Review of pharmacological effects, chemical constituents of Pien Tze Huang and prediction of its Q-markers

DONG Zhao-Min, WANG Hong, WANG Guang-Ji*

Jiangsu Provincial Key Laboratory of Drug Metabolism and Pharmacokinetics, Research Unit of PK-PD Based Bioactive Components and Pharmacodynamic Target Discovery of Natural Medicine of Chinese Academy of Medical Sciences, China Pharmaceutical University, Nanjing 210009, China

[ABSTRACT] Pien Tze Huang (PTH) was documented as an imperial prescription composed of Panax notoginseng, Calculus bovis, snake gallbladder and musk, is famous in China and Asian countries due to its excellent the functions of heat clearing, detoxifying, swelling reducing, and pain relieving. Modern pharmacological studies show that PTH shows excellent effects against various inflammatory diseases, liver diseases, and carcinomas. This review summaries and discusses its pharmacological effects, clinical application, and main chemical components. More importantly, its quality markers (Q-markers) are then forecasted and analyzed based on the “five core principles” of Q-markers, of Traditional Chinese Medicine, including transfer and traceability, specificity, efficacy, compatibility, and measurability. Ginsenosides Rb1, ginsenoside Rg1, ginsenoside Rd, ginsenoside Re, notoginsenoside R1, dencichine, bilirubin, biliverdin, taurocholic acid, and muscone are speculated as the Q-markers of PTH. This review provides a reference and basis for the construction of a quality control system for PTH.

[KEY WORDS] Pien Tze Huang; quality marker (Q-marker); chemical components; pharmacological effects; clinical application; quality control


Introduction

Pien Tze Huang (PTH) is a famous and precious Traditional Chinese Medicine Prescription (TCMP), with a history of more than 450 years since the Ming Dynasty. Nowadays, it is used to treat acute and chronic viral hepatitis, and various inflammatory diseases [1]. PTH is mainly composed of four Traditional Chinese Medicine (TCM) ingredients, including Panax notoginseng (Sanqi in Chinese), Calculus bovis (Niu-huang in Chinese), snake gallbladder (Shedan in Chinese) and musk (Shexiang in Chinese) [2], which together have functions of heat clearing, detoxifying, swelling reduced, and pain relieving. However, the complexity in chemical composition, instability of raw materials, and complexity of scientific connotation make its quality control more difficult than that of chemical drugs and biological drugs [3].

Lot of studies on active components of PTH have been carried out since 1990s. However, most of the previous studies follow the western medical research model, ignoring its scientific connotation under the guidance of TCM theory. Thus, it is difficult to fully reveal the active components of PTH. The quality marker (Q-marker) of Chinese medicine is a new concept proposed by Academician Changxiao Liu. It integrates the knowledge of TCM theory, compound compatibility, and clinical application [4, 5]. It analyzes the core theory and scientific connotation of TCM Q-marker from the five core principles of “efficacy correlation,” “specificity,” “traceability and transitivity” “measurability,” and “compatibility” [6]. Q-marker analysis is conducive to breaking the bottleneck of quality control of TCMs.

Here in this review, we provide a comprehensive review about the pharmacological effects and clinical application of PTH. Furthermore, following the “five core principles”, we analyze the Q-markers of PTH based on its active ingredients.

Pharmacological effects and clinical applications of PTH

PTH has a wide range of clinical applications, and its intrinsic pharmacological effects have always been of concern to researchers. Modern pharmacological studies have shown...
that PTH shows excellent effects against various inflammatory diseases, liver diseases, and carcinoma.

**Anti-inflammatory effects**

Inflammation is an adaptive response, occurs when the body is affected by harmful stimuli or factors, such as infection, injury, and tissue dysfunction. Recently, many studies support that PTH shows powerful anti-inflammatory effect. Due to this property, PTH has been used for the treatment of arthritis, colitis, cholecystitis, hepatitis, and myelitis. The underlying mechanisms involve inhibition of the nuclear factor kappa-B (NF-κB) pathway, NOD-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome, and Interleukin-6 (IL-6)/signal transducer and activator of transcription 3 (STAT3) signal transduction. In addition, PTH can downregulate tumor necrosis factor α (TNF-α), IL-1β and upregulate LC3, improving cholecystitis development. It can alleviate osteomyelitis by downregulating the percentages of Th1 and Th17 cells.

**Cerebrovascular protection**

Ischemic stroke is a process in which blood flow to the brain is blocked due to blocked cerebral arteries, sudden loss of brain function due to reduced blood flow, and the brain tissue shuts down as the ischemic cascades are gradually activated. Brain injury induced by cerebral ischemic stroke is the result of complex pathophysiological processes, such as excitotoxicity, oxidative stress, inflammation and apoptosis. PTH has been reported to improve brain injury caused by cerebral ischemia-reperfusion. The mechanisms may be associated with the inhibition of neuronal apoptosis, promotion of autophagy, suppression of neuroinflammation through NLRP3 and toll-like receptor 4/NF-κB/mitogen-activated protein kinase signaling pathways.

**Hepatoprotective effect**

PTH is well demonstrated to protect the liver from various injuries. PTH also reduced fibrosis markers (hyaluronic acid, laminin, procollagen III and collagen IV) and inflammatory cytokines (IL-1β, IL-6 and TNF-α) in a CCl4-induced liver fibrosis. In addition, reduction in NF-κB expression levels and liver protection may be associated with the liver protection of PTH.

In an alcoholic liver disease model, PTH reduces liver oxidative stress and liver steatosis via inhibiting oxidized lipid metabolite/Adenosine 5-monophosphate-activated protein kinase // acetyl-CoA carboxylase/ carnitine palmitoyl transferase 1A axis and RNA-activated protein kinase-like endoplasmic reticulum kinase // eukaryotic translation initiation factor 2 subunit alpha pathway.

In nonalcoholic fatty liver disease, PTH reduces AST, ALT, gamma-glutamyl transferase, triglyceride in serum and hepatic steatosis. Regulation of farnesoid X receptor-small heterodimer partner-sterol regulatory element binding protein-1c pathway contributes to its role in maintaining lipids homeostasis.

**Antitumor effect**

**Osteosarcoma**

Osteosarcoma, the most common primary bone malignancy in children and adolescents, is a malignancy of the mesenchymal tissue (made of spindle-shaped interstitial cells that produce bone-like tissue) that produces osteoclasts. Numerous studies support the application of PTH in fighting against osteosarcoma. Possible mechanisms include of proliferation, migration and invasion, as well as promotion of apoptosis of osteosarcoma cells. PTH reduces migration and invasion by decreasing the expression of survivin, vascular endothelial growth factor (VEGF), matrix metalloprotein 9, and CD44 variant isoform 6, while increasing the expression of tissue inhibitor of metalloproteinase-1. PTH induces apoptosis by downregulating B-cell lymphoma-2 (Bcl-2) while upregulating Bcl2 Associated X Protein (Bax), which enhancing mitochondrial permeability and then activating caspases. PTH inhibits proliferation by upregulating phosphatase and tensin homolog deleted on chromosome ten, while downregulating phosphoinositide 3-kinase, protein kinase B, and phosphorylation protein kinase B proteins.

**Colorectal cancer**

Colorectal cancer (CRC) is a common type of gastrointestinal tumor, and its conventional treatment is mainly surgical resection, supplemented with radiotherapy, chemotherapy, and other adjuvant treatments. Application of PTH in CRC treatment has drawn much attention. PTH induces apoptosis of CRC cells by inhibiting STAT3, which upregulates the Bax/Bcl-2 ratio, and the expression of cyclin D1 and cyclin dependent kinase 4 is downregulated. PTH also inhibits proliferation by increasing miR-22 and miR-34c-5p.

PTH inhibits tumor angiogenesis by reducing the expression of various angiogenesis factors, including inducible nitric oxide synthase, endothelial nitric oxide synthase, VEGF-A, basic fibroblast growth factor, and their specific receptors VEGFR2 and bFGFR, respectively. Besides, PTH also inhibits hypoxia inducible factor-1alpha /VEGF-A pathway.

PTH suppresses epithelial-mesenchymal transition and tumor metastasis by inhibiting the hypoxia inducible factor-1 (HIF-1) and transforming growth factor beta pathways. In addition, PTH inhibits lymphangial genesis by downregulating VEGF-C and exerting its molecular mechanisms of antitumor activity in CRC metastasis.

**Liver cancer**

Inhibitory effect of PTH on liver cancer has also been reported. PTH exerts this effect by downregulating IL-6, TNF receptor-1, TNF receptor-2, and G2/M check point. PTH promotes HepG2 apoptosis by regulating the ANXA1/VEGF signaling pathway. In addition, PTH inhibits the proliferation of liver cancer stem cells while promoting their apoptosis by regulating the expression levels of Bcl2/Bax, CyclinD1, cyclin dependent kinase 4, miR-483-5p and cyclin dependent kinase inhibitor 1A.
Other

PTH also has a certain inhibitory effect on other cancers, including breast cancer [65], lung cancer [66], ovarian cancer [67], tongue cancer [68] and myeloma [69] (Table 1, Fig. 1).

Table 1  pharmacological actions of PTH

<table>
<thead>
<tr>
<th>Pharmacology</th>
<th>Mechanism</th>
<th>Disease</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrovascular protection</td>
<td>Neuronal apoptosis ↓</td>
<td>Ischemia stroke</td>
<td>[24]</td>
</tr>
<tr>
<td></td>
<td>Autophagy ↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NLRP3 ↓</td>
<td></td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>TLR4/NF-κB/MAPK ↓</td>
<td></td>
<td>[25]</td>
</tr>
<tr>
<td>AMPK/ACC/CPT1A ↓, PERK/eIF2a ↓</td>
<td>ALD</td>
<td></td>
<td>[30, 31]</td>
</tr>
<tr>
<td>Hepatic protection</td>
<td>FXR-SHP-SREBP-1c ↑</td>
<td>NAFLD</td>
<td>[32]</td>
</tr>
<tr>
<td></td>
<td>NF-κB ↓</td>
<td>Liver fibrosis</td>
<td>[28, 29]</td>
</tr>
<tr>
<td></td>
<td>Survivin ↓, VEGF ↓, MMP-9 ↓</td>
<td></td>
<td>[39-41]</td>
</tr>
<tr>
<td></td>
<td>Bcl-2 ↓, Bax ↑; Caspase ↑</td>
<td>Osteosarcoma</td>
<td>[42, 43]</td>
</tr>
<tr>
<td></td>
<td>PTEN ↑, PIK/Akt ↓; PARP ↓</td>
<td></td>
<td>[44-46]</td>
</tr>
<tr>
<td></td>
<td>STAT3 ↓, Bax/Bcl-2 ↑, Cyclin D1 ↓, CDK4 ↓</td>
<td>Colorectal cancer</td>
<td>[48, 49]</td>
</tr>
<tr>
<td></td>
<td>Angiogenesis factor ↓</td>
<td></td>
<td>[53]</td>
</tr>
<tr>
<td></td>
<td>VEGF-C ↓</td>
<td></td>
<td>[58]</td>
</tr>
<tr>
<td></td>
<td>HIF-1 pathway ↓, TGF-β pathway ↓; EMT ↓</td>
<td></td>
<td>[56, 57]</td>
</tr>
<tr>
<td>Antitumor</td>
<td>IL-6 ↓, TNFR1 ↓, TNFR2 ↓, G2 /M check point</td>
<td>Liver cancer</td>
<td>[60]</td>
</tr>
<tr>
<td></td>
<td>ANXA1 ↑/VEGF ↓</td>
<td></td>
<td>[61, 62]</td>
</tr>
<tr>
<td></td>
<td>Bcl2/Bax ↓, CyclinD1 ↓, CDK4 ↓, mir-483-5p ↓, CDKN1A ↑</td>
<td>Breast cancer</td>
<td>[64]</td>
</tr>
<tr>
<td></td>
<td>ABCG2 ↓, ABCB1 ↓, TGF-β1 ↓</td>
<td>Lung cancer</td>
<td>[65]</td>
</tr>
<tr>
<td></td>
<td>Akt ↑</td>
<td></td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td>Akt ↓, PARP ↓, CDK6 ↓</td>
<td>Ovarian cancer</td>
<td>[67]</td>
</tr>
<tr>
<td></td>
<td>STAT3 ↓, Bcl-2 ↓</td>
<td>Tongue cancer</td>
<td>[68]</td>
</tr>
<tr>
<td></td>
<td>Bel-xl ↓, Bok ↑, Bak-1 ↑, Bax ↑, PI3K-AKT ↓</td>
<td>Myeloma</td>
<td>[69]</td>
</tr>
</tbody>
</table>

Chemical components of PTH

PTH is mainly composed of four precious TCMs: Panax notoginseng, Calculus bovis, snake gallbladder, and musk. Panax notoginseng is the dried root of Panax notoginseng (Burk.) F. H. Chen (Fig. 2). This TCM contains a variety of chemicals, such as saponins, flavonoids, cyclopeptides, sterols, saccharides, amino acids, and polyacetylenes. It is well-accepted that its main active ingredients include ginsenosides, notoginsenosides, and dencichine [70, 71]. Calculus bovis, also known as “Ugly Treasure,” is the dried gallstone of bovine (Bos taurus domesticus Gmelin). Components of Calculus bovis mainly include bile pigment, bile acid, cholesterol, and amino acids. Among them, bilirubin and bile acids are accepted as the main active components [73]. More than 90% of the bilirubin in Calculus bovis is present in the form of bilirubin calcium salts, that is, in the form of a combination of bilirubin and calcium [74]. Musk is the dry secretion in the male sachet of musk deer (Moschus berezovskii Flerov, M. sifaniacus, and M. moschiferus L). The main chemical components include macrocycles, pyridines, steroids, fatty acids, and amino acids. Snake gallbladder is the gallbladder that stores bile in snakes. The main chemical components are bile acids, among which taurocholic acid is an important component.

Fig. 1 Main pharmacological action and clinical application of PTH

Fig. 2 The material basis of PTH

Q-marker predictive analysis of PTH

The core definitions of Q-marker includes: some substances are secondary metabolites of the drug itself, or compounds formed after processing; some chemical components are unique to some medicinal materials; some components have clear biological activity and chemical structure; under the guidance of the theory of TCM, take the prescription "Jun" medicine as the first choice principle, and give consideration to the representative substances of "Chen", "Zuo" and "Shi" medicine; some substances can be qualitatively identified and quantitatively determined.

Prediction of Q-marker based on component transfer and traceability

The “prototype composition” in the formulation is an im-
Table 2 Pharmacological composition of PTH

<table>
<thead>
<tr>
<th>Pharmacological composition</th>
<th>Ref.</th>
<th>Pharmacological composition</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saponins</strong></td>
<td></td>
<td><strong>Bile acid</strong></td>
<td></td>
</tr>
<tr>
<td>Notoginsenoside R1</td>
<td>[75-80]</td>
<td>Cholic acid</td>
<td>[76, 78, 80]</td>
</tr>
<tr>
<td>Ginsenoside Rg1</td>
<td>[75-79]</td>
<td>Deoxycholic acid</td>
<td>[76, 78]</td>
</tr>
<tr>
<td>Ginsenoside Rb1</td>
<td>[75-79]</td>
<td>Hyodeoxycholic acid</td>
<td>[76, 80]</td>
</tr>
<tr>
<td>Ginsenoside Rg3</td>
<td>[76]</td>
<td>Ursodeoxycholic acid</td>
<td>[76, 80]</td>
</tr>
<tr>
<td>Ginsenoside Re</td>
<td>[78, 80]</td>
<td>Chenodeoxycholic acid</td>
<td>[76, 78, 80]</td>
</tr>
<tr>
<td>Ginsenoside Rd</td>
<td>[78, 80]</td>
<td>Taurocholic acid</td>
<td>[78]</td>
</tr>
<tr>
<td><strong>Bile</strong></td>
<td></td>
<td>Glycodeoxycholic acid hydrate</td>
<td>[78, 80]</td>
</tr>
<tr>
<td>Cholic acid</td>
<td>[76, 78, 80]</td>
<td>Glycocholic acid hydrate</td>
<td>[78]</td>
</tr>
<tr>
<td>Deoxycholic acid</td>
<td>[76, 78]</td>
<td>Sodium taurochenodeoxycholate</td>
<td>[76]</td>
</tr>
<tr>
<td>Hyodeoxycholic acid</td>
<td>[76, 80]</td>
<td>Sodium taouroursodeoxycholate</td>
<td>[76]</td>
</tr>
<tr>
<td>Ursodeoxycholic acid</td>
<td>[76, 80]</td>
<td>Sodium taurocholic acid</td>
<td>[75, 77]</td>
</tr>
<tr>
<td>Chenodeoxycholic acid</td>
<td>[76, 78, 80]</td>
<td>Taurochenodeoxycholic acid</td>
<td>[80]</td>
</tr>
<tr>
<td><strong>Phenols</strong></td>
<td></td>
<td><strong>Macrocycles</strong></td>
<td></td>
</tr>
<tr>
<td>Taurine</td>
<td>[78]</td>
<td>Muscone</td>
<td>[76, 78]</td>
</tr>
<tr>
<td>****</td>
<td></td>
<td>Thymol</td>
<td>[77]</td>
</tr>
<tr>
<td><strong>Macrocycles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Panax notoginseng</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculus bovis</td>
<td>[76]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snake gallbladder</td>
<td>[76]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>****</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver cancer as an example</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17672 disease targets of liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cancer were obtained through the</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genecards database.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>275 common targets of PTH and</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>liver cancer were obtained by</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>drawing a Venn diagram (Fig. 5).</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finally, we can use string to</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>draw the protein-protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>interaction networks of the</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>common targets, and use R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>language tools to analyze the</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KEGG and GO of these targets to</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>obtain the corresponding path</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>data, and then determine the Q-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>markers of PTH.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3 Venn of components’ screening of PTH

**Prediction of Q-marker based on component specificity**

Ingredient specificity refers to the components that have...
the same type of TCM and characteristics that are different from other types of TCM. It is a differential component, reflecting different TCMs in the same class [87].

Panax notoginseng is the dried root of the five-plus family plant Panax notoginseng, which mainly contains compounds, such as: saponins, volatile components, polysaccharides, and amino acids [88]. Among them, the saponins are often dammarane triterpenoid saponins (approximately 45), including notoginsenoside R1, ginsenoside Rg1, ginsenoside Rb1, ginsenoside Rd, ginsenoside Re and other [89-94]. The volatile components include terpenes, alkanes, aldehydes, alcohols, α-pinene, α-gurjunene, heptacosane, octanal, and panaxyanol [95-97]. The polysaccharide is sanchinan A [98, 99], and the amino acid components include dencichine, arginine, γ-aminobutyric acid, and tryptophan [100]. Dencichine, notoginsenoside R1, ginsenoside Rg1, ginsenoside Rb1, ginsenoside Rd, and ginsenoside Re are the main active components of Panax notoginseng, as well as the representative components of its pharmacodynamic material base, with certain properties [101, 102].

Calculus bovis is a stone in the gallbladder, bile duct, or hepatic duct of cows or buffalos. Main chemical constituents are bile pigments (bilirubin and biliverdin), bile acids (including cholic, taurocholic, deoxycholic, chenodeoxycholic, ursodeoxycholic, and glycocholic acids), amino acids (including glutamic acid, aspartic acid, proline, and leucine), fatty acids (including palmitic acid, stearic acid, arachidonic acid, and oleic acid), and minerals (including zinc, ferrum, sodium, manganese, and magnesium). Bilirubin, biliverdin, cholic acid and deoxycholic acid as its main active ingredients, with certain characteristics [103-110].

Snake gallbladder, the gallbladder of snakes; it mainly contains bile acids (including taurocholic, taurochenodeoxycholic, and taurodeoxycholic acids), bile pigments, cholesteryl, inorganic salt, mucin, and other. Taurocholic acid is the most abundant and characteristic [111-114].

Musk is a dry secretion, which is derived from the mature male sachets of Moschus berezovskii, M. chrysogaster, or M. moschiferus. Its main ingredients are macrocycles, steroids, pyridines, and proteins. It mainly contains muscone, cholesterol, androsterone, and cholestanol [115], of which muscone is the main active ingredient, with certain character-
istics \cite{116, 117}. Based on component specificity, we predict that Dencichine, notoginsenoside R1, ginsenoside Rg1, ginsenoside Rb1, ginsenoside Rd, ginsenoside Re, bilirubin, biliverdin, cholic acid, deoxycholic acid, taurocholic acid, and muscone can be used as Q-marker.

**Prediction of Q-marker based on the correlation between components and efficacy**

For TCMs with complex ingredients, their efficacy does not depend on all the ingredients contained in the medicine, but on some key ingredients with better “drug-like properties” \cite{118}; therefore, the efficacy is closely related to these ingredients, which is the basis for determining the quality markers of TCMs. PTH, as discussed above, has anti-inflammatory, hepatoprotective, and antitumor effects.

Components that contribute to the anti-inflammatory effects of PTH were analyzed. Twenty-seven chemical components in PTH were separated and identified using HPLC-Q/TOF MS. Among them, 9 components belong to saponins, and 11 belong to bile acids. Their activities against anti-inflammatory were then analyzed. Result showed that taurine and muscone show anti-inflammatory activities \cite{119}.

Components that contribute to the anti-tumor effects of PTH were intended to predicted and analyzed. PTH Sixteen active ingredients were screened from PTH using network pharmacology and molecular docking technology. Eight ingredients, including sodium taurodeoxycholate, taurochenodeoxycholic acid, glycodehydrocholic acid, chenodeoxycholic acid, and ginsenoside Rd, muscone, cholic acid, ginsenoside Rb1, may exhibit anti-tumor effect via estrogen receptor 1, tyrosine kinase receptor 2, insulin-like growth factor 1 receptor, androgen receptor, NOTCH1 and albumin \cite{196}. The contribution of these 8 components needs further validation. Besides, components that contribute to the pharmacological activities against others cancers, including osteosarcoma, CRC, and other, need further analysis.

In addition, PTH also has cerebrovascular protective effects, which might be achieved through ginsenosides and muscones \cite{120}. PTH is famous for its hepatoprotective effects. However, the active ingredients that contribute to these effects remain unclear.

Taken above together, notoginsenoside R1, ginsenosides (Re, Rg1, Rg2, Rf, Rg1, Rh1, Rd, Rb1, and Rd), taurocholic acid, taurochenodeoxycholic acid, cholic acid, and muscone play an important role in the pharmacological activities of PTH, and can be used as its Q-marker.\footnote{DONG Zhao-Min, et al. / Chin J Nat Med, 2023, 21(x): 1-13}

**Prediction of Q-marker based on compatibility of prescription**

A TCM compound is the basic form of clinical medicine in TCM, and its compatibility follows certain principles: the same medicinal flavor plays different roles in different compatibility environments, and the material basis of its efficacy is often different. Therefore, a “compatible environment” is the most important factor to be considered when selecting quality markers of Chinese medicine compounds \cite{121}.

Knockout/knock-in of potential active ingredients is one of the powerful approaches to screen for Q-markers in a compound compatibility environment. Contribution of target ingredients to the overall efficacy of a compound can be judged by knocking out the specific components and comparing the changes in efficacy \cite{122}. Currently, there are few studies on the compatibility of PTH compound and the knockout/knock-in of these components. Active ingredients in Calculus bovis were analyzed by this method. Bilirubin components, sodium taurocholate, glycodehydrocholic acid, taurine, cholic acid, deoxycholic acid, hyodeoxycholic acid, and chenodeoxycholic acid were knock out of Calculus bovis, and the efficient were compared. It was found that conjugated bilirubin, bilirubin, cholic acid, and glycodehydrocholic acid have protective effects against oxidative stress damage, suggesting that these are the main active ingredients of Calculus bovis \cite{123}.

**Prediction of Q-marker based on component measurability**

Ingredient measurability is a necessary condition as a quality marker. The 2020 edition of the Chinese Pharmacopoeia \cite{1} indicates that the components for the determination of Panax notoginseng, Calculus bovis, snake gallbladder, and musk are ginsenoside Rg1, ginsenoside Rb1, notoginsenoside R1, cholic acid, bilirubin, and muscone. Additionally, an HPLC method for the determination of the multi-index components of PTH was established, and the ginsenoside Rb1, ginsenoside Rg1, notoginsenoside R1, and sodium taurocholate contents were simultaneously determined under the same conditions \cite{79}. Another UPLC-QQQ-MS method for the determination of PTH was established, and the ginsenoside Rb1, ginsenoside Rg1, ginsenoside Rd, ginsenoside Re, notoginsenoside R1, cholic acid, taurocholic acid, glycocolichic acid, deoxycholic acid, glycodehydrocholic acid, chenodeoxycholic acid, taurine, and muscone were determined \cite{79}. Furthermore, a DI-MS/MS\textsuperscript{ALL} method was established to rapidly characterize and analyze the chemical components of PTH. Based on the results combined with the database and related literature information for matching, 53 compounds were obtained, of which 41 compounds, comprising those from Panax notoginseng (16), snake gallbladder and Calculus bovis (24), and musk (1) were designated as sources \cite{124}. The different polar compounds in PTH were measured comprehensively, which provided the reference for the qualitative analysis of PTH. In addition, the application of fingerprint measurement technology has become increasingly more extensive. For example, a correlation analysis technique for PTH fingerprint total statistical moment and similarity analysis has been established. The fingerprints of 10 batches of PTH samples were determined, and data processing methods, such as similarity and integration, were used to study the fingerprints of TCM chromatographic fingerprint samples. A total of 14 common feature peaks were found. Four characteristic peaks were identified through comparison with the reference substances, namely notoginsenoside R1, ginsenoside Rg1, ginsenoside Rb1, and sodium taurocholate \cite{125}, thereby implementing a Q-marker predictive analysis based on component measurability (Fig. 6).
Conclusion and future prospects

PTH, a famous TCMP, has the characteristics of “multi-component, multitarget, and extensive pharmacodynamic output” \cite{126}; therefore, it is of great significance to clarify chemical components that reveal the scientific connotation. In this review, we summarized and discussed the pharmacological effects and clinical application of PTH. Besides, we also provided a summary about its chemical components. Due to the complexity of its components, it is difficult to identify which ones are the chemical components of the corresponding pharmacological effects. Q-marker of PTH was then forecasted and analyzed based on the “five core principles” of TCM. Ginsenosides Rb1, ginsenoside Rg1, ginsenoside Rd, ginsenoside Re, notoginsenoside R1, dencichine, bilirubin, biliverdin, taurocholic acid, and muscone were speculated as the Q-marker of PTH (Table 3). It provides strong support for quality control, pharmacological mechanism investigation, and clinical application of PTH (Fig. 7). However, it should be recognized that Q-markers predicted in this study need further validation.

PTH is a famous and precious TCMP that has been used in China and Asian countries since the Ming Dynasty. Calculus bovis and musk in PTH are precious TCMs. In order to protect the endangered species, under the auspices of Ministry of Health of the Chinese Government, substitution of natural Calculus bovis and musk by artificial ones is proposed. However, whether the chemical components are same and whether the pharmacological efficacies are comparable for the natural and artificial ones remain unclear. System studies are needed to conclude the probability of the substitution, and Q-markers provide a useful tool for these studies. Clarification and validation of Q-markers, which of course contribute to the overall pharmacological efficacies, will pave the way to uncover the underlying mechanism.

Table 3 Information of Q-Markers of PTH

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Molecular Formula</th>
<th>source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ginsenoside Rb1</td>
<td>C_{54}H_{92}O_{23}</td>
<td>Radix notoginseng</td>
</tr>
<tr>
<td>Ginsenoside Rg1</td>
<td>C_{42}H_{72}O_{14}</td>
<td>Radix notoginseng</td>
</tr>
<tr>
<td>Ginsenoside Rd</td>
<td>C_{48}H_{82}O_{18}</td>
<td>Radix notoginseng</td>
</tr>
<tr>
<td>Ginsenoside Re</td>
<td>C_{48}H_{82}O_{18}</td>
<td>Radix notoginseng</td>
</tr>
<tr>
<td>Notoginsenoside R1</td>
<td>C_{47}H_{80}O_{18}</td>
<td>Radix notoginseng</td>
</tr>
<tr>
<td>Dencichine</td>
<td>C_{6}H_{8}N_{2}O_{5}</td>
<td>Radix notoginseng</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>C_{33}H_{38}N_{2}O_{6}</td>
<td>Calculus Bovis, Snake Gallbladder</td>
</tr>
<tr>
<td>Biliverdin</td>
<td>C_{33}H_{38}N_{2}O_{6}</td>
<td>Calculus Bovis, Snake Gallbladder</td>
</tr>
<tr>
<td>Taurocholic acid</td>
<td>C_{26}H_{45}N_{2}O_{7}</td>
<td>Calculus Bovis, Snake Gallbladder</td>
</tr>
<tr>
<td>Muscone</td>
<td>C_{16}H_{30}O</td>
<td>Musk</td>
</tr>
</tbody>
</table>
Fig. 7 Chemical structures of Q-Markers of PTH

References


[64] Chen X. Study on the Mechanism of Inhibiting the Growth of Hepatocellular Carcinoma Cells by Pien Tze Huang Based on the Regulation of Tumor Stem Cell miRNA [D]. Fujian Univ Tradit Chin Med, 2017.


[120] Zhang L, Lam WP, Lü L, et al. How would composite traditional Chinese medicine protect the brain-an example of the


**Cite this article as:** DONG Zhao-Min, WANG Hong, WANG Guang-Ji. Review of pharmacological effects, chemical constituents of Pien Tze Huang and prediction of its Q-markers [J]. *Chin J Nat Med*, 2023, 21(0): 1-13.