Gut microbiota-mediated metabolism of *Panax notoginseng* saponins and its role in pharmacokinetics and pharmacodynamics

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**Author contributions**
Xuan Zeng and Wei-Wei Su contributed to the conceptualization of this study. Yu-Ying Zheng, Yu-Ling Liu and Wei-Jian Zhang completed the information retrieval and contributed to writing the original draft. Xuan Zeng was responsible for compilation and editing of the manuscript. All authors read and approved the final version of the manuscript.

**Competing interests**
The authors declare no conflicts of interest.

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**Abbreviations**
PNS, Panax notoginseng saponins; PPD, protopanaxadiol; PPT, protopanaxatriol; TCM, traditional Chinese medicine; NA, not available; ADME, absorption, distribution, metabolism, excretion, and toxicity; ADMET, absorption, distribution, metabolism, exclusion, and toxicity; TNF, tumour necrosis factor; CASP3, Caspase 3; AKT1, serine/threonine kinase 1; TP53, tumor protein P53.

**Citation**

**Abstract**
*Panax notoginseng* saponins (PNS) are a class of effective ingredients in *Notoginseng Radix et Rhizoma*, a well-known herbal medicine called San-Qi in Chinese. After oral administration, PNS inevitably interacts with gut microbiota, and thus affect the pharmacokinetic profiles and pharmacological effects. To date, studies concerning gut microbiota-mediated metabolism of PNS have not been reviewed systematically. Herein, we outline the metabolic profiles of *Panax notoginseng* saponins mediated by gut microbiota, as well as its role in the pharmacokinetics and pharmacodynamics on the basis of reported data. The metabolic pathways of primary saponins are proposed, and step-by-step deglycosylation is found to be the primary degradation pathways of PNS mediated by gut microbiota. Specific microorganisms and enzymes involved in the metabolic processes were summarized. Gut microbiota is deeply involved in the metabolism of PNS, affects the pharmacokinetic profiles, and produces a series of active metabolites. These metabolites were documented to play an essential role in the efficacy of the parent compounds. Future studies should focus on strengthening the real-world evidence, defining the interaction between gut microbiota and PNS, and developing the strategy for modulating gut microbiota to enhance the bioavailability and efficacy of PNS. This information would be useful for further research and clinical application of PNS.

**Keywords:** *Panax notoginseng* saponins; gut microbiota; metabolism; pharmacokinetics; pharmacodynamics
The metabolism pathways of Panax notoginseng saponins (PNS) mediated by gut microbiota were profiled, as well as its role in the pharmacokinetics and pharmacodynamics. Deglycosylation is the primary mode of PNS metabolism mediated by gut microbiota. Metabolites flowed from gut microbiota are crucial for PNS to exert pharmacological activities.

**Medical History of objective**

PNS are a kind of effective ingredients in *Notoginseng Radix et Rhizoma*, a well-known herbal medicine called San-Qi in Chinese. San-Qi was originally a herbal medicine used by minority ethnic groups in southwest China around 1500 CE., and was more widely accepted after being recorded in the *Compendium of Materia Medica* (Ben Cao Gang Mu in Chinese, 1578 C.E.) compiled by Li Shi-Zhen. Modern pharmacological studies showed that PNS possessed bioactivities in maintaining blood circulation, improving myocardial ischemia, anti-arrhythmia, anti-shock, sedation, improving intelligence, anti-aging, anti-oxidation, anti-cell proliferation and anti-tumor.

**Background**

*Notoginseng Radix et Rhizoma*, called San-Qi in Chinese, is the dried root and rhizome of *Panax notoginseng* (Burk) F. H. Chen. It was originally a herbal medicine used by minority ethnic groups in southwest China around 1500 CE., and was more widely accepted after being recorded in the *Compendium of Materia Medica* (Ben Cao Gang Mu in Chinese, 1578 C.E.) compiled by Li Shi-Zhen [1]. To date, more than 200 chemical components have been detected in *Panax notoginseng*, including amino acids, saponins, polysaccharides, flavonoids, volatiles, and acetylenic alcohols [2]. Among these compounds, saponins have been documented as the main effective ingredients. So far, over 70 saponins have been isolated from different parts of *Panax notoginseng* (root, stem, leaf, flower, seed, etc.), but the main components are ginsenoside Rg1, Rb1, Rd, Re, Rf, and notoginsenoside R1 [3, 4]. Most of these monomeric saponins can be classified into two types, including protopanaxadiol (PPD) type and protopanaxatriol (PPT) type. Ginsenoside Rb1 is the primary PPD-type saponin in San-Qi, while ginsenoside Re, Rg1, and notoginsenoside R1 are the main PPT-type saponins. The proportions of these four components in the total PNS are 30%, 2.5%, 20% and 2.5%, respectively [3]. Ginsenosides Rg1, Rb1, and notoginsenoside R1 have shown pharmacological effects in maintaining blood circulation, improving myocardial ischemia, anti-arrhythmia, anti-shock, sedation, improving intelligence, anti-aging, anti-oxidation, anti-cell proliferation and anti-tumor [5-8].

*Panax notoginseng* is widely used in a variety of oral traditional Chinese medicine (TCM) formulas, therefore contained saponins inevitably interacts with gut microbiota [9]. Gut microbiota is the most important and diverse microbial community in human body. The micro-ecosystem composed of gut microbes and intestinal environment is involved in regulating a series of physiological processes, such as digestion, absorption, development, and immune defense of the host [10, 11]. In recent years, studies have shown that the pharmacologic activities of drugs are not only related to the host itself, but also affected by the microbial community inhabiting the human body. TCM formulas are diverse and complex in composition, and their efficacy is usually related to the specific active metabolites generated in the body, rather than the original compounds. After oral administration, some TCM components are metabolized by intestinal microorganisms in the gastrointestinal tract, and their metabolites are absorbed into the blood to exert pharmacological effects [12]. In some cases, the bioavailability of some TCM components with low fat solubility increase under the metabolic action of gut microbiota [13]. In other cases, intestinal microorganisms can also play a role of reducing or increasing toxicity by changing the functional groups of some molecules [14]. In addition, changes in gut microbiota caused by environment can also affect the metabolism of some TCM ingredients, indicating the import role of gut microbiota in the pharmacokinetic profiles and pharmacological effects [15, 16].

To date, some in vitro and in vivo studies have been conducted to clarify the interaction between PNS and gut microbiota. However, these results have not been summarized systematically. Herein, we outline the metabolic profiles of PNS mediated by gut microbiota, as well as its role in the pharmacokinetics and pharmacodynamics, so as to provide reference for further research and clinical application of PNS.

**Materials and methods**


**Gut microbiota and drug metabolism**

Gut microbiota is a large and complex microecosystem. Due to its key role in metabolism, gut microbiota has been widely concerned as an “invisible organ” in recent years. Studies have shown that the adult intestinal cavity area is about 300–400 square meters, colonized by about $10^{13}–10^{14}$ microorganisms, including bacteria, archaea, fungi, protists, and viruses. Among them, bacteria are the main residents, and more than 99% are anaerobic bacteria [17]. Affected by host genetic background, dietary habits and environmental factors, gut microbiota is diverse and specific [18]. From the phylum level, gut microbiota is mainly composed of Firmicutes (60%-65%), Bacteroides (20%-25%), Proteobacteria (5%-10%), and Actinobacteria (approximately 3%) [19]. From the species level, there are dominant and secondary bacterial groups, among which the dominant bacterial groups in the intestinal tracts of healthy adults are mainly *Bifidobacterium*, *Lactobacillus*, and obligatory anaerobic bacteria such as *Spirillum*, *Peptostreptococcus*, *Bacteroides*, etc. [20]. These gut microbes play a very important role in the host physiological functions, including digestion of food, production of important metabolites, promotion of immune system development and maturation, maintenance of gastrointestinal homeostasis, influence of brain function and behavior, and protection of host from pathogen infection [21-24].

Gut microbiota could secrete a wide variety of enzymes and has great potential to transform glycosides, which are an important class of active components in TCM [25]. The free sugar moieties generated by hydrolysis can be used as carbon sources required for microbial growth and reproduction, thus producing more enzymes [26]. Therefore, different microorganisms can play an important role in the biotransformation process of natural products as biocatalysts [27, 28]. A number of studies have pointed out the important contribution of glycosidase, nitroreductase, azoreductase, urease, sulfatase and methylase secreted by intestinal microorganisms in the transformation of glycosides [29]. Among them, α-hamnosidase, β-glucosidase, β-glucuronidase, β-xilosidase, nitroreductase and other glycosidases are the most critical enzymes for the metabolism of saponins, and deglycosylation catalyzed by these glycosidases is the main pathway to hydrolyze glycosides [30, 31]. Metabolites yielded by deglycosylation are generally more easily absorbed by the intestine and thus have better bioavailability [32]. In addition, gut microbiota

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can also drive other metabolic reactions of saponins such as hydroxylation, dehydrogenation, dehydration and demethylation.

**Gut microbiota-mediated metabolism of PNS**

PNS have high polarity, low fat solubility and low oral bioavailability. Mediated by gut microbiota, most saponins can be metabolized to more absorbable active metabolites [33]. The metabolites of total PNS degraded by human gut microbiota were identified with in vitro incubation method [34]. A total of 45 metabolites were detected, among which ginsenoside F₁, PPT, ginsenoside Rb₁, ginsenoside C-K and PPD had the highest content, and corresponding pharmacological activities were all higher. Deglycosylation is the primary metabolism pathways of PNS mediated by gut microbiota, which also involves hydration, dehydroxylation, oxidation and so on. 

Ginsenoside Rg₁, ginsenoside Rb₁, and notoginsenoside R₁, which are three saponins with highest content in Panax notoginseng, were incubated with gut microbiota isolated from female and male Sprague-Dawley rats [35]. The results showed that the degradation of ginsenoside Rb₁ by rat intestinal flora was a slow and progressive process, and the metabolic capacity of male rats was stronger than that of female rats, which may be related to the different proportions of various bacteria in feces of different genders. However, ginsenoside Rg₁ and notoginsenoside R₁ contents remained relatively stable during incubation, suggesting that gut microbiota had different capacity to degrade saponins with different structures. In another study, saponins in Panax notoginseng aqueous extract were degraded by human gut microbiota to produce metabolites PPD and PPT under in vitro incubation, while degradation product of rat gut microbiota were only PPT [36]. These results indicated the species differences of gut microbiota-mediated metabolism of PNS, but did not determine whether there are differences in metabolic pathways.

Influenced by diet and disease, the abundance of specific bacteria in the gut microbiota fluctuates dynamically, leading to changes in metabolic capacity of saponins. The metabolic rate of ginsenoside Rg₁ and ginsenoside Rb₁ in the gut microbiota of patients with liver cancer was significantly lower than that of healthy subjects, suggesting that changes in the intestinal microbes of patients with liver cancer had a significant impact on the metabolic rate of above-mentioned two ginsenosides [37]. In addition, different incubation conditions also had certain effects on the metabolic capacity of saponins mediated by gut microbiota. For example, Qian et al. compared the metabolism difference of ginsenoside Rb₁ in the anaerobic and aerobic conditions by step-by-step deglycoside under the action of rat gut microbiota [38]. The metabolic pathway of ginsenoside Rb₁ was proposed as Rb₁→Rd→F₁→C-K. There was no difference in the metabolic types of aerobic and anaerobic incubation, but the metabolic rate of aerobic incubation was faster than that of anaerobic condition. The Rd→F₂ process was considered to be the rate-limiting step of deglycosylation of Rb₁ mediated by gut microbiota.

**Table 1**

**Summary of specific microorganisms and enzymes involved in the metabolism of PNS**

<table>
<thead>
<tr>
<th>No.</th>
<th>Parent component</th>
<th>Microbial metabolite</th>
<th>Relate microorganism</th>
<th>Relate enzymes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total saponins</td>
<td>Ginsenoside C-K</td>
<td>Fusarium sp.</td>
<td>NA</td>
<td>[41, 49]</td>
</tr>
<tr>
<td>2</td>
<td>Total PNS</td>
<td>Ginsenoside Rb₁</td>
<td>Bifidobacterium adolescentis, Lactobacillus rhamnosus</td>
<td>NA</td>
<td>[50]</td>
</tr>
<tr>
<td>3</td>
<td>Ginsenoside Rb₁</td>
<td>Ginsenoside C-K</td>
<td>Ruminococcus sp., Bacteroides sp., Bifidobacterium longum H-1</td>
<td>β-Glucosidase</td>
<td>[51, 52]</td>
</tr>
<tr>
<td>4</td>
<td>Ginsenoside Rb₁</td>
<td>Gypenoside XVII</td>
<td>Bacillus sp., Fusobacterium K-60</td>
<td>NA</td>
<td>[44, 45, 53]</td>
</tr>
<tr>
<td>5</td>
<td>Ginsenoside Rb₁</td>
<td>Ginsenoside Rd</td>
<td>Bifidobacterium animalis GM1, Aspergillus niger, Penicillium sp., Bacillus sp., Lactobacillus sp., Eubacterium sp., Bifidobacterium longum</td>
<td>β-Glucosidase</td>
<td>[15, 39, 40, 42-45, 54, 55]</td>
</tr>
<tr>
<td>6</td>
<td>Ginsenoside Rb₁</td>
<td>Ginsenoside F₁</td>
<td>Bacillus sp.</td>
<td>NA</td>
<td>[44, 45]</td>
</tr>
<tr>
<td>7</td>
<td>Ginsenoside Rb₁</td>
<td>Ginsenoside Rg₁</td>
<td>Bacillus sp., Lactococcus lactis, Thermotoga thermarum</td>
<td>β-Glucosidase</td>
<td>[44-48]</td>
</tr>
<tr>
<td>8</td>
<td>Ginsenoside Rb₁</td>
<td>Ginsenoside Rb₂</td>
<td>Bifidobacterium breve K-110</td>
<td>β-Xylosidase</td>
<td>[56]</td>
</tr>
<tr>
<td>9</td>
<td>Ginsenoside Rb₁</td>
<td>Ginsenoside Rd</td>
<td>Bifidobacterium sp., Lactobacillus sp.</td>
<td>NA</td>
<td>[54]</td>
</tr>
<tr>
<td>10</td>
<td>Ginsenoside Rc</td>
<td>Ginsenoside Rd</td>
<td>Bifidobacterium sp., Lactobacillus sp.</td>
<td>NA</td>
<td>[54]</td>
</tr>
<tr>
<td>11</td>
<td>Ginsenoside Rb₁</td>
<td>Ginsenoside Rd</td>
<td>Aspergillus niger</td>
<td>NA</td>
<td>[39, 40]</td>
</tr>
<tr>
<td>12</td>
<td>Ginsenoside Rd</td>
<td>Ginsenoside F₁</td>
<td>Aspergillus niger, Penicillium sp., Bifidobacterium sp., Lactobacillus sp., Fusobacterium sp., Bifidobacterium K-103, Bacteroides JJ-6</td>
<td>NA</td>
<td>[39, 40, 42, 43, 53, 54]</td>
</tr>
<tr>
<td>13</td>
<td>Ginsenoside Rd</td>
<td>Ginsenoside Rg₁</td>
<td>Lactococcus lactis, Thermotoga thermarum, Bacteroides sp., Microbacterium sp.</td>
<td>β-Glucosidase</td>
<td>[46-48, 54]</td>
</tr>
</tbody>
</table>

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Table 1 Summary of specific microorganisms and enzymes involved in the metabolism of PNS (continued)

<table>
<thead>
<tr>
<th>No.</th>
<th>Parent component</th>
<th>Microbial metabolite</th>
<th>Relate microorganism</th>
<th>Relate enzymes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Ginsenoside Rg3</td>
<td>Ginsenoside Rh3</td>
<td>Eubacterium sp., Fusobacterium sp., Bacteroides sp.</td>
<td>β-Glucosidase</td>
<td>[16, 54]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Aspergillus niger, Penicillium sp., Bifidobacterium breve, Bifidobacterium sp., Bifidobacterium K-103, Lactobacillus sp., Eubacterium sp., Fusobacterium sp., Bacteroides sp., Bacteroides JY-6</td>
<td>NA</td>
<td>[39, 40, 42-44, 53, 54]</td>
</tr>
<tr>
<td>15</td>
<td>Ginsenoside F2</td>
<td>Ginsenoside C-K</td>
<td>Rosebacteria sp., Bifidobacterium sp.</td>
<td>β-Glucosidase</td>
<td>[54, 57]</td>
</tr>
<tr>
<td>16</td>
<td>Ginsenoside C-K</td>
<td>PPD</td>
<td>Bacteroides sp.</td>
<td>NA</td>
<td>[54]</td>
</tr>
<tr>
<td>17</td>
<td>Ginsenoside Rh2</td>
<td>PPD</td>
<td>Bacteroides sp.</td>
<td>NA</td>
<td>[54]</td>
</tr>
</tbody>
</table>

**PPT type saponins**

<table>
<thead>
<tr>
<th>No.</th>
<th>Parent component</th>
<th>Microbial metabolite</th>
<th>Relate microorganism</th>
<th>Relate enzymes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Notoginsenoside R1</td>
<td>Dehydrogenated, oxidative, and demethylated metabolites</td>
<td>Bacteroides sp.</td>
<td>Redox enzymes</td>
<td>[58]</td>
</tr>
<tr>
<td>19</td>
<td>Ginsenoside Re</td>
<td>Ginsenoside Rg1</td>
<td>Bifidobacterium K-103, Bacteroides JY6, Bacteroides HJ15, Eubacterium sp., Fusobacterium sp., Bacteroides sp.</td>
<td>NA</td>
<td>[54, 59]</td>
</tr>
<tr>
<td>20</td>
<td>Ginsenoside Re</td>
<td>Ginsenoside Rh3, Ginsenoside F1, PPT</td>
<td>Bacteroides JY-6</td>
<td>NA</td>
<td>[59]</td>
</tr>
<tr>
<td>21</td>
<td>Ginsenoside Rg1</td>
<td>PPT</td>
<td>Fusobacterium K-60</td>
<td>NA</td>
<td>[52, 60]</td>
</tr>
<tr>
<td>22</td>
<td>Ginsenoside Rg1</td>
<td>Ginsenoside F1</td>
<td>Bifidobacterium K-103, Bacteroides JY6, Bacteroides HJ15, Fusobacterium sp., Bacteroides sp.</td>
<td>NA</td>
<td>[54, 59]</td>
</tr>
<tr>
<td>23</td>
<td>Ginsenoside Rg1</td>
<td>Ginsenoside Rh1</td>
<td>Bifidobacterium K-103, Bacteroides JY6, Bacteroides HJ15, Bifidobacterium sp., Fusobacterium sp., Bacteroides sp.</td>
<td>NA</td>
<td>[54, 59]</td>
</tr>
<tr>
<td>24</td>
<td>Ginsenoside Rf</td>
<td>Ginsenoside Rh1</td>
<td>Bifidobacterium sp., Bacteroides sp.</td>
<td>NA</td>
<td>[54]</td>
</tr>
<tr>
<td>25</td>
<td>Ginsenoside Rh1</td>
<td>PPT</td>
<td>Bifidobacterium sp., Bacteroides sp.</td>
<td>NA</td>
<td>[54]</td>
</tr>
<tr>
<td>26</td>
<td>Ginsenoside F1</td>
<td>PPT</td>
<td>Bacteroides sp.</td>
<td>NA</td>
<td>[54]</td>
</tr>
<tr>
<td>27</td>
<td>PPT</td>
<td>Dehydropropanaxtriol</td>
<td>Oscilloba sp., Phascolarctobacterium sp.</td>
<td>NA</td>
<td>[34]</td>
</tr>
</tbody>
</table>

NA, not available; PNS, Panax notoginseng saponins; PPD, protopanaxadiol; PPT, protopanaxatriol.

In general, step-by-step deglycosylation is the primary metabolic pathway of PNS, metabolizing into secondary saponins and aglycones to exert pharmacological effects [61, 62]. Based on reported studies, the metabolic pathways of PPD-type and PPT-type saponins mediated by gut microbiota were plotted in Figure 1, 2, respectively [49, 63, 64].

**Role of gut microbiota on the pharmacokinetics of PNS**

Pharmacokinetic studies aim to understand the behavior of drugs in vivo, including absorption, distribution, metabolism, and excretion. PNS will inevitably interact with gut microbiota after oral ingestion, which will affect the absorption and metabolism profiles of saponins. Herein, we evaluated the effects of gut microbiota mediated metabolism on the pharmacokinetic properties of PNS using chemometrics approach. Based on database resources, the molecular properties of ginsenoside Rb1, PPD, notoginsenoside R1, and PPT were listed in Table 2 [65, 66]. Through the deglycosylation mediated by gut microbiota, the LogP value, solubility, surface area and polarity parameters of PNS were greatly transformed, which led to the change of pharmacokinetic properties.

Pseudo germ-free rodents are an effective strategy for evaluating the role of gut microbiota in metabolic processes in vivo, typically through antibiotic intervention to deplete their resident microbiota. Specifically, normal control and pseudo germ-free rats were used to investigate the in vivo metabolic profiles of PNS mediated by gut microbiota [13]. A total of 73 metabolites were identified in the feces of normal control rats, while only 11 PNS metabolites were detected in pseudo germ-free rats, highlighting the profound role of gut microbiota on the metabolism of PNS. In another study, a rat model of gut microbiota disturbance was established by lincomycin intervention, so as to investigated its effects on the pharmacokinetics of ginsenoside Rg3 [16]. Compared with the normal control group, the Cmax and area under the curve of ginsenoside Rg3 were significantly reduced in the dysbiosis rats. This may be due to the decrease of β-glucosidase activity in gut microbiota of rats treated with lincomycin, which affected the deglycosylation of ginsenoside Rg3 and then changed the pharmacokinetic behavior of ginsenoside Rg3 and ginsenoside Rb1.

These results suggest that the metabolism of PNS was found to be greatly affected by gut microbiota. For example, Proteobacteria may affect the deglycosylation of PNS by regulating the activity of glycosidase, and Bacteroides may promote the oxidation-reduction metabolism of PNS by improving the activity of corresponding enzymes [58]. In addition, individual differences in PNS metabolism have been observed to be related to the composition and abundance of gut microbiota [15]. The abundance of Bacteroidetes S24-7, Alcaligenaceae, and Erysipelotrichaceae were higher in the gut microbiota of individuals with fast metabolism. *Bifidobacterium animalis* GM1 was further isolated, which could convert ginsenoside Rb1 to ginsenoside Rd. These specific intestinal bacteria was deemed as the dominated microorganism in the metabolism of PNS. And the abundance changes of these gut microbiota can modulate the metabolism of ginsenosides in intestine, and subsequently affect the pharmacokinetic profiles.

**Role of gut microbiota on the pharmacodynamics of PNS**
The pharmacological activity of the compound is closely related to its structure. Mediated by gut microbiota, PNS underwent deglycosylation, dehydrogenation, oxidation, dehydroxylation, and other reactions, producing a series of metabolites with different activities from the parent compounds [67]. A number of studies have proved that PNS metabolites generated through the deglycosylation mediated by gut microbiota have stronger pharmacological activity [68].

Ginsenoside Rb1 and PPD were defined as the main metabolites of ginsenoside Rg1 and found to inhibit the proliferation of tumor cells with in vitro experiments [69]. Niu et al. determined the anti-tumor activity of ginsenosides Rb1 and its metabolites on A/J mouse metastatic lung cancer LM1 cells [70]. The metabolites ginsenosides F2 and CK were found to have significant anti-tumor activity, while prototype ginsenosides Rb1 did not have such activity. Another in vitro experiments confirmed that ginsenoside Rb1 has no obvious anti-tumor effect on human colon cancer HT-29 cells, but its deglycosized product ginsenoside CK has a strong anti-tumor effect [71]. Herein, Ginsenoside C-K, the metabolite of PPD-type saponins, has stronger anti-tumor activity in vivo and in vitro than that of parent compounds [27]. Similar results were observed for PPT-type saponins. Lee et al. compared the anti-tumor activity of ginsenoside Rg1, Re, and their metabolites PPT under different administration strategies [72]. All three ginsenosides were found to have strong anti-tumor cell metastasis effects after oral administration, while only PPT has anti-tumor activity when administered intravenously. The results indicated that the anti-tumor effect of ginsenoside Rg1, and Re after oral administration was contributed by their metabolites PPT.

Table 2 The molecular properties of ginsenoside Rb1, PPD, notoginsenoside R1, and PPT

<table>
<thead>
<tr>
<th>Molecular properties</th>
<th>Glnosides Rb1</th>
<th>PPD</th>
<th>Notoginsenoside R1</th>
<th>PPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALogP</td>
<td>−1.198</td>
<td>5.789</td>
<td>−0.11</td>
<td>4.62</td>
</tr>
<tr>
<td>Molecular solubility</td>
<td>−6.095</td>
<td>−8.328</td>
<td>−5.424</td>
<td>−7.19</td>
</tr>
<tr>
<td>Molecular volume</td>
<td>773.12</td>
<td>372.15</td>
<td>663.36</td>
<td>374.89</td>
</tr>
<tr>
<td>Molecular surface area</td>
<td>1077.6</td>
<td>500.93</td>
<td>917.35</td>
<td>513.89</td>
</tr>
<tr>
<td>Molecular polar surface area</td>
<td>377.29</td>
<td>60.69</td>
<td>298.14</td>
<td>80.92</td>
</tr>
<tr>
<td>ADMET absorption level</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>ADMET blood-brain barrier level</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>ADMET solubility</td>
<td>−8.235</td>
<td>−5.769</td>
<td>−6.783</td>
<td>−4.532</td>
</tr>
</tbody>
</table>

PPD, protopanaxadiol; PPT, protopanaxatriol; ADMET, absorption, distribution, metabolism, excretion, and toxicity.

Figure 1 Proposed metabolism pathways of PPD-type saponins mediated by gut microbiota
In vitro incubation experiments showed that PNS had no obvious inhibitory effect on Akkermansia and Bacteroides, but the main metabolite generated by gut microbiota, ginsenoside C-K could inhibit the growth of Bacteroides hetaoatomicon and Bacteroides virgatus [73]. Ginsenoside C-K has therapeutic effects on colitis and is one of the pharmacodynamic substances of PNS metabolized by gut microbiota. In another study, ginsenoside Rh₃, a metabolite of ginsenoside Re degraded by gut microbiota, was found to be more quickly absorbed into the body to exert estrogen-like effects [59]. These studies indicate that PNS metabolites produced by gut microbiota have a variety of pharmacological activities, and play an essential role in the efficacy of the parent compounds.

In addition, taking insenoside Rh₂, PPD, notoginsenoside R₁, and PPT as examples, we evaluated the effects of gut microbiota mediated deglycosylation on the pharmacodynamic properties using network pharmacology and molecular docking strategies [74, 75]. The corresponding targets of mentioned four compounds were obtained from SwissTargetPrediction (http://www.swisstargetprediction.ch/), and the potential targets of PNS for disease treatment were acquired from DisGeNET (https://www.disgenet.org/search) using the keywords of “Angina pectoris”, “Coronary arteriosclerosis”, “Coronary heart disease”, and “Coronary thrombosis”. There were 410 targets corresponding to the four compounds and 1,819 disease-related targets, among which 159 targets were common (Supplementary Figure S1). The protein-protein interaction network was constructed using the String (https://string-db.org/) in combination with Cytoscape, so as to identify the crucial targets. Subsequently, to reveal the interactions between mentioned four compounds and target proteins at the molecular level, molecular docking was performed on five crucial targets, i.e. TNF (tumour necrosis factor), CASP3 (Caspase 3), AKT1 (serine/threonine kinase 1), TP53 (tumor protein P53), and STAT3 (signal transducer and activator of transcription 3) (Supplementary Table S1). The molecular docking was conducted with Autodock Tools (Version 1.4.6). Calculated docking affinity were listed in Supplementary Table S2. The docking scores of ginsenoside Rh₃, protopanaxadiol, notoginsenoside R₁ and protopanaxatriol to TNF, CASP3, AKT1, TP53, and STAT3 proteins were ≤ -5.0 kJ/mol, indicating that mentioned four compounds bind closely to the crucial target proteins. A lower docking score suggests a stronger binding force. The docking affinity of protopanaxadiol to TNF, CASP3, AKT1, and TP53 were lower than that of ginsenoside Rh₂, especially the TNF. Similar results were also observed in protopanaxatriol and notoginsenoside R₁, which again confirmed the effect of deglycosylation mediated by gut microbiota on the bio-activities of PNS. The molecular spatial structure changes after deglycosylation make the compound bind more closely to some target proteins and show better biological activity. The 3D diagrams of molecular docking for ginsenoside Rh₂, notoginsenoside R₁, protopanaxadiol, and protopanaxatriol to TNF were shown in Supplementary Figure S2. The docking affinity of PPD to TNF, CASP3, AKT1, and TP53 were lower than that of ginsenoside Rh₂, suggesting a stronger binding force. Similar results were also observed in PPT and notoginsenoside R₁, which again confirmed the effect of deglycosylation mediated by gut microbiota on the bio-activities of PNS. The molecular spatial structure changes associated with deglycosylation make the compound bind more closely to some target proteins and show better biological activity. Therefore, future studies pharmacodynamic studies of PNS should fully take into account the role of gut microbiota.

Summary and future prospects

Herein, the metabolic profiles of PNS mediated by gut microbiota were summarized systematically, as well as its role in the pharmacokinetics and pharmacodynamics. Mediated by gut microbiota, PNS were extensively engaged in deglycosylation, giving rise to a series of metabolites. Gut microbiota could modulate the metabolism of PNS in the intestine, and then affect their pharmacokinetic and pharmacodynamic profiles. However, there were some issues to be settled in the future.

Firstly, current researches on PNS metabolism mediated by gut microbiota were mainly conducted in vitro models or rodent animals, as well as other studies on pharmacokinetics and pharmacodynamics. The real-world evidence about these biological processes in the human body is limited. For example, the differences in PNS metabolism among different enterotype microbiota are not yet clear, and the effects of gastrointestinal diseases and antibiotic interventions on the pharmacokinetics and pharmacodynamics of PNS are also uncertain.

Secondly, in-depth and systematic studies are necessary to define the interaction between gut microbiota and PNS. Germ-free rodent animals have been used to investigate the role of gut microbiota on the pharmacokinetics and pharmacodynamics of PNS, but the main focus is on the impact of gut microbiota abundance. Information concerning the effects of specific microorganisms or enzymes on the metabolism of certain saponins is still scarce, as well as corresponding regulatory mechanisms. Meanwhile, more researches are called for defining the modulation effects of PNS on gut microbiota, especially on its impacts on pharmacokinetics and efficacy after long-term ingestion. Given that PNS metabolites produced by gut microbiota have a variety of pharmacological activities, follow-up studies should pay more attention to the role of gut microbiota when exploring the
pharmacological mechanism of PNS.
Thirdly, there is a lack of precise strategies for regulating gut microbiota to enhance the bioavailability and efficacy of PNS. At present, probiotics and prebiotics have been used to improve the gut microbiota of patients, but their effects on PNS metabolism and efficacy are still unclear. It is worth exploring how modulating gut microbiota through convenient and safe intervention strategies, so as to overcome the low bioavailability of PNS and improve treatment effectiveness.

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