Progress on the pharmacological research of puerarin: a review

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[ABSTRACT] Contemporary pharmacological research has demonstrated that puerarin, the most important phytoestrogen extracted from Pueraria lobata (Willd.) Ohwi, has protecting functions on the cardiovascular system, nervous system, osteoporosis, liver injury, and inflammation in vivo and in vitro. Most of these research studies focused on inhibiting oxidative stress and apoptosis through regulating various bioactivators and signal pathways. Among these, superoxide dismutase (SOD), endothelial nitric oxide synthase (eNOS) and malondialdehyde (MDA), and PI3K/Akt, MAPK, and NF-κB are of great importance. The data cited in this review were mainly obtained from articles listed in PubMed and Elsevier SDOL published from 1959 to 2013, and the search term used was “puerarin”.

[KEY WORDS] Puerarin; Cardiovascular protection; Neuroprotection; Osteoporosis; Liver injury; Inflammation


Introduction

Since the most abundant phytoestrogen, puerarin (Pur), was isolated from kudzu root in the late 1950s [1], researchers have concentrated on the pharmacological activities of this “novel” component possessing a series of beneficial activities on hangover [2], cardiovascular diseases [3], osteoporosis [4], neurological dysfunction [5], fever [6], and liver injury [7] in clinical treatment and experimental research. Pur injection and other preparations have been extensively used in the clinic in China [8-9], but due to the limited availability of well-designed clinical trials, its beneficial outcomes on human health are still unclear [10]. Many of the experimental cytoprotective effects of Pur cannot be translated into curative effects. How to correctly assess the cytoprotection of Pur and relate that to clinical treatment remains a major challenge for research on this attractive component. This review summarizes the pharmacological activities and mechanisms of Pur, and aims to provide a consolidated platform for the further study of this active component. These main mechanisms are summarized in Fig. 1.

Cardiovascular protection

Protection of cardiomyocytes

The cardioprotection of Pur was partly attributed to effects on Ca2+, Na+ and K+ current of cardiomyocytes. Pur (both 0.1 mmol·L–1 and 1 mmol·L–1) demonstrated its blocking effect on L-type calcium channels in ventricular myocytes isolated from Langendorff guinea pig hearts by collagenase, and inhibited the amplitude of peak calcium channel current by 63.2% and decreased the level of calcium channel current form -40 mV to +10 mV [11]. In isolated ventricular myocytes, Pur prevented hydrogen peroxide-induced cell death, which was inhibited by paxilline, indicating that these protective effects were associated with opening the calcium-activated potassium channel [13], and in isolated rat hearts subjected to 30 min regional ischemia and 120 min reperfusion, Pur inhibited mitochondrial permeability

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transition pore opening and activated the mitochondrial ATP-sensitive potassium channel [14]. However, in aconitine-induced arrhythmias in rats, Pur was found to be an open-channel blocker of inward rectifier potassium channel, and inhibition of the potassium channel was shown to prolong the action potential duration and refractory period in cardiomyocytes to prevent arrhythmia development [15].

Other data indicated that the significant cardioprotective effects of Pur were associated with its antioxidant and antilipid peroxidation properties. In rats with myocardial ischemia, Pur induced the gene or protein expression of eNOS [16], which might be through activation of an estrogen receptor-mediated PI3K/Akt and CaMKII/AMPK-dependent signal pathway [17], in isoprenaline-induced myocardial fibrotic mice, Pur reduced transforming growth factor (TGF-β1, β2, β3) expression through activation of the peroxisome proliferator-activated receptor α/γ and subsequent inhibition of factor-kappaB (NF-κB) in myocardial tissue [18], and in myocardium of spontaneously hypertensive rats, Pur reduced mRNA expression of TGF-β1 and Smad3, but increased that of Smad7, which might be the mechanism of myocardial protection from hypertension [19].

The combined use of Pur and Danshensu increased serum activity of SOD and reduced ischemic size, serum levels of creatine kinase-MB, lactate dehydrogenase and MDA in a dose-dependent manner in rats with hyperhomocysteinemia induced by methionine [20], and in lipopolysaccharide (LPS)-induced peripheral blood mononuclear cells of patients with unstable angina pectoris, Pur had similar functions [21]. In heart failure rats with coronary artery ligation for 4 weeks, Pur improved myocardial microcirculation and cardiac performance through down-regulating the endothelin system, and normalizing the expression of SERCA2a and phospholamban [22]. Pur also induced angiogenesis in the myocardium of rats with myocardial infarction through activating vascular endothelial growth factor (VEGF) and hypoxia-inducible factor (HIF)-1α [23].

**Effect on vessels**

Pur induced an endothelium-independent relaxation in rat aortic rings [24-26]. Pur (100 mmol·L⁻¹) completely relaxed contractions of endothelium-intact rat aorta induced by phenylephrine (PE) through activating potassium channels, which was significantly inhibited by the potassium channel antagonists tetraethylammonium and 4-aminopyridine [24], as well as glibenclamide and Ba²⁺ [25], and in Ca²⁺-free solution, the anti-vasoconstriction of Pur on PE was totally deprived [25]. Pur also activated BKCa channels, especially BKα1.1 channels in a concentration-dependent manner, which was significantly inhibited by iberiotoxin, a specific blocker of BKα channels [26].

In other studies, Pur displayed its antagonistic and cytoprotection through regulating signal pathways. Pur (10–160 μmol·L⁻¹) concentration-dependently induced the nitric oxide (NO) production in rat aortic cells, and 8-bromo-cGMP significantly strengthened this action, suggesting that the NO/NO-cGMP signal pathway appeared to contribute to the anti-vasoconstriction of Pur [25]. In rings from porcine coronary artery, Pur enhanced sodium nitroprusside-induced relaxation through the cAMP-dependent pathway [27]. In addition, Pur (0.1–3 mmol·L⁻¹) augmented the proliferative, migratory, adhesive, and vasculogenesis capacity of endothelial progenitor cells through increasing activity of telomerase and the phosphorylation of Akt, a downstream effector of PI3K, and was attenuated by pretreatment with PI3K blockers, either wortmannin or LY294002 [26-29]. In high glucose-induced acute dysfunction of glomerular mesangial cells [30] and vascular [31], Pur increased heme oxygenase (HO)-1 protein expression and HO activity of thoracic aorta, which was inhibited by ZnPP (an inhibitor of heme oxygenase-1) [31].
**Protection of hyperlipidemia**

Pur had hypocholesterolemic effects *in vivo*. Four weeks treatment with Pur (300 mg·kg⁻¹·d⁻¹, p.o.) suppressed the impairment of eNOS over-expression and reduced the atherogenic properties in diet-induced hypercholesterolemic rats [32]. In lead-induced liver injury and hyperlipidemia rats, Pur reduced ROS production, renewed the activities of the hepatic glutathione antioxidant system, and influenced expression of hepatic lipid biosynthesis and metabolism genes, such as cholesterol 7α-hydroxylase, 3-hydroxy-3- methylglutaryl-CoA reductase, and low density lipoprotein (LDL) receptor [33], and in the livers of C57 BL/6J mice, Pur increased LDL uptake, reduced cholesterol biosynthesis, and possibly enhanced cholesterol degradation [34].

**Protection of diabetes**

The protection of Pur on diabetes was attributed to its functions on inhibiting apoptosis and oxidative stress. In hydrogen peroxide (H₂O₂)-induced pancreatic islets separated from rats, Pur (100 μmol·L⁻¹) significantly inhibited the production of free radicals and the apoptosis of islets, restored glucose-stimulated insulin secretion and increased catalase and SOD activities [35]. In streptozotocin (STZ)-induced diabetic rats, Pur enhanced the uptake of radioactive glucose in a concentration-dependent manner, increased the mRNA and protein levels of glucose transporter-4 (GLUT4) in soleus muscle [36], up-regulated apoptosis-related genes, including Bcl-2 and SOD, and down-regulated the NF-κB and TNFRα/FADD/caspase signal pathways [37]. Pur regulated the gene expressions of retinal VEGF and HIF1 stimulated by STZ [38], inhibited the expression of inducible nitric oxide synthase (iNOS) mRNA, and decreased nitrotyrosine, peroxynitrite, and Fas/FasL protein expressions on retina pigment epithelial cells in diabetic rats [39-42]. In addition, Pur exerted an inhibitory effect on NADPH oxidase-related ROS pathways by inhibiting the phosphorylation of p47phox and Rac1 and blocking NF-κB activation, thereby ameliorating retinal microvascular dysfunction [43].

The molecular mechanism of Pur on improving insulin resistance was associated with improving body weight gain, glucose/insulin intolerance and adipokine expression [43], inactivating the Cb₁ binding protein path, promoting GLUT4 transposition to cell membrane to increase the transportation of glucose [44], and inhibiting inflammation and attenuating endothelial stem cell resistance in an IKKKβ/IRS-1-dependent manner in *in vivo* and *in vitro* experiments [45].

**Neuroprotection**

**Protection in Alzheimer’s disease models**

Alzheimer’s disease (AD) is a progressive, neurodegenerative disorder mostly caused by apoptosis of hippocampus neurons, and characterized by an age-dependent loss of memory. Pur had protective effects on learning-memory behavior in aging mice treated with D-galactose, when Pur (60 mg·kg⁻¹·d⁻¹, p.o.) was used for six weeks, the spontaneous behavior, learning-memory behavior, and activity of SOD increased significantly compared with the D-galactose group [46]. Further studies demonstrated that long-term treatment of Pur ameliorated learning and memory deficits of ovariectomy (OVX) mice through normalizing the glutamatergic/GABAergic system and causing synaptic structural modifications in the hippocampus [47], and decreasing the width of the synaptic cleft, as well as enlarging the thickness of postsynaptic density in the hippocampus CA1 area [48]. In global cerebral ischemia-reperfusion rats, Pur had a protective effect on learning-memory disorders based on up-regulating the expression of Bcl-2, which resulted in a decrease of apoptotic cells [49].

Amyloid beta (Aβ) peptides play a major role in the pathogenesis of AD, intrahippocampal injection of Aβ in rats induced a spatial memory deficit, apoptosis, and caspase-9 activation in hippocampal neurons, while four weeks treatment with Pur (100 mg·kg⁻¹·d⁻¹, p.o.) ameliorated this cognitive impairment and reversed the increase of apoptosis in the hippocampus, which was due to the activation of Akt and phosphorylation of Bad [50]. In *in vitro* experiments, Pur attenuated Aβ-induced oxidative stress in hippocampus neuronal cultures from rats characterized by scavenging ROS and inhibiting lipid peroxidation based on the GSK-3β/Nrf2 signaling pathway [51], and activated PKB/Akt, an important upstream kinase of glycogen synthase kinase (GSK)-3β, so as to promote GSK-3β inhibition [5]. The protection by Pur to Aβ-induced PC12 cells apoptosis was related to the increasing expression of p-Akt, Bcl-2, and p-Bad, as well as the decreasing expression of Bax, which were abolished by wortmannin (an inhibitor of PI3K phosphorylation) [52]. Similar functions were found in H₂O₂-induced PC12 cells, and these protections were associated with regulating PI3K/Akt signalling pathway [53], and in 6-hydroxydopamine (6-OHDA)- induced nerve growth factor (NGF)-differentiated PC12 cells, Pur inhibited caspase-8 and partially inhibited caspase-3 activation [54].

Mitochondrial oxidative stress and calcium overload had been strongly associated with the pathogenesis of AD. In oxygen/glucose deprivation model of primary hippocampal cultures, Pur (100 μmol·L⁻¹) significantly inhibited the glutamatergic transmitter, intracellular Ca²⁺ elevation and neuronal NO synthesis [55], but other studies found that application of Pur (30 μmol·L⁻¹) had no effect on the basal intracellular calcium concentration ([Ca²⁺]), in primary rat hippocampal neurons, but potentiated the Ca²⁺-induced Ca²⁺ release via cAMP/PKA signaling pathway, and could be completely inhibited by H89 (a membrane-permeant inhibitor of protein kinase A) [56]. In addition, Pur also blocked transient outward K⁺ current and delayed rectifier K⁺ current in mice hippocampal CA1 neurons [57]. In mitochondrial transgenic neuronal cell cybrid models of sporadic AD, Pur protected neurons against oxidative stress-in-
stress-induced apoptosis through down-regulating the Bax/ Bcl-2 ratio subsequently blocking the activation of JNK, p38, and caspase-3 [58].

Protection in Parkinson’s disease models

Parkinson’s disease (PD), characterized by reduction and degradation of dopaminergic neurons in the substantia nigra, and apoptosis, is a widely accepted pathogenesis of PD. In nigral neurons of OVX rats, after using Pur, the cells positive for tyrosine hydroxylase increased and the numbers of apoptosis cells decreased, indicating that this protective effect might be associated with apoptosis [59]. In 6-OHDA-induced substantia nigra, Pur decreased the level of Bax, restored the contents of dopamine and its metabolites, and increased the expression level of glial cell line-derived neurotrophic factor, which suggested that Pur developed its neuroprotective effects through the inhibition of apoptotic signaling pathways and the up-regulation of glial cell line-derived neurotrophic factor expression [60].

In an in vitro experiment, the neuroprotective effects of Pur against 1-methyl-4-phenylpyridinium iodide (MPP+) -induced toxicity of PC12 cells were attributed to a decrease in the phosphorylation of MKK7, JNK, and c-Jun, and in the levels of cytochrome c, which indicated the inhibition of the JNK signaling pathways [61], and reduction of caspase-3-like activity, which indicated a mitochondria-dependent caspase cascade pathway [62]. In MPP+-induced human neuroblastoma SH-SY5Y cells, Pur activated the PI3K/Akt pathway through inhibiting nuclear p53 accumulation and subsequently caspase-3-dependent programmed cell death [63], reduced the accumulation of ubiquitin-conjugated proteins, attenuated caspase-3 activity and decreased the ratio of Bax/Bcl-2, so as to regulate the function of ubiquitin proteasome system [64].

Protection of ischemia-reperfusion and other injuries

Pur (100 mg·kg⁻¹) significantly decreased infarct size in rats subjected to transient middle cerebral artery occlusion (MCAO), and also reduced apoptosis and necrosis induced by glutamate (Glu) in cultured hippocampal neurons [65]. Moreover, the protective effects of Pur on MCAO rats were mediated by inhibiting the activities of HIF-1α, TNF-α, caspase-3, and neutrophil [66-67], decreasing expression of IL-1β-mRNA, MDA, and NO [67-68], increasing activities of SOD [69], improving expression of synaptophysin [69], HSP70 [70], and erythropoietin [70]. 3-Methoxy Pur increased the level of prostaglandin I₂ (PGI₂) in cerebral tissue and the activity of plasma tissue plasminogen activator, and also inhibited the activity of plasma plasminogen activator inhibitor and the expression of endothelin-1 mRNA in cerebral tissue [71]. Pur had protective functions on transient spinal cord ischemia-reperfusion injury in rabbits and rats. Pur significantly ameliorated the neural function and the histopathological damage in rabbit models [72], and reduced ischemia-reperfusion injury through increasing the transcription of thioredoxin and reducing apoptosis in rat models [73]. Pur had protective effects on neurons damaged by glutamic acid, asparaginic acid, and kainic acid [74], and prevented hippocampal cells from acidosis-induced death through decreasing the amplitude and accelerating the desensitization of acid-sensing ion channels [75]. Pur alleviated neuropathic pain mediated by P2X₃ receptors in dorsal root ganglion neurons [76], protected against Glu-induced neurofilament axonal transport impairment in rat primary hippocampal neurons by inhibiting the increased [Ca²⁺], and decreasing the activation of cyclin-dependent kinase 5 [77], reduced microglia activation induced by LPS through inhibiting expression of iNOS via regulating O-GlcNAcylation [78], and promoted nerve growth in cultured Schwann cells, when treated with Pur (1 μmol·L⁻¹) for eight weeks, a significantly higher density of myelinated axons, greater evoked action potential area, and a larger nerve conductive velocity were found compared to the controls [79].

Anti-osteoporosis

In in vitro experiments, Pur had a stimulatory effect on increasing new bone formation in both New Zealand White rabbits [4] and rats [80]. Pur increased trabecular bone volume and trabecular thickness, decreased trabecular separation [81], and decreased bone absorption through a non estrogen receptor mediated-pathway in OVX mice [82]. Pur prevented osteonecrosis through protecting narrow necrosis and trabecule thinner, decreasing empty osteocyte lacunae in the subchondral region and increasing activity of alkaline phosphatase (ALP) and the content of osteocalcin in femoral osteonecrosis rats induced by alcohol [83]. In streptozocin (STZ)-induced diabetic rats, Pur protected diabetic osteoporosis through the reduction of caspase-3 expression [84]. In cultured osteoblasts treated with Pur (40 or 80 μmol·L⁻¹), there was a higher rate of bone formation and mineral modules than in the control group [80], and these functions maybe associated with co-activation of NO and BMP-2/MAPK pathways [85] and activation of the PI3K/Akt pathway [86]. In osteoblast-like UMR106 cells, Pur increased the mRNA expression of ALP and osteoprotegerin [87]. In primary osteoblasts cultures, the stimulating functions of Pur on osteoblast differentiation and bone formation were attributed to the MAPK and Wnt/β-catenin pathways, and could be inhibited by ICI 182780, an estrogen receptor (ER) antagonist [88]. However, in an ER-antagonist human breast cancer cell line, Pur was shown not to bind to ERα or β, and exerted its anti-osteoproteic action independently of the ER-mediated pathway [82].

Protection of liver injuries

Pur had protective effects on liver injury induced by alcohol through reducing alcohol intake [2] and cytoprotection [7]. In acute alcoholic liver injured Wistar rats, treatment with Pur (200 mg·kg⁻¹·d⁻¹, i.g.) for five days decreased the level of MDA in plasma and liver, and increased the level of SOD and
glutathione peroxidase, so as to inhibit oxidative stress [7]. In alcohol and CCl₄-induced liver fibrosis Wistar rats, after treated with Pur (400 mg·kg⁻¹·d⁻¹, i.g.) for four weeks, serum alanine aminotransferase and aspartate aminotransferase, apoptosis of activated hepatic stellate cells and the expression of Bcl-2 mRNA were reduced, whereas no significant effect was observed in the levels of ALP and γ-glutamyltransferase activities [89].

In high fat diet-induced male rats, Pur exerted a therapeutic effect on non-alcoholic fatty liver disease by improving leptin signal transduction through the JAK2/STAT3 pathways [90]. In addition, Pur reduced lipid accumulation by activating autophagy through the AMPK/mTOR-mediated pathways in ethanol-treated hepatocytes [91]. Pur significantly decreased the activity of glutamic-pyruvic transaminase and glutamic-oxalacetic transaminase in CCl₄ and 4AC [99] and inhibited the production of interleukin and total protein, attenuated TNF-α CYP2D6 [61, 85, 88-99], and protein levels, lowered serum levels of albumin and total protein, attenuated TNF-α/α-NF-κB pathway and improved metabolic function [93], reduced ROS production and DNA oxidative damage [98] in liver tissue.

**Anti-inflammation**

Pur exerted an antipyretic effect on LPS-induced fever in rats through inhibition of pyrogen production from macrophages [6]. In LPS-induced RAW264.7 macrophage cells, Pur inhibited the expression of iNOS, COX-2, and C-reactive protein, due to a dose-dependent inhibition of phosphorylation and a reduction of p65NF-κB nuclear translocation [97]. Some of other active components isolated from *Pueraria lobata* (Willd.) Ohwi [98], as well as some other compounds [99], such as lupenone, lupeol, and Pur derivative (4AC), had similar anti-inflammatory functions. Furthermore, Pur [6, 21] and 4AC [99] inhibited the production of interleukin (IL)-1β, IL-6, TNF-α, PGE₂, and NO, and regulated the transcriptional level through suppression of the NF-κB and MAPK signal pathways. Pur also exhibited anti-inflammatory activity by suppressing IL-8 production from the co-culture of human bronchial epithelial cells and neutrophils, treatment with Pur could significantly down-regulate the expression of IL-8 mRNA in these co-culture cells [100]. In mouse mesangial cells, Pur suppressed N-carboxymethyllysine-induced inflammation through inducing HO-1 expression mediated by the PKC δ-Nrf-2/HO-1 pathway [101].

**Summary**

In conclusion, a large number of research studies have shown that Pur has extensive pharmacological activities, and these functions are mainly manifested in animal models and cell experiments, the clinical curative effects are still not obvious [2]. The molecular mechanisms of Pur’s pharmacological activities are very complex, current research mainly focuses on regulating the signal pathways of individual cells, such as PKB/Akt/GSK-3β/Nrf2 [5, 51], CaMKII/AMPK [17], PI3K/Akt [17, 28-29, 53, 63, 86], NF-κB [18, 21, 37, 42, 93, 97, 99], NO/NO-cGMP [25], TNFR1/FADD/caspase [37], cAMP/PKA [56], MAPK [61, 85, 88-99], Wnt/β-catenin [89], JAK2/STAT3 [90], AMPK/mTOR [91], and PKC/δ-Nrf-2/HO-1 [101] signal pathways, and different research studies led to different conclusions. This status might be attributed to different doses and treatment times of Pur used in different animal and cell models. Another reason which is usually ignored is that these signal pathways connect with each other to form networks, and some of them can be influenced by each other, when one of these bioactivators are regulated by compounds such as Pur, others might be modulated too. So the present data can only indicate that Pur can regulate different signaling pathways, the binding sites, and the real mechanism are still poorly understood, which is the main question for further researches to address in order to improve the clinical effects and utilization of this attractive constituent.

**References**


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