of the investigational agents produced dose-dependent vasodilation and increased cGMP release without systemic side effects. The agents also increased the duration and magnitude of treatment with inhaled NO.[194] These preliminary studies demonstrate the short-term efficacy of sGC in PAH treatment. More extensive studies are needed to confirm the long-term effects of sGC stimulators and activators.

IX.G. Fasudil (Rho-kinase Inhibitor)

Rho-kinase (ROCK) signaling plays an important role in PAH prognosis. Intravenous Rho-kinase inhibitors have been demonstrated to ameliorate PAH symptoms. However, very few studies have investigated the efficacy of inhaled fasudil, a potent Rho-kinase inhibitor, in PAH treatment. One of the seminal studies was reported by Nagaoka et al.[106] The authors showed that fasudil, when administered as an aerosol solution at a concentration of 100 mM, produced selective

pulmonary vasodilation. Significant reductions in MPAP in three animal models of PAH (hypoxia, fawn-hooded, and monocrotaline-induced) were observed with no adverse effect on heart rate or cardiac index.[106] Indeed, there has been increasing interest in pulmonary applications of Rho-kinase inhibitors, especially fasudil, in PAH treatment. Fasudil will soon be entering into clinical trials.

X. NOVEL SYSTEMS FOR CONTROLLED DELIVERY OF ANTI-PAH DRUGS

As discussed above, many of the current therapeutic agents approved for PAH treatment have the shortcomings of very short biological half-lives and systemic side effects. To circumvent the systemic side effects, the pulmonary route has been proposed for delivery of anti-PAH drugs. However, the short biological half-lives of many anti-PAH drugs, which necessitate multiple inhalations (6–9 per day in the case of iloprost®) or continuous infusions (for epoprostenol® and treprostinil®), remain the major impediment that must be overcome. This problem can be addressed by using novel controlled-release technology for delivering anti-PAH agents directly to the lungs. Inhalable controlled-release drug-delivery systems offer several advantages over conventional drug formulations, including: (i) selective short- and long-term vasodilation of the pulmonary arteries; (ii) elimination of catheters or needles required to administer the drugs; (iii) higher bioavailability of drugs compared to other mucosal routes of administration because of the lungs' relatively large absorptive surface area and thin epithelium; (iv) close proximity of the alveolar epithelium, that is, the short distance between the site of absorption and the pulmonary artery makes this avenue of drug administration particularly suited for PAH treatment; (v) self-administration of the formulations by patients as a dry powder by means of an inhaler device similar to that used for inhaled insulin. Administration of anti-PAH medication directly into the lungs will give a relatively high concentration of the drug in the lungs that can reduce the total dose required; and (vi) portability and self-administration of the formulation will allow patients to carry on a more normal lifestyle, including going to work or school. However, controlled delivery systems for anti-PAH agents are an entirely novel idea, and very little information is available in the scientific literature regarding the use of controlled-release formulations in PAH therapy. We have summarized below some very novel findings that should be studied in more detail for accomplishing the goal of sustained release of medications to hypertensive lungs.

X.A. Polymeric Micro and Nano Particulate Drug Delivery Systems

Polymeric carriers have been extensively studied as carriers for inhalable, controlled-release formulations of drugs. The major features of the polymeric micro- and nano-sized carriers that make them attractive for controlled-delivery technology include: (i) degradability of the polymers in endogenous fluid, (ii) low systemic toxicity, (iii) biocompatibility, and (iv) flexibility to refine the drug-

release properties. A number of biodegradable polymers have been investigated for their ability to achieve sustained delivery of various therapeutic agents, including poly (lactic-co-glycolic acid) (PLGA), polyanhydrides, polyketals, hydroxypropyl methacrylate (HPMA), and hydropropyl cellulose (HPC).[195,196] Some of the controlled-release PLGA-based formulations approved by the FDA include Lupron Depot®, Prostap® 3, Enantone Depot®, Decapeptil®, Trelstar®, Neutropin Depot®, and Somatuline® L.

Although there are little data on the use of polymeric particles for controlled release of anti-PAH drugs, this approach has tremendous potential for producing prolonged, localized delivery of anti-PAH agents. PLGA has chiefly been used to prepare micro- and nanoparticles for inhalable controlled-release formulations.

Very recently, our lab showed that pulmonary delivery of PGE_1, a selective pulmonary vasodilator and an investigational drug for PAH treatment, encapsulated in biodegradable PLGA microparticles resulted in a tremendous increase in the biological half-life of the drug: 582.39 ± 70.3 minutes as compared to 3.49 ± 0.41 minutes following administration of plain PGE_1 via the pulmonary route (Fig. 11A). In addition, encapsulation of PGE_1 into PLGA microparticles increased the metabolic stability of the drug up to 8 hours compared to 60 to 120 minutes with the plain drug, when studied in rat lung homogenates (Fig. 11B). Also, the microparticles were found to be safe for pulmonary administration, as demonstrated by acute cytotoxicity studies in the Calu-3 lung epithelial cell line and by studies of bronchoalveolar lavage (BAL) fluid (Fig. 12). However, the efficacy of the formulations in providing a sustained reduction in MPAP has yet to be tested in rodent models of PAH.[197]

In another recent study, Ishihara et al. showed that encapsulation of PGE_1 in nanoparticles prepared by a blend of polylactic acid (PLA) homopolymer and pegylated (PEG)-PLA block copolymers results in retention of the drug in the nanoparticles and extension of the blood-residence time of PGE_1, with preferential accumulation of PGE_1 in vascular lesions.[198] Although that study did not use inhalational drug delivery, it still provides new insights regarding the use of PEG-PLA nanoparticles for delivery of anti-PAH agents. Very recently, Huang et al. reported that PLGA microspheres of PGE_1 provide sustained release of the drug, thus potentiating the impaired angiogenesis by basic fibroblast growth factor (bFGF) in ischemic limbs of diabetic mice. Although not related to PAH treatment, that study utilized controlled drug-delivery technology, which can be directed toward developing controlled-release systems for anti-PAH medications.[199] The information gained with PGE_1 as a model drug can be employed in developing sustained-release formulations of currently approved anti-PAH prostacyclin analogues, which are structurally similar to PGE_1.

Several other investigational anti-PAH treatments have also been studied using a polymeric controlled-release approach. Very recently, Harada-Shiba et al. reported successful intratracheal delivery of polymeric nanomicelles of the human ADM gene in monocrotaline-induced PAH rats. The authors utilized a poly (aspartamide) derivative of PEG-based block catiomers. They reported a

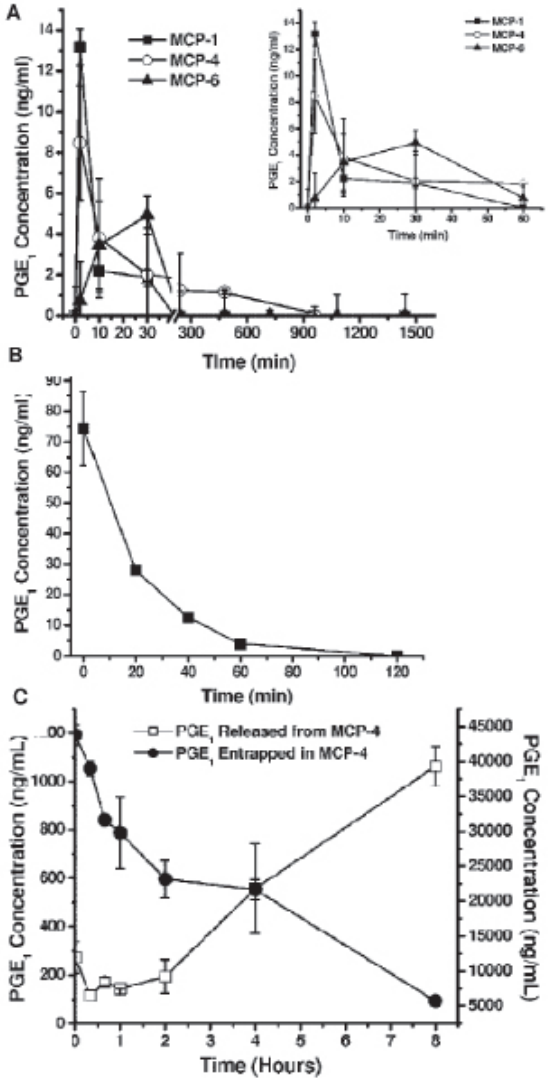

FIGURE 11. Pulmonary delivery of PLGA-microparticulate formulations of PGE_1 in rats. (A) *In vivo* performance of the formulations [MCP-1 (PLGA 50:50, 1% PVA), MCP-4 (PLGA 85:15, 1% PVA), and MCP-6 (PLGA 85:15, 5% PVA)] following pulmonary administration at a dose of 80 mg/kg (n = 6–8); and metabolic degradation of PGE_1 in rat lung homogenate after addition of (B) plain PGE_1 (80 ng/mL) and (C) MCP-4 (5 mg) (n = 3). Data represent mean ± standard error of the mean. PLGA, poly (lactic-co-glycolic acid); PVA, polyvinyl alcohol. (Reproduced from Gupta et al.[197] with permission from Wiley InterScience.)

decrease in right ventricular pressure 3 days after administration of the ADM gene with a notable increase in pulmonary human ADM mRNA levels.[200] In another study, Kimura et al. reported that pulmonary delivery of nuclear factor kappa-B (NF-κB) oligodeoxynucleotides (ODNs) encapsulated in PEG-block-

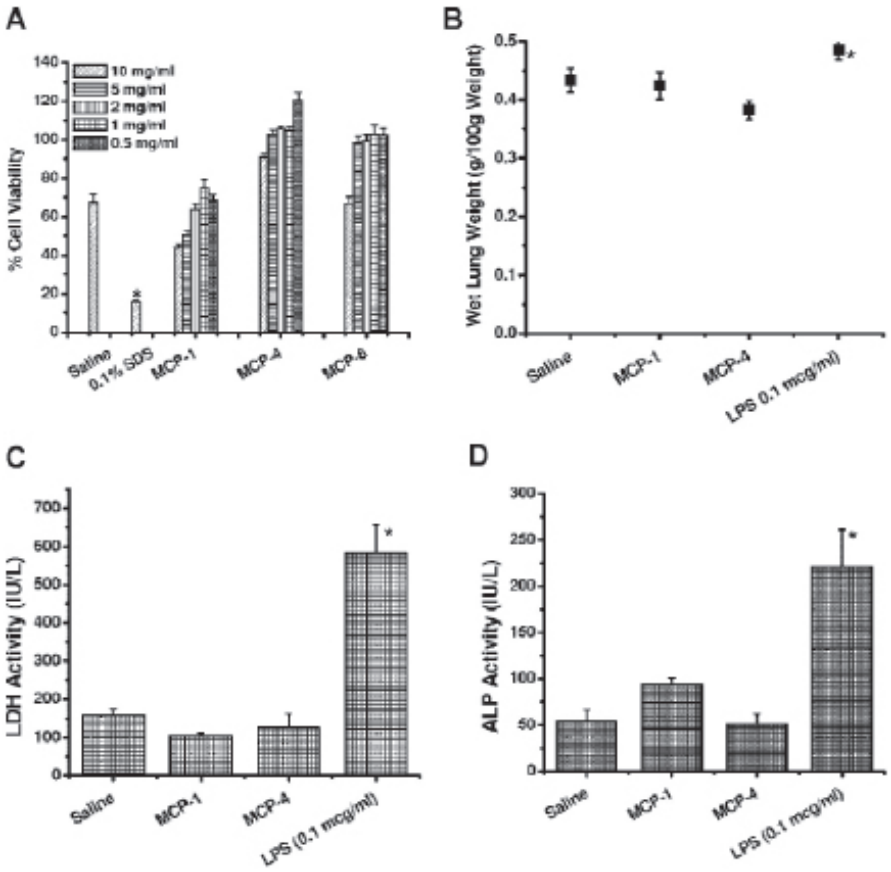

FIGURE 12. (A) Effects of microspheric formulations (MCP-1, -4, and -6) on the viability of Calu-3 cells. The test samples contained 0.5, 1.0, 2.0, 5.0, or 10.0 mg/mL of microspheres (n = 16). (B–D) Safety studies with bronchoalveolar lavage fluid analysis 12 h after administration of the formulations. (B) Corrected wet lung weights. (C) Levels of lactate dehydrogenase (LDH), and (D) alkaline phosphatase (ALP). Data represent mean ± standard error of the mean (n = 4). LPS, lipopolysaccharide positive control. (Reproduced from Gupta et al.[197] with permission from Wiley InterScience.)

lactide/glycolide copolymer nanoparticles in monocrotaline-induced PAH rats produced a significant reduction in PH and pulmonary arterial remodeling.[201]

The above findings suggest that polymeric micro/nanocarriers may offer a viable alternative for delivery of anti-PAH medications directly to the lungs with sustained therapeutic benefits. However, there are some practical limitations regarding the use of these delivery systems. In terms of nanoparticulate delivery, most of the inhaled nanoparticles are exhaled from the respiratory tract rather than being deposited into the lungs, thus making nanoparticle-based systems unsuitable for inhalational delivery. On the other hand, microparticle-based delivery systems, although capable of reaching small bronchi, are susceptible to

being recognized and eliminated by the lungs' clearance mechanisms. To overcome these limitations and to take advantage of both systems, it is necessary to develop a nanocomposite microsized delivery system, which will decompose into nanoparticles after reaching the small bronchi and thereby bypass the clearance mechanisms and avoid the risk of being exhaled.[202]

X.B. Liposomes

For many years liposomes have been studied as carriers for pulmonary delivery of various therapeutic agents. Liposomes offer several advantages for pulmonary drug delivery, including minimal adverse effects, minimal local irritations in the lungs, and prolonged release rates following pulmonary administration. Liposomes can be prepared from a variety of phospholipids and can be customized for specific requirements of the molecule to be delivered based on charge, size, and composition of phospholipids. In the pulmonary circulation, liposomes are phagocytosed by macrophages, thus resulting in rapid clearance of the encapsulated drug from the circulation. To overcome this limitation, sterically stabilized or stealth liposomes were developed. These liposomes have a hydrophilic polymeric (polyethylene glycol) coating, which attracts water to the liposomal surface, and, in turn, prevents recognition of the liposomes by macrophages, allowing their subsequent uptake.

Several studies have reported the use of liposomal drug-delivery systems for inhalational delivery of anti-PAH medications. In a pioneering study, Kleemann et al. described preparations of liposomes of iloprost, an FDA-approved inhaled prostacyclin analogue for pulmonary delivery.[203] The authors reported that DPPC/CH (dipalmitoyl-phosphatidylcholine/cholesterol) and DPPC/CH/DPPE-PEG liposomes of iloprost are attractive vehicles for therapeutic delivery of anti-PAH medications. Their data suggest that liposomal iloprost is suitable for delivery via both vibrating-mesh and ultrasonic nebulizers.[203] The study by Kleemann et al. was the first to investigate liposomal formulations of prostacyclin analogues, which should now be tested for pharmacokinetic and therapeutic efficacy. In another study, Stark et al. reported the development of a liposome-based inhalational therapy for VIP for PAH treatment. Nebulized VIP provides sustained release of VIP, thus extending the drug's pharmacological effects.[204] In a more recent study from the same group, Hajos et al. reported that sustained release of VIP from VIP-loaded liposomes (VLL) improved the biological and pharmacological activity of VIP.[205] A schematic illustration of the mechanism of action of VIP-loaded liposomes is shown in Figure 13. Although, at this time, there are only a very few studies reporting the efficacy of liposomal drug-delivery systems in PAH treatment, we anticipate that liposomal delivery systems of anti-PAH medications may eventually play a significant role in PAH treatment and in targeting the diseased cells of the pulmonary circulation.

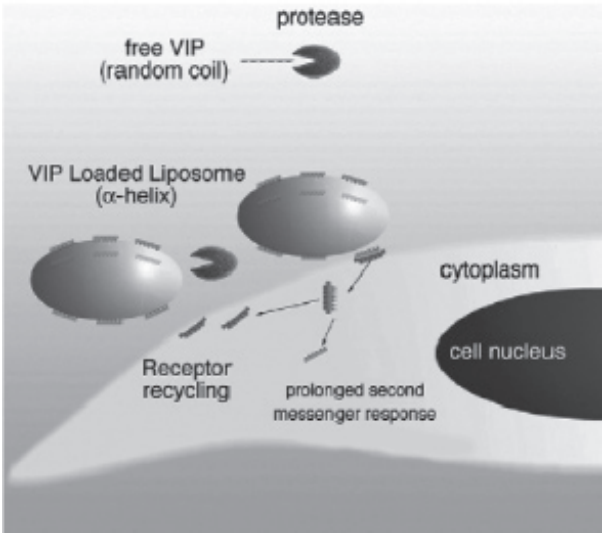

FIGURE 13. Suggested functional model of VIP-loaded liposomes (VLL). Free VIP (random coiled) can easily be degraded by proteases on the way to the receptor. VIP from VLL is protected against proteases. Following internalization of the ligand–receptor complex, and intracytoplasmic disintegration of the complex, the receptor is recycled to the cell surface to bind new incoming VIP molecules. In addition to the protection by liposomes per se, the alpha helical conformation of VIP induced by negatively charged liposomes may convey further protection from degradation; moreover, it is the preferred VIP-conformation for receptor binding. (Reproduced from Hajos et al.[205] with permission from Elsevier.)

X.C. Other Approaches

Although there are no reports regarding the use of any other system for delivery of anti-PAH drugs to the pulmonary vasculature, there are several new and exciting approaches that are being investigated for the development of inhalational sustained-release drug-delivery systems. These include lipid microspheres, solid lipid nanoparticles, nanocomposite microsized particles, and large porous particles. Several laboratories, including ours, are working on inhaled controlled-release alternatives to the current therapeutic agents for PH, and we expect to see some important developments in the near future.

XI. CONCLUDING REMARKS

PAH, a devastating disorder of the pulmonary circulation, is associated with the imbalance of various neurochemical mediators, inflammatory cell-derived and locally generated cytokines, and growth factors. The current treatment approaches have been focused on restoring normal vascular tone via prostacyclin replacement, endothelin inhibition, NO inhalation, and PDE-5 inhibition. Despite the efforts of many investigators and the existence of many FDA-approved anti-PAH

medications, there is still no cure for PAH. This situation prompted exploration of other pathways responsible for PAH progression and led to the emergence of various investigational PAH treatments such as tyrosine kinase inhibitors, ADM, and statins. Furthermore, efforts have also been made to develop novel approaches to deliver anti-PAH drugs to overcome the limitations of current PAH therapy, including increasing the biological half-life of currently available medications. The use of the pulmonary route for anti-PAH drug delivery has emerged as an excellent alternative to the current injectable therapy, because it provides controlled and targeted delivery of the medications to the diseased region of the body (i.e., the lungs) and can also circumvent the systemic side effects associated with current therapy. Several studies have reported successful development of novel drug-delivery systems such as polymeric micro/nanoparticles, liposomes, and nanomicelles all loaded with currently available anti-PAH drugs to treat PAH in rodent models. These studies have generated immense interest within the PAH research community. Based on the interest generated, it is reasonable to expect that a safe and effective drug-delivery system will be developed in the near future that will produce selective vasodilation of the pulmonary arteries and provide a better quality of life to patients with PAH.

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