Research progress on the pharmacological effects and chemical constituents of Pien Tze Huang and its potential Q–markers

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Research progress on the pharmacological effects and chemical constituents of Pien Tze Huang and its potential Q-markers

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[ABSTRACT] Pien Tze Huang (PTH) was documented as an imperial prescription composed of Notoginseng Radix, Calculus Bovis, Snake Gallbladder, and Musk. It is famous in China and Asian countries due to its excellent effects in heat clearing, detoxifying, swelling reduction, and pain relieving. Modern pharmacological studies demonstrate that PTH shows excellent effects against various inflammatory diseases, liver diseases, and cancers. This review summaries the pharmacological effects, clinical applications, and main-chemical components of PTH. More importantly, its potential quality markers (Q-markers) were then analyzed based on the “five principles” of Q-markers under the guidance of Traditional Chinese Medicine theory, including transfer and traceability, specificity, efficacy, compatibility, and measurability. As a result, ginsenosides Rb1, ginsenoside Rg1, ginsenoside Rd, ginsenoside Re, notoginsenoside R1, dencichine, bilirubin, biliverdin, taurocholic acid, and muscone are considered as the Q-markers of PTH. These findings will provide guidance and assistance for the construction of a quality control system for PTH.

[KEY WORDS] Pien Tze Huang (PTH); 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 Notoginseng Radix (Sanqi in Chinese), Calculus Bovis (Niuhuang in Chinese), Snake Gallbladder (Shedan in Chinese) and Musk (Shexiang in Chinese) [2], which together have functions in heat clearing, detoxifying, swelling reduction, and pain relieving. However, its quality control is more difficult than chemical and biological drugs due to complex chemical composition, instable raw materials, and complicated scientific connotation [3].

Since 1990s, scientific studies on the active components of PTH have been carried out. However, most of them followed Western medical research model. The scientific connotation of PTH under the guidance of TCM theory was ignored. Thus, it is difficult to fully reveal the active components of PTH. Notably, a new concept for a TCM quality marker (Q-marker) was proposed by Academician LIU Changxiao. This concept integrates the knowledge of TCM theory, compound compatibility, and clinical application [4, 5]. The Q-marker 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.

In this review, we provide a comprehensive summary of the pharmacological effects and clinical applications of PTH. Furthermore, following the “five core principles”, we analyzed the potential 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 showed that PTH exhibits excellent effects against various inflammatory diseases, liver diseases, and cancers.

**Anti-inflammatory effects**

Inflammation is an adaptive response, which occurs when the body is affected by harmful stimuli or factors, such as infection, injury, and tissue dysfunction. Recently, many studies have demonstrated that PTH exerts powerful anti-inflammatory effects. Due to this property, PTH has been used for the treatment of arthritis, colitis, cholecystitis, hepatitis, and myelitis. The underlying mechanisms involved in inhibition of 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 downregulated tumor necrosis factor α (TNF-α) and IL-1β, and upregulated LC3, improving cholecystitis development. It also alleviated osteomyelitis by downregulating the percentages of Th1 and Th17 cells.

**Cerebrovascular protective effects**

Ischemic stroke is a process in which blood flow to the brain is impaired due to blocked cerebral arteries, brain function is suddenly lost 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 inhibition of neuronal apoptosis, promotion of autophagy, suppression of neuroinflammation via NLRP3, and inhibition of the toll-like receptor 4/NF-xB/mitogen-activated protein kinase signaling pathways.

**Hepatoprotective effects**

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

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

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

**Antitumor effects**

**Osteosarcoma**

Osteosarcoma, the most common primary bone malignancy in children and adolescents, is a malignancy of the mesenchymal tissue (composed of spindle-shaped interstitial cells that produce bone-like tissue). Numerous studies supported the application of PTH in the treatment of osteosarcoma. Possible mechanisms include inhibition of proliferation, migration and invasion, as well as promotion of apoptosis of osteosarcoma cells. PTH reduced migration and invasion of osteosarcoma cells by decreasing the levels of survivin, vascular endothelial growth factor (VEGF), matrix metalloprotein 9, and CD44 variant isosform 6, while increasing the levels of tissue inhibitor of metalloproteinase-1 (TIMP-1), PTH induced apoptosis of osteosarcoma cells by downregulating B-cell lymphoma-2 (Bcl-2) while upregulating Bcl2 associated X protein (Bax), which enhanced mitochondrial permeability and then activated caspases. PTH inhibited proliferation by upregulating phosphatase and tensin homolog deleted on chromosome ten (PTEN), 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, which is mainly treated by surgery, supplemented with radiotherapy, chemotherapy, and other adjuvant treatments. Application of PTH in CRC treatment has drawn much attention. PTH induced the apoptosis of CRC cells by inhibiting STAT3, while the Bax/Bcl-2 ratio was upregulated, and the expression of cyclin D1 and cyclin dependent kinase 4 was downregulated. PTH also inhibited proliferation of CRC cells by increasing miR-22 and miR-34c-5p.

**PHT inhibited 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. Moreover, PTH inhibited the VEGF-C pathway.

PTH suppressed epithelial-mesenchymal transition and tumor metastasis by inhibiting the hypoxia inducible factor-1 and transforming growth factor beta pathways. In addition, PTH inhibited lymphangial genesis by downregulating VEGF-C to exert its molecular mechanisms of antitumor activity in CRC metastasis.

**Liver cancer**

The inhibitory effect of PTH on liver cancer has also been reported. PTH exerted this effect by downregulating IL-6, TNF receptor-1, TNF receptor-2, and G2/M check point. PTH promoted the apoptosis of HepG2 cells by regulating the ANXA1/VEGF signaling pathway. In addition, PTH inhibited the proliferation of liver cancer stem cells while promoting their apoptosis by regulating the levels of Bcl2/Bax, cyclin D1, cyclin dependent kinase 4, miR-483-5p and cyclin dependent kinase inhibitor 1A.
PTH also exhibit inhibitory effects on other cancers, such as breast cancer [65], lung cancer [66], ovarian cancer [67], tongue cancer [68] and myeloma [69] (Table 1, Fig. 1).

### Table 1 Pharmacological action of PTH

<table>
<thead>
<tr>
<th>Pharmacological effect</th>
<th>Mechanisms</th>
<th>Diseases</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-inflammatory effect</td>
<td>NF-κB ↓, NLRP3 ↓</td>
<td>Arthritis</td>
<td>[16]</td>
</tr>
<tr>
<td></td>
<td>IL-6/STAT3 ↓</td>
<td>Colitis</td>
<td>[17]</td>
</tr>
<tr>
<td>Cerebrovascular protective effect</td>
<td>Neuronal apoptosis ↓</td>
<td>Ischemia stroke</td>
<td>[24]</td>
</tr>
<tr>
<td></td>
<td>Autophagy ↑</td>
<td></td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>NLRP3 ↓</td>
<td></td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>TLR4/NF-κB/MAPK ↓</td>
<td>Alcoholic liver diseases (ALD)</td>
<td>[30, 31]</td>
</tr>
<tr>
<td>Hepatoprotective effect</td>
<td>AMPK/ACC/CPT1A ↓, PERK/eIF2a ↓</td>
<td>Nonalcoholic fatty liver diseases (NAFLD)</td>
<td>[32]</td>
</tr>
<tr>
<td></td>
<td>FXR-SHP-SREBP-1c ↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NF-κB ↓</td>
<td>Liver fibrosis</td>
<td>[28, 29]</td>
</tr>
<tr>
<td>Antitumor effect</td>
<td>Survivin ↓, VEGF ↓, MMP-9 ↓, CD44v6 ↓, Timp-1↑</td>
<td>Osteosarcoma</td>
<td>[39-41]</td>
</tr>
<tr>
<td></td>
<td>Bcl-2 ↓, Bax ↑, Caspase ↑</td>
<td></td>
<td>[42, 43]</td>
</tr>
<tr>
<td></td>
<td>PTEN ↑, PIK/Akt ↓, PARP ↓</td>
<td>Colorectal cancer</td>
<td>[44-46]</td>
</tr>
<tr>
<td></td>
<td>STAT3 ↓, Bax/Bcl-2 ↑, Cyclin D1 ↓, CDK4 ↓</td>
<td></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></td>
<td>IL-6 ↓, TNFR1 ↓, TNFR2 ↓, G2/M check point</td>
<td></td>
<td>[60]</td>
</tr>
<tr>
<td></td>
<td>ANXA1 ↑, VEGF ↓</td>
<td>Liver cancer</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>ANXA1 ↑, 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: Notoginseng Radix, Calculus Bovis, Snake Gallbladder, and Musk. Notoginseng Radix is the dried root of Panax notoginseng (Burk.) F. H. Chen (Fig. 2). It contains a variety of chemicals, such as saponins, flavonoids, cyclopeptides, sterols, saccharides, amino acids, and polyacetylenes, where its main active ingredients are considered to be ginsenosides, notoginsenosides, and dencichine [70, 71]. Calculus Bovis, also known as “Ugly Treasure”, is the dried gallstone of bovine (Bos taurus domesticus Gmelin). The components of Calculus Bovis mainly include bile pigments, bile acids, cholesterol, and amino acids [72]. Among them, bilirubin and bile acids are the main active components [73]. More than 90% of the bilirubin in Calculus Bovis is present in the form of bilirubin calcium salts, a combination of bilirubin and calcium [74]. Musk is the dry secretion in the male sachet of musk deer (Moschus berezovskii Flerov, M. sifanicus, and M. moschiferus L.). Its main chemical components include macrocyclcs, pyridines, steroids, fatty acids, and amino acids. Snake gall-
bladder is the gallbladder that stores the bile in snakes. Its main chemical components are bile acids, among which taurocholic acid is an important component.

Taken together, PTH contains saponins, organic acids, macrocyclic compounds, and others. Specifically, the components include notoginsenoside R1, ginsenoside Rg1, ginsenoside Rg3, ginsenoside Rb1, ginsenoside Re, ginsenoside Rd, taurine, taurocholic acid, cholic acid, glycocholic acid hydrate, glycineoxycholic acid hydrate, deoxycholic acid, hyodeoxycholic acid, ursodeoxycholic acid, chenodeoxycholic acid, taurochenodeoxycholic acid, sodium tauroursodeoxycholate, muscone, and thymol (Table 2) [89, 75-80].

Due to the complexity of its components, it is difficult to determine the material basis of the corresponding pharmacological effects; new methods are then required. Notably, predictive analysis of Q-markers is a novel way to investigate the material basis for pharmacological effects of various TCMs and TCMPs.

**Predictive Analysis of Q-markers in PTH**

The core definitions of Q-marker are listed as follows. Some substances are the secondary metabolites of the drug itself, or compounds that are formed after processing. Some chemical components are unique to some medicinal materials. Some components have clear biological activities and chemical structures. According to the TCM theory, 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 [81, 82].
The prototype component in the formulation is an important component of mass transfer and traceability. It is not only the result of acquisition and transfer of active ingredients through extraction, purification, and preparation of Chinese herbal decoction pieces, but also the source of agents entering into the bloodstream and metabolites traced back to the origin of medicinal materials. It is an important indicator of quality control. A total of 73 chemical components of PTH were detected using online pressurized liquid extraction-UPLC-Q-TOF, and 71 components were identified and attributed. Among them, 36 components were derived from Notoginseng Radix, 15 components from Snake Gallbladder, 9 components from Calculus Bovis, and 11 components from Calculus Bovis and Snake Gallbladder.

The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform was used to search for active ingredients in PTH. The four TCMs, namely Notoginseng Radix, Calculus Bovis, Snake Gallbladder and Musk in PTH, were used as the keywords, while drug-like properties > 0.18 and oral bioavailability > 30% were used as the filter criteria. The number of active ingredients was discovered to be 21. Among them, eight components belong to Notoginseng Radix (including ginsenoside Rh2, ginsenoside F2, and quercetin), five components belong to Calculus Bovis (including deoxycholic acid, methyl deoxycholate, and ZINC01280365), six components belong to Snake Gallbladder (including taurocholic acid, taurodeoxycholic acid, and cholic acid, etc.), and two components belong to Musk (muscone and 8-cyclohexadecen-1-one) (Fig. 3). Possible protein targets were further predicted (Fig. 4). Then, liver cancer was taken as an example. Briefly, 17672 disease targets of liver cancer were obtained using the Genecards database, whereas 275 common targets of PTH and liver cancer were obtained as displayed in a Venn diagram (Fig. 5). Finally, the STRING database was used to establish a protein-protein interaction network of the overlaps, and R language tools were utilized to analyze the genes based on KEGG pathways and GO terms, in order to determine the Q-markers of PTH. According to network pharmacology approach, these 21 compounds or some of them are the Q Markers 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>Saponins</strong></td>
<td></td>
</tr>
<tr>
<td>Notoginsenoside R1</td>
<td>[75-80]</td>
<td>Ginsenoside Rf</td>
<td>[80]</td>
</tr>
<tr>
<td>Ginsenoside Rg1</td>
<td>[75-79]</td>
<td>Ginsenoside Rg2</td>
<td>[80]</td>
</tr>
<tr>
<td>Ginsenoside Rb1</td>
<td>[75-79]</td>
<td>Ginsenoside F2</td>
<td>[80]</td>
</tr>
<tr>
<td>Ginsenoside Rg3</td>
<td>[76]</td>
<td>Ginsenoside Rg3</td>
<td>[80]</td>
</tr>
<tr>
<td>Ginsenoside Re</td>
<td>[78, 80]</td>
<td>Ginsenoside Rh2</td>
<td>[80]</td>
</tr>
<tr>
<td>Ginsenoside Rd</td>
<td>[78, 80]</td>
<td><strong>Bile acids</strong></td>
<td></td>
</tr>
<tr>
<td>Cholic acid</td>
<td>[76, 78, 80]</td>
<td>Glycodeoxycholic acid hydrate</td>
<td>[78, 80]</td>
</tr>
<tr>
<td>Deoxycholic acid</td>
<td>[76, 78]</td>
<td>Glycocholic acid hydrate</td>
<td>[78]</td>
</tr>
<tr>
<td>Hyodeoxycholic acid</td>
<td>[76, 80]</td>
<td>Sodium taurochenodeoxycholate</td>
<td>[76]</td>
</tr>
<tr>
<td>Ursodeoxycholic acid</td>
<td>[76, 80]</td>
<td>Sodium tauroursodeoxycholate</td>
<td>[76]</td>
</tr>
<tr>
<td>Chenodeoxycholic acid</td>
<td>[76, 78, 80]</td>
<td>Sodium taurocholic acid</td>
<td>[75, 77]</td>
</tr>
<tr>
<td>Taurocholic acid</td>
<td>[78]</td>
<td>Taurochenodeoxycholic acid</td>
<td>[80]</td>
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<tr>
<td><strong>Phenols</strong></td>
<td></td>
<td><strong>Phenols</strong></td>
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<tr>
<td>Taurine</td>
<td>[78]</td>
<td>Thymol</td>
<td>[77]</td>
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<td><strong>Macrocycles</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Muscone</td>
<td>[76, 78]</td>
<td></td>
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</tbody>
</table>

**Prediction of Q-markers based on component transfer and traceability**

The “prototype component” in the formulation is an important component of mass transfer and traceability. It is not only the result of acquisition and transfer of active ingredients through extraction, purification, and preparation of Chinese herbal decoction pieces, but also the source of agents entering into the bloodstream and metabolites traced back to the origin of medicinal materials. It is an important indicator of quality control. A total of 73 chemical components of PTH were detected using online pressurized liquid extraction-UPLC-Q-TOF, and 71 components were identified and attributed. Among them, 36 components were derived from Notoginseng Radix, 15 components from Snake Gallbladder, 9 components from Calculus Bovis, and 11 components from Calculus Bovis and Snake Gallbladder.

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![Fig. 3 Venn diagram of the components in PTH.](image-url)
**Prediction of Q-markers based on component specificity**

Ingredient specificity refers to the components that share the same type of a class of TCMs and distinguish them from other classes of TCMs. It is a differential component, reflecting different TCMs in the same class [86].

Notoginseng Radix is the dried root of the Araliaceae family plant *Panax notoginseng*, whose main components are saponins, volatile components, polysaccharides, and amino acids [87]. Among them, the saponins are often dammarane triterpenoid saponins (approximately 45), such as notoginsenoside R1, ginsenoside Rg1, ginsenoside Rb1, ginsenoside Rd, and ginsenoside Re [88-93]. The volatile components include terpenes, alkanes, aldehydes, alcohols, α-pinene, α-gurjunene, heptacosane, octanal, and panaxynol [94-96]. The polysaccharide is sanchinan A [97, 98], whereas the amino acids include dencichine, arginine, γ-aminobutyric acid, and tryptophan [99]. Dencichine, notoginsenoside R1, ginsenoside Rg1, ginsenoside Rb1, ginsenoside Rd, and ginsenoside Re are the main active components as well as the characteristic components of *Panax notoginseng* [100, 101].

Calculus Bovis is a stone in the gallbladder, bile duct, or hepatic duct of cows or buffalos. Its main chemical constituents are bile pigments (bilirubin and biliverdin), bile acids (such as cholic, taurocholic, deoxycholic, chenodeoxycholic, ursodeoxycholic, and glycocholic acids), amino acids (such as glutamic acid, aspartic acid, proline, and leucine), fatty acids (such as palmitic acid, stearic acid, arachidonic acid, and oleic acid), and minerals (such as zinc, ferrum, sodium, manganese, and magnesium). Bilirubin, biliverdin, cholic acid and deoxycholic acid act as the main active ingredients and characteristic components [102-109].

Snake Gallbladder mainly contains bile acids (including taurocholic, taurochenodeoxycholic, and taurodeoxycholic acids), bile pigments, cholesterol, inorganic salt, mucin, and others. Taurocholic acid is the most abundant and characteristic component [110-113].

Musk is a dry secretion derived from the mature male sachets of *Moschus berezovskii*, *M. chrysogaster*, or *M. moschiferus*. Its main ingredients are macrocycles, steroids, pyridines, and proteins and often contains muscone, cholesterol, androsterone, and cholestanol [114], where muscone is the main active ingredient and characteristic component [115, 116].
Based on component specificity, it is assumed 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-markers.

**Prediction of Q-markers 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” [113]. 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 have been analyzed. For example, 27 chemical components in PTH were separated and identified by HPLC-Q/TOF MS. Among them, 9 components belong to saponins, and 11 belong to bile acids. Their anti-inflammatory activities were then analyzed. The results showed that taurine and muscone exhibited anti-inflammatory effect [114].

Components that contribute to its anti-tumor effects have been predicted and analyzed. For instance, 16 active ingredients were screened from PTH through network pharmacology and molecular docking. The results indicated that 8 ingredients, including sodium taurodeoxycholate, taurochenodeoxycholic acid, glycodeoxycholic acid, chenodeoxycholic acid, ginsenoside Rd, muscone, cholic acid and ginsenoside Rb1, might exhibit anti-tumor effect via estrogen receptor 1, tyrosine kinase receptor 2, insulin-like growth factor 1 receptor, androgen receptor, NOTCH1 and albumin. The contribution of these ingredients needs further validation. Moreover, components that contribute to the pharmacological activity against other cancers, such as osteosarcoma and CRC, need further analysis.

In addition, PTH exhibited cerebrovascular protective effects, which might be achieved through ginsenosides and muscones [119]. PTH is famous for its hepatoprotective effects. However, the active ingredients that contribute to these effects remain unclear.

In sum, 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-markers.

**Prediction of Q-markers based on the compatibility of prescription**

A TCM compound is the common form of clinical medicine in TCM. Its compatibility follows certain principles, that is 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 during selection of the quality markers of Chinese medicine compounds [120].

Knockout/knock-in of potential active ingredients is one of the powerful approaches to screen for Q-markers in a compound compatibility environment. The 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 [113]. Currently, there are few studies about the compatibility of PTH compounds and the knockout/knock-in of these components. Active ingredients in Calculus Bovis were analyzed by this method. Bilirubin components, sodium taurocholate, glycocholic acid, taurine, cholic acid, deoxycholic acid, hyodeoxycholic acid, and chenodeoxycholic acid were knocked out from Calculus Bovis, and their efficacy were compared. The results demonstrated that conjugated bilirubin, bilirubin, cholic acid, and glycocholic acid exhibited protective effect against oxidative stress damage, suggesting that they are the main active ingredients of Calculus Bovis [122].

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

Ingredient measurability is a necessary condition as a quality marker. The 2020 edition of the Chinese Pharmacopoeia [1] indicates that the components for determination of Notoginseng Radix, Calculus Bovis, Snake Gallbladder, and Musk are ginsenoside Rg1, ginsenoside Rb1, notoginsenoside R1, cholic acid, bilirubin, and muscone. Furthermore, an HPLC method for determination of the multi-index components of PTH was established, where the contents of ginsenoside Rb1, ginsenoside Rg1, notoginsenoside R1, and sodium taurocholate were simultaneously determined under the same conditions [75]. 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, glycodeoxycholic acid, deoxycholic acid, glycocholic acid, glycocholic acid, deoxycholic acid, chenodeoxycholic acid, taurine, and muscone were determined [78]. Furthermore, a DI-MS/MS [114] 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 Notoginseng Radix (16), Snake Gallbladder and Calculus Bovis (24), and Musk (1) were designated as sources [123]. 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 [124] (Fig. 6).

**Conclusion and Future Prospects**

PTH, a famous TCMP, has the characteristics of “multi-
component, multitarget, and extensive pharmacodynamic output; therefore, it is of great significance to clarify chemical components to reveal the scientific connotation. In this review, we summarized and discussed the pharmacological effects and clinical applications 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 theory. Ginsenosides Rb1, ginsenoside Rg1, ginsenoside Rd, ginsenoside Re, notoginsenoside R1, dencichine, bilirubin, biliverdin, taurocholic acid, and muscone were speculated as the Q-markers of PTH (Table 3). These findings provide strong support for quality control, investigation of pharmacological mechanisms, and clinical applications of PTH. However, it should be recognized that the Q-markers predicted in this study need further validation.

**Table 3 Information of Q-markers of PTH**

<table>
<thead>
<tr>
<th>Compound names</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>Notoginseng Radix</td>
</tr>
<tr>
<td>Ginsenoside Rg1</td>
<td>C_{42}H_{72}O_{14}</td>
<td>Notoginseng Radix</td>
</tr>
<tr>
<td>Ginsenoside Rd</td>
<td>C_{48}H_{82}O_{18}</td>
<td>Notoginseng Radix</td>
</tr>
<tr>
<td>Ginsenoside Re</td>
<td>C_{48}H_{82}O_{18}</td>
<td>Notoginseng Radix</td>
</tr>
<tr>
<td>Notoginsenoside R1</td>
<td>C_{47}H_{80}O_{18}</td>
<td>Notoginseng Radix</td>
</tr>
<tr>
<td>Dencichine</td>
<td>C_{47}H_{80}O_{18}</td>
<td>Notoginseng Radix</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>C_{53}H_{42}N_{2}O_{6}</td>
<td>Calculus Bovis, Snake Gallbladder</td>
</tr>
<tr>
<td>Biliverdin</td>
<td>C_{53}H_{42}N_{2}O_{6}</td>
<td>Calculus Bovis, Snake Gallbladder</td>
</tr>
<tr>
<td>Taurocholic acid</td>
<td>C_{26}H_{30}NO_{5}S</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>

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 support of the Ministry of Health of the People’s Republic of China (now the National Health Commission of the People’s Republic of China), substitution of natural Calculus Bovis and Musk by artificial ones is proposed. However, it is still unclear whether these artificial sources share the same chemical components and exert similar pharmacological effects compared to the natural ones. Systematic studies are needed to evaluate the probability of the substitution, where Q-markers can act as a useful tool. Clarification and validation of Q-markers will contribute to the overall pharmacological effects, and...
provide substantial assistance to uncover the underlying mechanisms.

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