Pharmacological profiles and therapeutic applications of pachymic acid (Review)

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Abstract. *Poria cocos* is a saprophytic fungus that grows in diverse species of *Pinus*. Its sclerotium, called fu-ling or hoelen, has been used in various traditional Chinese medicines and health foods for thousands of years, and in several modern proprietary traditional Chinese medicinal products. It has extensive clinical indications, including sedative, diuretic, and tonic effects. Pachymic acid (PA) is the main lanostane-type triterpenoid in *Poria cocos*. Evidence suggests that PA has various biological properties such as cytotoxic, anti-inflammatory, antihyperglycemic, antiviral, antibacterial, sedative-hypnotic, and anti-ischemia/reperfusion activities. Although considerable advancements have been made, some fundamental and intricate issues remain unclear, such as the underlying mechanisms of PA. The present study aimed to summarize the biological properties and therapeutic potential of PA. The biosynthetic, pharmacokinetic, and metabolic pathways of PA, and its underlying mechanisms were also comprehensively summarized.

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Contents

1. Introduction

Pachymic acid (PA), a lanostane-type triterpenoid, was first isolated and characterized from the sclerotium of *Poria cocos* (Fig. 1) in 1958 (1). PA is mainly derived from the wood-rotting fungus *Poria cocos* (2-4), a saprophytic fungus that grows in diverse species of *Pinus*. Its sclerotium, known as fu-ling or hoelen, has been widely used as a health food and in traditional Chinese medicine with pharmacological properties, including sedative, diuretic, and tonic effects (5-7). Overall, >160 terpenoids have been identified in *Poria cocos* (8). As an important bioactive terpenoid, PA has been reported to exhibit numerous pharmacological effects, such as cytotoxic (9), anti-inflammatory (10), antihyperglycemic (11), antibacterial and antiviral (12), sedative-hypnotic (13) and anti-ischemia/reperfusion (14) effects. Thus, PA has potential for the treatment of various diseases. However, it is still in the preclinical development stage, possibly owing to limited *in vivo* data. To provide comprehensive data for further study, to the best of our knowledge, the current study systematically summarized the current progress in the research and development of PA for the first time, including its sources, structure and properties, pharmacological activities, and pharmacokinetics. It aimed to improve the current knowledge and development potential of PA, and improve its development for potential clinical applications.

PA was first discovered in the sclerotium of *Poria cocos* from which its name is derived (1). Since then, it has been observed as the active compound in several fungi, such as the sclerotium (15-24) and epidermis (25) of *Poria cocos* and the

fruiting bodies of *Fomitopsis nigra* (26,27) and *Fomitopsis pinicola* (28,29), which are used in traditional medicine. In addition to fungi, PA is extracted from *Heterosmilax chinensis* (30), a traditional Chinese medicine used for the treatment of cancer. Zhu *et al* (31) reported a possible biosynthetic pathway from lanostane to PA (Fig. 2). PA is a white powder and highly insoluble in water (32). Therefore, the poor solubility of PA results in its low bioavailability *in vivo*, which may limit its further clinical applications. Cai *et al* (32) demonstrated that glycyrrhizin (a triterpenoid glycoside) increases the solubility of PA in an aqueous solution, thereby improving its bioavailability.

2. Pharmacological activities

Cytotoxic effect. At present, cancer is a major disease that seriously affects human health (33). Table I presents the cytotoxic effects and underlying molecular mechanisms of PA. PA has been reported to against various cancer cell lines, including colon cancer (16), leukemia (34), bladder cancer (35,36), nasopharyngeal carcinoma (37), prostate cancer (38), primary osteosarcoma (39), breast cancer (40,41), lung cancer (42,43), gastric cancer (44,45) and pancreatic cancer (46). Several molecular mechanisms are involved in this process.

Notably, PA has been demonstrated to be a novel retinoid X receptor (RXR)-specific agonist that induces the differentiation of leukemia HL-60 cells (34). Moreover, it displays antiproliferative activity against SK-BR-3 cells by specifically activating pyruvate kinase muscle isozyme M2 (PKM2), inhibiting hexokinase 2 (HK2), and decreasing glucose uptake and lactate production (40). PA can inhibit the proliferation of several cancer cell lines by downregulating the expression of CDK1, CDK2, CDK4 and cyclin E to block cell cycle arrest, including in MDA-MB-231, SGC-7901 and MKN-49P cells (41,44,45). Apoptosis also plays an important role in PA's cytotoxicity. PA induces apoptosis in various cancer cells, including bladder EJ cells, breast SK-BR-3 cells, pancreatic PANC-1 cells, MIA paca-2 cells and prostate DU145 cells, through the generation of reactive oxygen species (ROS) and endoplasmic reticulum (ER) stress, upregulation of cleaved caspase-3,-8 and -9, cleaved poly (ADP-ribose) polymerases, Bax and Bad, phosphorylated (p)-ataxia telangiectasia mutated protein, p-ataxia telangiectasia-mutated and Rad3-related kinase, p-checkpoint kinase 1, p-histone H2A.X, X-box-binding protein-1s, activating transcription factor 4 (ATF4), heat shock protein 70, C/EBP homologous protein and p-eukaryotic initiation factor-2α and downregulation of Bcl-2 and B-cell lymphoma-extra large (36,37,38,44,46).

Studies on animal models have further demonstrated the *in vivo* anticancer activities of PA. For example, Kaminaga *et al* (47) revealed that PA suppresses the promotion of skin tumor formation by 12-O-tetradecanoylphorbol-13-acetate (TPA) in 7,12-dimethylbenz[a]anthracene-treated mice. Ma *et al* (43) demonstrated that PA significantly inhibits the growth of NCI-H23 xenograft tumors by inhibiting cell proliferation and inducing apoptosis without causing toxicity. Sun and Xia (44) reported that PA significantly suppresses the growth of SGC-7901 tumors in nude mice. In addition, PA pretreatment before animal xenograft model construction resulted in a significant decrease in tumor volume and weight (45). In addition, PA pretreatment increased xenograft survival and median times *in vivo*. This suggested that cancer cells pretreated with PA possessed weaker tumorigenicity and lethality. In a study by Cheng *et al* (46), PA was demonstrated to significantly inhibit MIA PaCa-2 tumor growth via the induction of apoptosis and the expression of ER stress-related proteins in tumor tissues; in addition, no major toxicity is observed in animals treated with 25 mg/kg PA. However, some toxic effects have been observed in mice treated with 50 mg/kg PA, including obvious pathological changes in the liver, kidney and spleen. Moreover, PA also demonstrates a sensitization effect. For example, Shan *et al* (48) reported that PA has no cytotoxicity at a concentration of 12.5 μg/ml, but does significantly enhance vincristine-induced cytotoxicity in drug-resistant KBV200 cells. PA was revealed to sensitize SGC-7901 and MKN-49P cells to radiation therapy by upregulating Bax under hypoxic conditions *in vitro* and *in vivo* (49).

Anti-invasion effect. In addition to its cytotoxic effect, PA also has an anti-invasion effect (Table I). It was reported to inhibit the invasion and adhesion ability of GBC-SD cells by affecting the ERK and AKT signaling pathways (50), HO-8910 cells by decreasing β-catenin and COX-2 expression and increasing E-cadherin expression (51), MDA-MB-231 cells via suppression of NF-κB-dependent MMP-9 expression and phosphorylation of membrane-associated phosphatidylinositol transfer protein 3 (52,53), and BxPc-3 cells via downregulation of MMP-7 expression (54).

Anti-inflammatory effect. Inflammation underlies numerous physiological and pathological processes involved in numerous diseases such as cancer, obesity and cardiovascular diseases (55). A number of studies have reported that PA has anti-inflammatory effects, and its mechanisms of action have been partially identified (Table I). Cuéllar *et al* (56) demonstrated that PA exhibits inhibitory activity against phospholipase A2 *in vitro*. PA has been observed to inhibit leukotriene B4 release, which is involved in chronic inflammation of skin pathologies (57). In addition, PA reduces lipopolysaccharide (LPS)-induced apoptosis, attenuates LPS-induced increases in mRNA expression levels of IL-1, IL-6 and TNF-α and inhibits LPS-induced apoptosis via the ERK1/2 and p38 pathways in H9c2 cells (58). Moreover, PA has been reported to inhibit inflammation in oral cells. For example, Kim *et al* (26) demonstrated that PA restores resin sealer AH Plus-damaged cell viability and alkaline phosphatase activity, suppresses the secretion of nitric oxide, TNF-α and IL-1β and reduces ROS formation and NF-κB translocation in MC-3T3 E1 cells. In addition, this study demonstrated that PA inhibits inflammation and induces odontoblast differentiation by increasing heme oxygenase 1 (HO-1) expression, suppressing NF-κB translocation and inducing nuclear factor erythroid 2-related factor 2 (Nrf2) translocation in human dental pulp cells (27). Furthermore, PA has been observed to inhibit TPA-induced mouse ear edema and phospholipase A2-induced mouse paw edema *in vivo* (59-61). Feng *et al* (62) revealed that, in animal models, PA reduces intravesical IL-1, IL-6 and lactate dehydrogenase levels, downregulates TNF-α and upregulates TP53 proteins in bladder samples. In cecal ligation and puncture (CLP)-induced septic rats, PA increases

Figure 1. Pachymic acid isolated from *Poria cocos*.

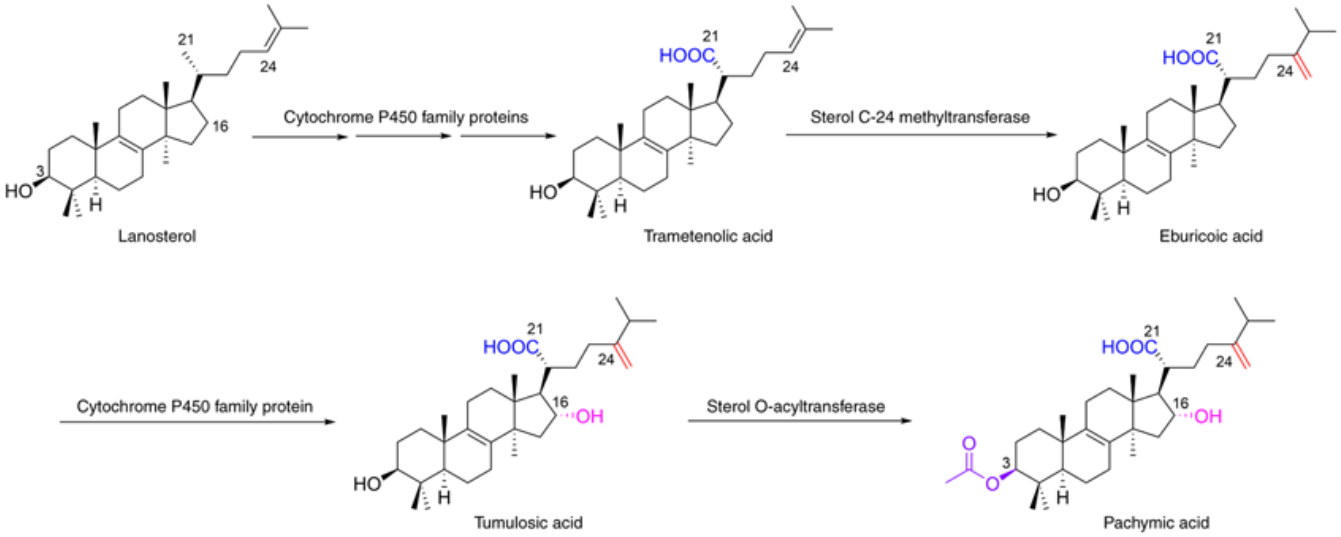

Figure 2. Putative biosynthetic pathway from lanostane to pachymic acid.

the survival of rats and attenuates CLP-induced acute lung injury by downregulating the serum levels of TNF-α, IL-1β and IL-6, decreasing malondialdehyde and myeloperoxidase levels and increasing superoxide dismutase levels (63). Gui *et al* (64) reported that PA alleviates LPS-induced lung injury by relieving LPS-inflammation, such as TNF-α, IL-6, monocyte chemoattractant protein 1 and IL-1β, reducing cell numbers in bronchoalveolar lavage fluid, and inhibiting LPS-induced apoptosis. Additionally, PA ameliorates renal injury *in vivo* by inhibiting renal inflammation, reducing the levels of TNF-α, IL-6 and inducible nitric oxide synthase, enhancing the expression of Nrf2 and HO-1, and preventing renal Wnt/β-catenin signaling (65,66).

Antihyperglycemic effect. Previously, several studies reported that PA has a significant antihyperglycaemic effect (67-69) (Table I). For example, Huang *et al* (67) revealed that PA induces glucose transporter type 4 (GLUT4) expression, stimulates GLUT4 redistribution from intracellular vesicles to the plasma membrane by upregulating the insulin-independent AMPK and insulin receptor substrate-1-PI3K-AKT pathways and induces triglyceride accumulation. Similarly, Chen *et al* (68) revealed that PA increases the glucose uptake by 50% in 3T3-L1 cells. PA has been further observed to decrease blood glucose levels in streptozocin-treated mice by enhancing insulin sensitivity irrespective of peroxisome proliferator-activated receptor-γ (69).

Table I. Biological activities of PA.

Biological effects	Details	Cell lines/Model	Dose	Application	(Refs.)
Cytotoxic effect	Inhibit proliferation with an IC_{50} value of 29.1 μM and inhibit DNA topoisomerase I and II	Colon HT-29 cells	20-100 μM	*In vitro*	(16)
	A novel RXR-specific agonist and induces differentiation of HL-60 cells with an EC_{50} value of 6.7±0.37 μM	Leukemia HL-60 cells	100 μM	*In vitro*	(34)
	Inhibit proliferation and induce apoptosis via mitochondrial-mediated intrinsic pathway and death receptor-mediated extrinsic pathway	Bladder T24 cells	5-30 μM	*In vitro*	(35)
	Inhibit proliferation and induce apoptosis via ROS generation, mitochondrial-mediated intrinsic pathway, and DR5-mediated extrinsic pathway	Bladder EJ cells	2.5-30 μM	*In vitro*	(36)
	Inhibit proliferation, induce apoptosis, and upregulate the levels of PARP, p-ATM, p-ATR, p-Chk-1, p-Chk-2, and p-histone H2A.X	Nasopharyngeal carcinoma CNE-1, CNE-2 cells	10-30 μM	*In vitro*	(37)
	Inhibit proliferation and induce apoptosis through mitochondria dysfunction by decreasing the phosphorylation of Bad and Bcl-2, and activating caspases-9 and -3	Prostate DU145 cells	10-40 μg/ml	*In vitro*	(38)
	Inhibit proliferation, and induce PTEN and caspase 3/7-dependent apoptosis	Primary osteosarcoma cells	10-50 μg/ml	*In vitro*	(39)
	An activator of PKM2 and an inhibitor of HK2, decrease glucose uptake and lactate production, and induce mitochondrial dysfunction, ATP depletion, and ROS generation	Breast SK-BR-3 cells	10-100 μM	*In vitro*	(40)
	Inhibit proliferation with an IC_{50} value of 2.13±0.24 μg/ml, induce cell cycle arrest and apoptosis through mitochondria-related and death receptor-mediated pathway	Breast MDA-MB-231 cells	1-5 μM	*In vitro*	(41)
	Inhibit the proliferation, induce apoptosis, and disrupt mitochondrial membrane potential, decrease IL-1β-induced activation of cPLA2 and COX-2 via inhibition of MAPK and the NF-κB signaling pathway	Lung A549 cells	1-200 μM	*In vitro*	(42)
	Inhibit proliferation, induce apoptosis, cell cycle arrest and ROS generation, and suppress tumor growth	Lung NCI-H23, NCI-H460 cells	20-180 μM	*In vitro*	(43)
		Nude mice bearing NCI-H23 xenograft tumors	10-60 mg/kg	*In vivo*	
	Inhibit proliferation, induce cell cycle arrest, apoptosis, and ROS generation via upregulation of Bax, cytochrome c and caspase 3, and suppress tumor growth	Gastric SGC7901 cells	20-80 μM	*In vitro*	(44)
		Nude mice bearing SGC-7901 xenograft tumors	10-60 mg/kg	*In vivo*	
	Inhibit proliferation, induce apoptosis via regulating the expression levels of apoptosis-related proteins and suppressing the mitochondrial capacity, and suppress tumor growth	Gastric SGC-7901, MKN-49P cells	15-240 μM	*In vitro*	(45)
		Nude mice bearing SGC-7901 and MKN-49P xenograft tumors	15-60 mg/kg	*In vivo*	

Table I. Continued.

Biological effects	Details	Cell lines/ Model	Dose	Application	(Refs.)
	Induce apoptosis and ER stress by increasing expression of XBP-1s, ATF4, Hsp70, CHOP and p-eIF2α, and suppress the tumor growth	Pancreatic PANC-1, MIA paca-2 cells Nude mice bearing MIA paca-2 xenograft tumors	15-30 μM 25-50 mg/kg	*In vitro* *In vivo*	(46)
	Inhibit the promotion of skin tumor formation by TPA in DMBA-treated mice	ICR mice	0.2 μM/mouse	*In vivo*	(47)
	Have no cytotoxicity but enhance the cytotoxicity of vincristine	Epidermoid carcinoma KBV200 cells	12.5-25 μg/ml	*In vitro*	(48)
	Sensitize cancer cells to radiation therapy *in vitro* and *in vivo* by upregulating Bax through HIF1α	Gastric SGC-7901, MKN-49P cells Nude mice bearing SGC-7901 and MKN-49P xenograft tumors	60 μM 60 mg/kg	*In vitro* *In vivo*	(49)
Anti-invasive effect	Inhibit proliferation, migration, invasion and adhesion ability, and induce cell cycle arrest involving AKT and ERK signaling pathways	Gallbladder GBC-SD cells	10-50 μg/ml	*In vitro*	(50)
	Inhibit proliferation, induce cell cycle arrest and suppress migration and invasion via decreasing β-catenin and COX-2 expression and increasing E-cadherin expression	Ovarian HO-8910 cells	0.5-2 μM	*In vitro*	(51)
	Inhibit proliferation and cell invasion by suppressing NF-κB-dependent MMP-9 expression	Breast MDA-MB-231 and MCF-7 cells	2.5-40 μM	*In vitro*	(52)
	Inhibit migration and invasion via suppressing the phosphorylation of PITPNM3	Breast MDA-MB-231 cells	10-40 μg/ml	*In vitro*	(53)
	Inhibit proliferation with an IC_{50} value of 0.26 μM and suppress invasion by downregulating MMP-7 expression	Pancreatic bxpc-3 cells	0.125-25 μM	*In vitro*	(54)
Anti-inflammatory effect	Restore AH Plus-damaged cell viability and ALP activity, suppress secretion of NO, TNF-a and IL-1β, reduce ROS formation and NF-κB translocation	MC-3T3 E1 cells	15 μM	*In vitro*	(26)
	Inhibit inflammatory effect, induce odontoblast differentiation through increasing HO-1 expression, show cytoprotection and mineralization, suppress NF-κB translocation and induce Nrf2 translocation	Human dental pulp cells	15 μM	*In vitro*	(27)
	Inhibit the activity of phospholipase A2 with an IC_{50} value of 2.897 mM	Phospholipase A2	0.5-4 mM	*In vitro*	(56)
	Inhibit leukotriene B4 release	Human leukocytes	100 μM	*In vitro*	(57)
	Reduce LPS-induced apoptosis, attenuate LPS-induced increased mRNA expression levels of IL1, IL6 and TNFα, and inhibit LPS-induced apoptosis via ERK1/2 and p38 pathways	H9c2 cells	0.125-5 μM	*In vitro*	(58)
	Inhibit TPA-induced mouse ear edema with an ID_{50} value of $4.7x10^{-3}$ μM/ear	Mouse	$1x10^{-2}$-$1x10^{-4}$ mg/ear	*In vivo*	(59)

Table I. Continued.

Biological effects	Details	Cell lines/ Model	Dose	Application	(Refs.)
	Inhibit TPA-induced mouse ear edema with an ID_{50} value of 0.044 mg/ear	Mouse	-	*In vivo*	(60)
	Inhibit serotonin-induced mouse paw edema, TPA-induced mouse ear swelling and PLA2-induced mouse paw edema	Swiss mice	0.5 mg/ear and 50 mg/kg	*In vivo*	(61)
	Reduce intravesical IL-1, IL-6 and LDH levels, downregulate TNF-α and upregulate TP53 proteins	ICR mice	20-40 mg/kg	*In vivo*	(62)
	Improve the survival of septic rats and attenuate CLP-induced acute lung injury, downregulate the serum levels of TNF-a, IL-1β and IL-6, decrease malondialdehyde and myeloperoxidase contents and increase SOD level	Wistar rats	1-10 mg/kg	*In vivo*	(63)
	Alleviate LPS-induced lung injury, relieve LPS-inflammation such as TNF-α, IL-6, MCP-1 and IL-1β, reduce cell numbers in the BALF, inhibit LPS-induced cell apoptosis and suppress NF-κB and MAPK signaling pathways	Rats	10-20 mg/kg	*In vivo*	(64)
	Decrease the kidney index, drop the contents of Cre and BUN, inhibit the renal inflammation via reducing the levels of TNF-α, IL-6 and iNOS and enhance the expression of Nrf2 and HO-1	SD rats	5-5 mg/kg	*In vivo*	(65)
	Ameliorate renal injury markers, improve renal inflammation, restore renal klotho levels and ameliorate renal Wnt/β-catenin signaling, improve renal tissue structure, and ameliorate renal fibrosis in doxorubicin-induced nephropathy	Wistar albino rats	10 mg/kg	*In vivo*	(66)
Antihyperglycemic effect	Induce GLUT4 expression, stimulate GLUT4 redistribution from intracellular vesicles to the plasma membrane, increase the phosphorylation of IRS-1, AKT and AMPK, induce triglyceride accumulation and inhibit lipolysis	3T3-L1 cells	0.01-1 μM	*In vitro*	(67)
	Increase glucose uptake by 50%	3T3-L1 cells	5 μM	*In vitro*	(68)
	Decrease blood glucose levels in streptozocin-treated mice via enhanced insulin sensitivity irrespective of PPAR-γ	Db/db mice	1-10 mg/kg	*In vivo*	(69)
Antibacterial and antiviral effects	Exhibit inhibitory effecton the SARS-CoV-2 3CL hydrolytic enzyme with an IC_{50} value of 18.607 μM	Mpro protease	10-50 μM	*In vitro*	(12)
	Inhibit EBV-EA activation induced by TPA	Raji cells	-	*In vitro*	(17)
	Inhibit the biofilm formation of *E. coli*	*E. coli*	32-256 μg/ml	*In vitro*	(70)
Sedative-hypnotic effect	Suppress locomotion activity, prolong sleeping time, and enhance hypnotic effect in pentobarbital-treated mice via chloride channel activation and GABA-ergic mechanisms	ICR mice	1-5 mg/kg	*In vivo*	(13)

Table I. Continued.

Biological effects	Details	Cell lines/Model	Dose	Application	(Refs.)
	Increase total sleep time and non-rapid eye movement sleep and reduce numbers of sleep/wake cycles	SD rats	5 mg/kg	*In vivo*	(72)
Anti-ischemia/ reperfusion injury	Increase cerebral blood flow, reduce infarct volume and brain water content and decrease neuronal damage and neuronal apoptosis via upregulation of p-PTEN, p-PDK1, p-Akt and p-BAD, and downregulation of cleaved caspase protein expression	SD rats	12.5-100 mg/kg	*In vivo*	(14)
	Exhibit protective effect on ischemia-reperfusion induced acute kidney injury through inhibition of ferroptosis, activation of NRF2, and upregulation of the expression of the downstream ferroptosis related proteins, GPX4, SLC7A11 and HO1	C57BL/6 mice	5-20 mg/kg	*In vivo*	(74)
Other pharmacological effects	Suppress 5-HT-stimulated inward current and inhibit I5-HT in Xenopus oocytes expressing human 5-HT3A receptor with an IC_{50} value of 5.5±0.6 μM	*Xenopus* oocytes	0.1-100 μM	*In vitro*	(75)
	Inhibit I_{ACh} in oocytes expressing nicotinic type α3β4 acetylcholine channel receptors with an IC_{50} value of 24.9 μM, via a concentration dependent and reversible manner	*Xenopus* oocytes	3-300 μM	*In vitro*	(76)
	Decrease allograft rejection, protect PBLs from apoptosis involving stabilization of the mitochondrial transmembrane potential, and reduce the percentage of $CD8^+$ lymphocyte	SD rats	1-10 mg/kg	*In vivo*	(77)
	Induce autophagy via upregulation of the LC3-II, Beclin 1 and Atg7 expression levels, and negative modulation of IGF-1 signaling pathway	WI-38 cells	1-4 μM	*In vitro*	(78)
	Reduce the cytotoxicity of root canal sealers	L929 cells	300 mg/ml	*In vitro*	(79)
	Maintain the physiochemical properties of AH Plus sealer, reduce the flow, film thickness and setting time of AH Plus and improve the sealing ability of the modified sealer with time	Ah plus	0.5%	*In vitro*	(80)
	Inhibit KLK5 protease activity with an IC_{50} value of 5.9 μM	Human kallikrein 5	1-100 μM	*In vitro*	(81)
	Decrease free fatty acid-induced increase in intracellular triglyceride levels and induce the phosphorylation of AMPK	Hepatoma hepg2 cells	0.63-1.25 μM	*In vitro*	(82)
	Improve the abnormal metabolism, increase the potential of GV oocytes, reduce the number of abnormal MII oocytes and damaged embryos, downregulate the expression ofovarian-related genes in ovarian tissue and pro-inflammatory cytokines in adipose tissue	ICR mice	50 mg/kg	*In vivo*	(83)

Table I. Continued.

Biological effects	Details	Cell lines/Model	Dose	Application	(Refs.)
	Reverse right ventricular hypertrophy and pulmonary vascular remodeling, suppress proliferation and induce apoptosis in hypoxia-induced pulmonary artery smooth muscle cells, downregulate the peroxy-related factor expression	SD rats	5 mg/kg	*In vivo*	(84)
	Demonstrates improvements in weight and kidney damage, and lower fasting blood glucose, Scr, BUN, U-Pro, p-AKT, p-PI3K levels and higher SOD activity	C57BL/6J mice	50 mg/kg	*In vivo*	(85)
	PA alone or as an adjuvant therapy with losartan lower serum BNP and improve systolic function and cardiac fiber diameter via suppressing miR-24 and preserving cardiac junctophilin-2	Albino rats	10 mg/kg	*In vivo*	(86)
	Ameliorates doxorubicin-induced renal injury via regulation of serum cystatin-C, and urine albumin/creatinine ratio, renal content of podocin and klotho, TNF-α, IL-6 and IL-1β	Wistar albino rats	10 mg/kg	*In vivo*	(66)

RXR, retinoid X receptor; ROS, reactive oxygen species; p-, phosphorylated; DR5, death receptor 5; PARP, poly (ADP-ribose) polymerases; ATM, ataxia telangiectasia mutated protein; ATR, ataxia telangiectasia-mutated and Rad3-related kinase; Chk, checkpoint kinase 1; PTEN, phosphatase and tensin homolog; PKM2, pyruvate kinase muscle isozyme; HK2, hexokinase 2; cPLA2, cytosolic phospholipase A2; COX-2, cyclooxygenase 2; ER, endoplasmic reticulum; XBP-1s, X-box-binding protein-1s; ATF4, activating transcription factor 4; Hsp70, heat shock protein 70; CHOP, C/EBP homologous protein; eIF2α, eukaryotic initiation factor-2α; TPA, 12-O-tetradecanoylphorbol-13-acetate; DMBA, 7,12-dimethylbenz[a]anthracene; HIF1α, hypoxia-inducible factor 1α; MMP, matrix metalloproteinase; PITPNM3, membrane-associated phosphatidylinositol transfer protein 3; ALP, alkaline phosphatase; NO, nitric oxide; Nrf2, nuclear factor erythroid 2-related factor 2; LPS, lipopolysaccharise; LDH, lactate dehydrogenase; CLP, cecal ligation and puncture; SOD, superoxide dismutase; BALF, bronchoalveolar lavage fluid; Cre, creatine; BUN, blood urea nitrogen; iNOS, inducible nitric oxide synthase; HO-1, heme oxygenase 1; GLUT4, glucose transporter type 4; IRS-1, insulin receptor substrate-1; PPAR-γ, peroxisome proliferator-activated receptor-γ; PDK1, pyruvate dehydrogenase kinase 1; BAD, Bcl-2-associated death promoter; GPX4, glutathione peroxidase 4; SLC7A11, solute carrier family 7 member 11; PBL, peripheral blood lymphocyte; BNP, B-type natriuretic peptide; miR, microRNA.

Antiviral and antibacterial effects. As presented in Table I, PA displays inhibitory effects on the SARS-CoV-2 3CL hydrolytic enzyme *in vitro*, with an IC_{50} value of 18.607 μM (12). Akihisa *et al* (17) demonstrated that PA exhibits antiviral activity against Epstein-Barr virus early antigen-induced TPA in Raji cells, with an IC_{50} value of 286 mol ratio/32 pmol TPA. In addition, PA at concentrations ranging from 32 to 256 μg/ml demonstrate antibacterial activity against *Escherichia coli* by inhibiting biofilm formation (70).

Sedative-hypnotic effect. *Poria cocos* is used in traditional Chinese medicine to treat various sleep disorders, such as insomnia (71). As presented in Table I, PA at a dose of 5 mg/kg suppresses locomotor activity, prolongs sleep time and enhances hypnotic effects in pentobarbital-treated mice via chloride channel activation and GABA-ergic mechanisms (13). Their subsequent study further indicated that PA increases total sleep time and non-rapid eye movement sleep, and reduces the number of sleep/wake cycles and wakefulness (72). To the best of our knowledge, no lanostane-type triterpenoids have been reported to show sedative-hypnotic effects, except for PA.

Anti-ischemia/reperfusion injury. Ischemia/reperfusion injury is a serious clinical problem that causes several diseases, including myocardial hibernation and cerebral dysfunction (73). Pang *et al* (14) reported that PA exhibits neuroprotective effects on brain ischemia/reperfusion injury *in vivo* by increasing cerebral blood flow, reducing infarct volume and brain water content and decreasing neuronal damage and apoptosis. Jiang *et al* (74) revealed that PA exhibits a dose-dependent protective effect on ischemia-reperfusion-induced acute kidney injury *in vivo* through the inhibition of ferroptosis in the kidneys, activation of Nrf2 and upregulation of the expression of the downstream ferroptosis-related proteins, glutathione peroxidase 4, solute carrier family 7 member 11 and HO1. These studies indicate that PA has the potential to treat ischemia/reperfusion injury (Table I).

Table II. Pharmacokinetic information of PA.

Model	Dose	Administration method	Quantitative method	Details	(Refs.)
SD rats	200 mg/kg (*Poria cocos* extract)	Oral gavage	LC-ESI-MSn method	PA is the only one detected both in rat urine and plasma	(87)
Caco-2 cells	10-50 μM	Mixed system	RP-HPLC method	PA is transported through the Caco-2 cell monolayer in a concentration-dependent manner and the Papp values of PA are $(9.50\pm2.20)10^{-7}$ cm/sec from AP side to BL side, and $(11.30\pm5.90)10^{-7}$ cm/sec from BL side to AP side, respectively	(88)
SD rats	30 mg/kg	Intravenous administration	HPLC method	$t_{1/2}$ =8.79±6.80 h; CL=0.53±0.28 l/h; $AUC_{0-\infty}$=18.90±9.39 μg h/ml; $MRT_{0-\infty}$=12.58±9.95 h	(89)
Wistar rats	10 mg/kg	Oral administration	LC-MS/MS method	$t_{1/2\beta}$=4.96±1.33 h; $AUC_{0-\infty}$=1466.9±361.7 ng h/ml; CL=6.82±1.73 l/h	(90)
SD rats	12.3 mg/kg	Oral administration	UPLC-Q-Orbi-trap MS method	$t_{1/2z}$=11.51±9.90 h; $AUC_{0-\infty}$=336.29±161.99 ng h/ml; CL=45.07±73.64 l/h	(91)
Human liver microsomes	100 μM	Mixed system	HPLC method	Inhibit CYP3A4, 2E1, and 2C9 in a concentration-dependent manner, with IC_{50} values of 15.04, 27.95 and 24.22 μM, respectively	(92)
SD rats	5 mg/kg	Oral administration	LC-MS/MS method	Increase AUC and $t_{1/2}$, decrease CL, enhance metabolic stability, and inhibit transport of bavachin via the inhibition of CYP2C9 and P-gp	(93)

PA, pachymic acid; AP, apical; BL, basolateral; CL, clearance; $t_{1/2}$, half-life; CYP3A4, cytochrome P450 3A4; CYP2E1, cytochrome P450 2E1; CYP2C9, cytochrome P450 2C9; AUC, area under the curve; P-gp, P-glycoprotein.

Other pharmacological effects. As presented in Table I, PA has been revealed to have several other pharmacological effects. Specifically, it suppresses 5-hydroxytryptamine (5-HT)-stimulated inward currents, and inhibits 5-HT-stimulated inward current in *Xenopus* oocytes expressing the human 5-HT3A receptor. It also inhibits the acetylcholine-induced inward current in oocytes expressing nicotinic type α3β4 acetylcholine channel receptors (75,76). PA decreases allograft rejection in rats, protects peripheral blood lymphocytes from apoptosis by stabilizing the mitochondrial transmembrane potential and reduces the percentage of $CD8^+$ lymphocytes (77). Lee and Kim (78) reported that PA could induce WI-38 cell autophagy to delay the aging process. Furthermore, PA can reduce the cytotoxicity of root canal sealers (79), maintain the physiochemical properties of AH Plus sealer, reduce the flow, film thickness and setting time of AH Plus, and improve the sealing ability of the modified sealer over time (80). PA has been revealed to affect the skin barrier function via inhibition of KLK5 protease activity (81) and to ameliorate hepatic steatosis by decreasing the free fatty acid-induced increase in intracellular triglyceride levels (82). Fu *et al* (83) reported that PA can protect oocytes by improving abnormal metabolism, increasing the potential of GV oocytes and reducing the number of abnormal metaphase II oocytes and damaged embryos in mice with polycystic ovary syndrome. Recently, He *et al* (84) demonstrated that PA significantly reverses right ventricular hypertrophy and pulmonary vascular remodeling, suppresses proliferation, induces apoptosis in hypoxia-induced pulmonary artery smooth muscle cells, downregulates the expression of peroxy-related factors and upregulates the expression levels of antioxidant-related factors. PA can protect against kidney injury in mice with diabetic nephropathy by inhibiting the PI3K/AKT pathway (85). PA alone or as adjuvant therapy with losartan can lower serum B-type natriuretic peptide and improve systolic function and cardiac fiber diameter to attenuate doxorubicin-induced heart failure *in vivo* (86). Moreover, PA ameliorates doxorubicin-induced renal injury by regulating of serum cystatin-C, urine albumin/creatinine ratio, renal podocin and klotho content, TNF-α, IL-6 and IL-1β (66).

3. Pharmacokinetics

Several analytical methods have been reported for the determination of PA *in vitro* and *in vivo*. The pharmacokinetic properties of PA, including its absorption, distribution, metabolism and excretion are summarized in Table II. Using the LC-ESI-MSn method, Ling *et al* (87) reported that seven out of 34 compounds in the extract of *Poria cocos* can be detected in rat urine after oral administration, whereas only PA can

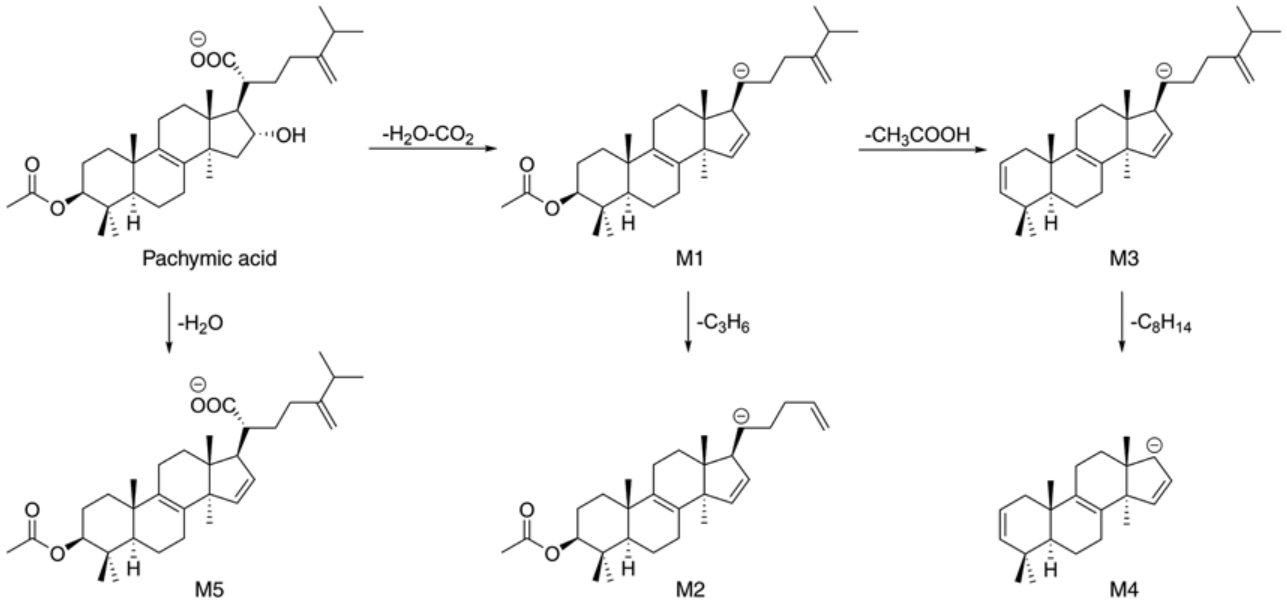

Figure 3. Proposed metabolic pathways of pachymic acid. M1, fragmentation molecule 1; M2, fragmentation molecule 2; M3, fragmentation molecule 3; M4, fragmentation molecule 4; M5, fragmentation molecule 5.

be detected in rat urine and plasma. *In vitro* determination of PA, using Caco-2 cell monolayers as an intestinal epithelial cell model with reversed-phase-high performance liquid chromatography, revealed that PA is transported through the Caco-2 cell monolayer in a concentration-dependent manner, and that the Papp values of PA are $(9.50\pm2.20)10^{-7}$ cm/sec from the apical (AP) side to the basolateral (BL) side, and $(11.30\pm5.90)10^{-7}$ cm/sec from the BL side to the AP side, respectively.

In addition to the passive diffusion of PA, ATP partially participates in its transport (88). After sublingual vein injection of PA at a dose of 30 mg/kg, the pharmacokinetic parameters in rat plasma were obtained using HPLC with half-life ($t_{1/2}$) at 8.79±6.80 h, clearance (CL) at 0.53±0.28 l/h, area under the curve $(AUC)_{0-\infty}$ at 18.90±9.39 μg h/ml and mean residence $time_{0-\infty}$ at 12.58±9.95 h (89). Following oral administration of PA (10 mg/kg), the main pharmacokinetic parameters in rat plasma using liquid chromatography tandem mass spectrometry (MS) were elimination half-life at 4.96±1.33 h, $AUC_{0-\infty}$ at 1466.9±361.7 ng h/ml and CL at 6.82±1.73 l/h (90). Determination of PA (12.3 mg/kg) in rats after oral administration using UPLC-Q-Orbi-trap MS demonstrated that the pharmacokinetic parameters were terminal half-life at 11.51±9.90 h, $AUC_{0-\infty}$ at 336.29±161.99 ng h/ml and CL at 45.07±73.64 L/h. PA is mainly distributed in the intestine and stomach and is considered to be further converted into other molecules *in vivo* (91). In addition, treatment of human liver microsomes with PA (100 μM) demonstrated that PA inhibits cytochrome P450 (CYP)3A4, 2E1 and 2C9, with IC_{50} values of 15.04, 27.95 and 24.22 μM, respectively, indicating potential drug-drug interactions (92). Moreover, after oral administration of PA (5 mg/kg) in rats, Zhang *et al* (93) indicated that PA increases the AUC and $t_{1/2}$, decreases CL, enhances metabolic stability and further inhibits transport of natural flavonoid bavachin via regulation of CYP2C9 and P-glycoprotein.

As presented in Fig. 3, three metabolic pathways are proposed for PA (87). It may undergo loss of $H_2O + CO_2$ to generate fragment M1, which is converted to fragment M2 by the loss of C_3H_6, and forms fragment M3 via the loss of CH_3COOH (87). A very low-abundance ion had been detected in the fragment M4 spectra via the loss of C_8H_{14} from M3 (87). Finally, PA is converted to fragment M5 via dehydration (87).

4. Conclusions

PA can be obtained from a number of natural sources, and has attracted great attention owing to its various pharmacological activities in the potential treatment of several diseases. In addition, progress has been made in exploring the pharmacological profiles and underlying molecular mechanisms of PA. In particular, PA has been demonstrated to be a novel RXR-specific agonist and can induce the differentiation of leukaemia HL-60 cells (34). In addition, PA has been demonstrated to be an activator of PKM2 and an inhibitor of HK2, and can inhibit the proliferation of breast cancer SK-BR-3 cells (40). It demonstrates significant anti-inflammatory effects by inhibiting the activity of PLA2 (56). To the best of our knowledge, no lanostane-type triterpenoids have been reported to show sedative-hypnotic effects, except PA (72).

Although considerable progress has been made in previous years regarding PA, tremendous challenges still lie ahead owing to the shortcomings of its low content in nature, complex structure, physical and chemical properties and pharmacokinetic profiles. Its low content in nature hinders further investigation and clinical applications. For example, the percentage of PA in *Poria cocos* crude extract was 0.053‰ (69). The bioavailability of PA is relatively low due to its poor water solubility after oral administration. Regarding intravenous administration, the dissolution of PA is complex (89). Mixed solubilizers, including DMSO, PEG-400 and 1, 2-propylene glycol are used to solubilize PA

in physiological saline, which may increase the risk of side effects and adverse reactions (89).

A limited amount of research on the pharmacological activity, underlying molecular mechanisms and other new biological effects of PA warrants further investigation (8). Investigators use different methods including experimental animals (*in vivo*), tissue and cell cultures (*in vitro*) in order to investigate novel therapies of human diseases (94). The mentioned procedures have their own advantages and disadvantages. *In vitro* models are used in biomedical fields for the advantages of low cost, efficiency and ease of quantification (94). The disadvantage of *in vitro* methods is they are usually conducted on cell lines. Although animal models (*in vivo*) provide some drawbacks such as high cost, inefficiency and a difference in biokinetics parameters in comparison with humans, they are more credible compared with *in vitro* tests (95). The majority of studies are focused on *in vitro* experiments (Table I); hence, it is necessary to validate the *in vivo* effectiveness and efficacy of PA, such as its anticancer and anti-inflammatory effects in different animal models. In addition to the above challenges, another critical concern is the safety assessment of PA (46). The main natural source of PA is *Poria cocos*, which is homologous to medicine and food (22). Evidence suggests that PA may be toxic *in vivo* (46). However, at present, the toxicity and relationship between dose and toxicity remains unaddressed.

To address these challenges, five strategies and suggestions aimed at enhancing the development and clinical application of PA are proposed in the present review. First, it is necessary to improve the efficiency of PA separation or efficiently prepare PA via chemistry and biocatalytic technologies. Second, the adoption of appropriate pharmaceutical or chemical methods would improve bioavailability. For example, some suitable formulation technologies (solid dispersion and micronization), chemical modifications with water-solubilizing groups, and PA derivatives designed as prodrugs or prepared in the form of sodium salts could be adopted to improve solubility and bioavailability. Third, regarding the stage of current research on the pharmacology and molecular mechanism of PA, further investigation of the *in vivo* effectiveness and efficiency of PA should be performed. Fourth, special pharmacological profiles of PA, such as its sedative-hypnotic effect, should be given more attention, as no other lanostane-type triterpenoids have been reported to exhibit a sedative-hypnotic effect (13,72). Fifth, novel PA derivatives library for exploring more promising candidates with higher pharmacological activities and improved drug-like properties should be performed. PA has a 33-carbon skeleton with five available sites at the C-3, C-8, C-16, C-21, and C-24 positions for modification (Fig. 1), which might promote the synthesis of novel molecules with higher potency and selectivity, fewer side effects and gradual expansion of the scope of patent protection. At present, to the best of our knowledge, only one study has reported the synthesis of novel PA derivatives with *in vitro* pharmacological evaluation (96). Finally, more attention should be paid to investigating the toxicity and underlying mechanism of PA in animals, and drug-drug interactions, to elucidate the relationship between dosage and toxic effects and decrease or avoid side effects. Although the awareness of PA has grown in recent years, it is necessary to further investigate its safety, efficacy, mechanism and pharmacokinetics.

Overall, the present review focused on comprehensive research on the biological properties and therapeutic potential of PA, and will be beneficial for the development and utilization of PA in the future.

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Availability of data and materials

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Authors' contributions

CW, HW, XS and JW carried out the literature search, summarized data and wrote the paper. GB, YX and LZ reviewed and edited the manuscript and analyzed the manuscript contents. QY contributed to manuscript reviewing and design of tables and figures. LZ and ZB revised the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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