Enhancement of oral bioavailability and immune response of Ginsenoside Rh2 by co-administration with piperine

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[ABSTRACT] Ginsenoside Rh2 (Rh2) is one of the major bioactive ginsenosides in Panax ginseng. However, the oral bioavailability of Rh2 is low, with P-glycoprotein (P-gp) and CYP3A4 being reported to be the main factors. The purpose of the present study was to determine the enhancing effect of piperine on the oral bioavailability as well as bioactivity of Rh2. The inhibitory effect of piperine on P-gp and CYP3A4 was determined using a Caco-2 monolayer model and a recombinant CYP3A4 metabolic system, respectively. The pharmacokinetics of oral Rh2 (10 mg·kg\(^{-1}\)) administered alone or in combination with piperine (10 and 20 mg·kg\(^{-1}\)) was performed in rats. The immune boosting effect of Rh2 was assessed in rats by measuring IL-12 level after treated by Rh2 alone or co-administered with piperine. The results indicated that piperine significantly increased the permeability of Rh2 and inhibited the metabolism of Rh2. The pharmacokinetic study results showed that the AUC of Rh2 was significantly increased in combination with piperine at high dose (20 mg·kg\(^{-1}\)) when compared to the control group, with relative bioavailability of 196.8%. The increase of Rh2 exposure led to increased serum levels of IL-12. In conclusion, piperine may be used as a bioenhancer to improve pharmacological effect of Rh2 when given orally.

[KEY WORDS] Panax Ginseng; Ginsenoside Rh2; Piperine; Immune modulation; Bioenhancer

[Introduction] Radix Ginseng, a very popular traditional herbal medicine used in eastern Asia for thousands of years, has been utilized to modulate immune system and ameliorate pathological hemostasis \([1]\). Ginsenosides are the main active components of ginseng, and more than 100 ginsenosides have been identified so far \([2]\). Ginsenoside Rh2 (Rh2) is one of the major active ginsenosides and possesses multiple functions such as anti-inflammation, anti-proliferation, and immune modulation via suppressing NF-xB and JNK signaling pathways \([3-5]\).

However, the oral bioavailability of Rh2 has been found to be low (less than 5%) and the underlying mechanisms have been extensively studied by many research groups \([6-8]\). P-glycoprotein mediated efflux is reported to be one possible reason, although there is controversy over this conclusion. Shi and co-workers have reported that Rh2 is not a substrate but an inhibitor of P-glycoprotein (P-gp) in an in vitro cell line model \([7]\). While in another study, Yang and co-workers have found that Rh2 is a substrate of P-gp with high affinity and Rh2 plasma concentrations are increased significantly in the P-gp knockout mice, compared with that of wildtype mice \([6]\). The discrepancy between these two studies may be due to the different P-gp expression levels in Caco-2 cells among laboratories. The results from the in vivo animal study are considered to be more convincing and reliable. Thus P-gp is very likely to be the main factor that reduces the oral absorption of Rh2 through intestinal epithelia.

In addition to P-gp, CYP3A4 has been found to be the predominant enzyme involved in the oxidative metabolism of Rh2, indicating that first-pass extraction may also further reduce the bioavailability of Rh2 \([9]\). Many studies have been carried out to increase the bioavailability of Rh2 \([6, 10]\). Because P-glycoprotein and CYP3A4 are considerably important for Rh2 absorption when...
given orally, the inhibitor of CYP3A4 and P-gp might be able to increase Rh2 absorption when given in combination with Rh2. Although many CYP3A4 and P-gp inhibitors have been reported, most of them are clinical medications or have not been approved for human use. Ideally, absorption enhancers should be safe with reversible effect [11]. The substances from nutraceuticals and dietary supplements offer a good source for such absorption enhancers. Piperine, an alkaloid obtained from *Piper longum* (Piperaceae), commonly used as a spice and adjuvant in various traditional medicines, has been known as a bioavailability-enhancer by inhibiting multiple drug metabolic enzymes and P-gp [12]. The co-administration of piperine and docetaxel results in improved anti-tumor effect in an *in vivo* xenograft mice model of Castrate-resistant prostate cancer (CRPC) by inhibiting CYP3A4 activity [13]. Piperine increases the permeability of rifampin and phenytoin, which are P-gp substrates by inhibiting P-gp [14].

Therefore in the present study, piperine was selected to increase the bioavailability of Rh2. The permeability enhancing effect was determined using the Caco-2 monolayer model, while the CYP3A4 inhibitory effect was evaluated using an *in vitro* rCYP3A4 reaction system. A rat pharmacokinetic study was further conducted to verify the enhancement effect on Rh2 bioavailability by co-administration of piperine.

Many literatures have reported that ginseng regulates immune responses through the modulation of cytokine secretion. Long-term oral administration of the ginseng extract appears to potentiate the immune response [15-16]. In the present study, the IL-12 level was measured and compared in rats following multiple doses of Rh2 alone or in combination with piperine.

**Materials and Methods**

**Materials**

Piperine was purchased from National Institute for Food and Drug Control (Beijing, China), while 20(S)-Ginsenoside Rh2 obtained from Shanghai Source Leaf Biological Technology Co., LTD. (Shanghai, China). Caco-2 cell line was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Cell culture medium RPMI-1640, DMEM, fetal bovine serum (FBS), pyruvic acid, non-essential amino acids, penicillin-streptomycin, and 0.25% trypsin-EDTA solution were purchased from Invitrogen Corp. (Carlsbad, CA, USA). Human recombinant CYP3A4 (rCYP3A4) was obtained from BD Biosciences (San Jose, CA, USA).

**CYP3A4 inhibition assay**

The metabolic study of Rh2 was conducted using the recombinant cytochrome CYP3A4 enzyme (rCYP3A4) in a reaction mixture containing an NADPH-generating system (3.3 mMol·L⁻¹ of G-6-P, 1.3 mMol·L⁻¹ of NADP, 3.3 mMol·L⁻¹ of MgCl₂, and 0.4 U of G-6-PDH, and 25 μg·mL⁻¹ of alamethicin). The final concentration of rCYP3A4 was 0.5 mg·mL⁻¹. The reaction mixture was preincubated for 2 min at 37 °C. The stock solution of Rh2 was then added to the reaction buffer to final concentration of 1 μmol·L⁻¹ in presence of piperine at concentrations of 5, 20, and 50 μmol·L⁻¹. The reaction was quenched by the addition of 3 volume of methanol after 60-min incubation. The incubation mixture was vortexed for 1 min and then centrifuged at 10 000 r·min⁻¹ for 5 min.

The residual Rh2 concentrations were detected using an HPLC-MS/MS method. Briefly, to 0.1 mL of solution, 0.1 mL of methanol and 10 μL of internal standard (docetaxel, IS, 1 μg·mL⁻¹) were added and mixed well. After centrifuging at 10 000 min⁻¹ for 5 min, 10 μL of the supernatant was injected onto the HPLC-MS/MS system for analysis. The HPLC-MS/MS system consists of an API 3000 mass spectrometer (AB Scix, Foster City, CA, USA) coupled with an HPLC system consisted of a binary SPD-10Avp pump (Shimadzu, Kyoto, Japan) and a CTC autosampler (CTC Analytics AG, Zwingen, Switzerland). Chromatographic separation was performed using a Zorbax SB C18 column (150 mm × 2.1 mm, 5 μm, Zorbax, Agilent, Santa Clara, CA, USA). The mobile phases were (A) acetonitrile and (B) 0.1% formic acid containing 2 mMol·L⁻¹ of ammonium acetate. A linear gradient elution was carried out with flow rate of 0.3 mL·min⁻¹. The gradient began with 30% eluent A and 70% eluent B for 3 min. The ratio was changed linearly to 20% eluent B in 2 min and remained at 20% eluent B for 3 min. The gradient was then changed back to 70% eluent B in 0.5 min and kept at this percentage for 2 min. The autosampler was set at room temperature and the injection volume was 10 μL. Multiple reaction monitoring (MRM) scanning in positive ionization mode was used to quantify Rh2 by monitoring the transition of m/z 622.9–408.2 for Rh2 and 830.1–304.2 for IS, respectively.

To determine enzyme kinetic parameters, different concentrations of Rh2 (0.01 to 2 μmol·L⁻¹) were incubated in presence of 0, 5, 20, and 50 μmol·L⁻¹ of piperine for 30 min. The reaction system was the same as mentioned above. The velocity of reaction was expressed as the amount of Rh2 disappearance for a unit of time. The samples were prepared in triplicate.

\[ V_{\text{max}} \] and \[ K_m \] values were calculated using Michaelis-Menten curve with aid of GraphPad Prism (Version 6.0, San Diego, CA, USA). The apparent dissociation constants of the enzyme-inhibitor complex (Ki) of piperine were determined using non-competitive inhibition model [17].

**Determination of permeability of Rh2 in presence of piperine**

The enhancement on Rh2 permeability by piperine was evaluated using a Caco-2 monolayer model. Caco-2 cells were seeded onto the Transwell® inserts (Corning Costar Co., Corning, NY, USA) at a density of about 3 × 10⁵ cells/well and cultured for 21 days. Transepithelial electrical resistance was measured to monitor the integrity of the monolayer. The permeability studies were performed in transport buffer containing phosphate buffer saline (PBS) (pH 7.4) with supplement of calcium (0.9 mMol·L⁻¹) and potassium chloride (0.9 mMol·L⁻¹). Rh2 was initially dissolved in ethanol and diluted to PBS to final concentration of 10 μmol·L⁻¹. The
permeability of Rh2 was tested in presence of piperine with concentrations of 5, 20, and 50 μmol·L⁻¹. The loading buffer was then pre-warmed and added to the loading chamber. At different time intervals (0, 15, 30, 60, and 90 min), 0.2 mL of solution from basal site (bottom) was precisely withdrawn and replaced with equal amount of fresh PBS. Rh2 concentrations were analyzed using the HPLC-MS/MS method as mentioned above.

The apparent permeability coefficient (Papp, cm·s⁻¹) was calculated as following equation.

\[
P_{app} = \frac{dc/dt \times t}{A \times C_0}
\]

where “dc/dt” is the change of the drug concentration in the receiver chambers versus time, “V” is the volume of the solution in the receiver chambers (cm³); “A” represents the membrane surface area (4.7 cm²); while “C₀” is the initial concentration in the donor chamber.

**Animal studies**

The animal studies were approved by the Institutional Animal Care and Use Committee of Sichuan University (Chengdu, Sichuan, China).

**Pharmacokinetic study**

The pharmacokinetics of Rh2 following oral administration alone or in combination with piperine was carried out in cannulated Sprague-Dawley (SD) rats (Animal Facility at Sichuan University, Chengdu, China) weighing 180 to 220 g (8 to 10 weeks). The rats were housed one per cage at room temperature (22 ± 1 °C) with 50%-60% relative humidity and an automatic day-night rhythm (12 h-12 h cycle).

Rh2 was dissolved in 50% ethanol (50 : 50, V/V) and diluted using same amount of saline to generate a concentration of 10 mg·mL⁻¹ right before dosing. Piperine was dissolved in PEG400 and diluted in saline to generate a concentration of 2 mg·mL⁻¹. Heparin was dissolved using saline to generate a concentration of 2 mg·mL⁻¹ (200 IU·mL⁻¹). The heparin solution was further diluted to 40 IU·mL⁻¹ and filter sterilized which was used as the block solution.

Prior to each experiment, eighteen rats were fasted overnight but with free access to tap water and randomly divided into three groups (n = 6). The rats were then undergone jugular catheter implantation surgery. The rats received one of the following three treatments: (1) PO (oral gavage) dose of 10 mg·kg⁻¹ Rh2; (2) PO dose of 10 mg·kg⁻¹ Rh2 in combination with 10 mg·kg⁻¹ piperine, and (3) PO dose of 10 mg·kg⁻¹ Rh2 in combination with 20 mg·kg⁻¹ piperine. Piperine was given 15 min before dosing Rh2. The 15-min time interval was determined based on a preliminary pharmacokinetic study, which indicated that 15-min time interval resulted in higher plasma concentration of Rh2. The rats were kept fasted for another 2 h but have access to tap water.

The blood samples were collected at 0, 0.083, 3, 0.25, 0.5, 1, 2, 3, 4, 6, and 8 h post Rh2 dose. The blood samples were drawn via cannula and placed into a centrifuge tube prefilled with 10 μL of heparin (200 IU·mL⁻¹). The cannula was flushed with 300 μL of saline and blocked with 100 μL of heparin (40 IU·mL⁻¹). The plasma was separated from the blood by centrifuging the blood samples at 10 000 r·min⁻¹ for 5 min. All the plasma samples were stored at −80 °C until analysis. The Rh2 concentration in plasma was determined using the HPLC-MS/MS method above with slight modification. To 0.1 mL of plasma sample, 10 μL of IS working solution was added followed by addition of 0.3 mL of tertiary butyl methyl ether. The mixture was vortexed for 3 min. The upper organic layer was separated and evaporated to dryness. The residue was reconstituted using 0.1 mL of 80% acetonitrile and 10 μL was injected into an HPLC-MS/MS system for quantification. The method was linear over a concentration range of 10 to 500 ng·mL⁻¹ with precision and accuracy of < 12.8% and 89.2%–109.8%, respectively.

The plasma concentration-time data were analyzed by a non-compartmental method. The following pharmacokinetic parameters were calculated. Cmax (peak drug concentration) and Tmax (time to peak drug concentration) were obtained directly from the original concentration-time data. AUC₀–₈h (area under the concentration–time curve from time zero to 8 h) was calculated using a linear/log trapezoidal method, while AUC₀–∞ (area under the concentration-time curve from time zero to infinity) was calculated as \( AUC_{0-\infty} = AUC_{0-8h} + C_{last}/K_e \), where \( K_e \) refers to the terminal phase elimination rate constant and \( C_{last} \) the concentration of last plasma sample. \( T_{1/2} \) (terminal elimination half-life) was calculated as 0.693/\( K_e \). CL/F (oral clearance) was calculated as Dose/AUC.

**Assays for immune modulation effects**

Of the numerous cytokines produced by the immune system, interleukin-12 (IL-12) possesses unique and distinctive features such as activation of cellular responses [16]. Interleukin-12 (IL-12) has an essential role in regulating inflammatory responses, innate resistance to infection, and adaptive immunity. Endogenous IL-12 is found required for resistance to many pathogens as well as transplanted and chemically induced tumors [15]. Ginseng extract could modulate the immune response by induction of interleukin-12 production [18]. Thus in the present study, IL-12 level was used as a pharmacodynamics marker to assess Rh2 activity in rat immune system.

In the present study, 24 healthy SD rats were used (weighing180–220 g and 8–10 weeks). The animals were kept in individual cages and fed ad libitum. The rats were randomly divided into four groups (n = 6) and received one of the following treatments for two weeks: (1) control group (saline), (2) Rh2 group (PO Rh2, 10 mg·kg⁻¹ daily), (3) piperine group (PO piperine, 20 mg·kg⁻¹, daily), and (4) the drug combination group (PO Rh2 10 mg·kg⁻¹ + piperine 20 mg·kg⁻¹, daily). For the drug combination group, piperine was given 15 min before Rh2. On the 14th days of the study, the blood samples were collected from the tail vein and serum samples were separated for measurement of IL-12. The IL-12 level was measured using a rat IL-12 ELISA kit (ThermoFisher Scientific, Waltham, MA, USA).
Statistical analysis

Analysis of variance (ANOVA) was utilized to compare the differences among multiple groups, followed by the post-hoc Bonferroni test for multiple comparisons, while the difference between two independent groups was compared by Student’s t test. Difference in $T_{max}$ was compared using a Wilcoxon signed-rank test. $P < 0.05$ was considered statistically significant. All analysis was performed with GraphPad Prism (GraphPad, San Diego, CA, USA).

Results

The permeability of Rh2 was increased by piperine as the Papp (apical to basal) value was increased from $0.56 \pm 0.21$ (Rh2 alone) to $3.21 \pm 1.56$ (Rh2 + piperine ($\times 10^{-6}$) cm$^{-1}$s$^{-1}$, see Table 1). The results indicated the potential increase of Rh2 absorption in vivo when given orally by co-administration with piperine.

Table 1  Papp values of Rh2 when given alone or in combination with piperine

<table>
<thead>
<tr>
<th>Papp ($\times 10^{-6}$ cm$^{-1}$s$^{-1}$)</th>
<th>Rh2 recovery</th>
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<tbody>
<tr>
<td>Rh2 alone</td>
<td>0.56 ± 0.21</td>
</tr>
<tr>
<td>Rh2 + 5 μmol·L$^{-1}$ Piperine</td>
<td>1.68 ± 1.87</td>
</tr>
<tr>
<td>Rh2 + 20 μmol·L$^{-1}$ Piperine</td>
<td>2.61 ± 0.86</td>
</tr>
<tr>
<td>Rh2 + 50 μmol·L$^{-1}$ Piperine</td>
<td>3.21 ± 1.56</td>
</tr>
</tbody>
</table>

As expected, piperine showed a dose-dependent inhibition of Rh2 metabolism when incubated with rCYP3A4 (see Fig. 1A). The degradation of Rh2 was significantly slower in the combination groups than that of control. The enzymatic metabolic profiles of Rh2 are shown in Fig. 1B with metabolic parameters being listed in Table 2. The results showed piperine may non-competitively inhibit CYP3A4 activity on Rh2, since no statistical significance was observed in $K_m$ values of Rh2 when incubated alone or in combination with piperine (concentration ranged from 5 to 50 μmol·L$^{-1}$), while $V_{max}$ was significantly reduced from 64.6 to 23.6 μmol·min$^{-1}$·mg$^{-1}$. The $K_i$ value was calculated to be 30.5 μmol·L$^{-1}$ (Table 2).

The concentration profiles of Rh2 following oral administration of Rh2 alone or in combination are shown in Fig. 2. Rh2 plasma concentration was found to be very low in Rh2 alone group indicating minimum amount of Rh2 reached body circulation when given orally. When combined with piperine at a low dose (10 mg·kg$^{-1}$), the area under the curve of Rh2 increased slightly but without statistical significance. However, the Rh2 concentrations were found significant higher in combination with 20 mg·kg$^{-1}$ of piperine with relative bioavailability of 196.8%. The pharmacokinetic parameters are shown in Table 3. The $C_{max}$ in the drug combination group is significant greater than Rh2 alone group (270.8 ± 62.4 vs 156.8 ± 58.8 ng·mL$^{-1}$ for Rh2 alone and Rh2 + Piperine groups, respectively). $T_{max}$ was found to be slight shorter in the combination group than Rh2 alone group, although there was no statistical significance (1.63 vs 1.83 h). Piperine significantly increased the half-life of Rh2 ($T_1/2$ of 2.16 ± 0.32 vs 1.41 ± 0.48 h, for high dose combination group and Rh2 alone group, respectively) due to the decrease in $K_e$. The decreased $K_e$ also resulted in a decreased $CL/F$ (Table 3), since there was no statistical significance was observed in volume distribution. The pharmacokinetic results suggested that piperine increased Rh2 exposure by increasing its absorption and inhibiting its elimination as well.
The IL-12 level was significantly higher in piperine and Rh2 combination group than that of the control group as well as Rh2 alone group (P < 0.05) (Fig. 3). The IL-12 concentration in drug combination group was 389 ± 72 pg·mL⁻¹ in comparison to 102 ± 20 pg·mL⁻¹ of control group, while there was no statistical significance observed between piperine and Rh2 alone group. The results indicated that piperine could enhance the immune response by oral Rh2.

Discussion

In the present study, the bioavailability of Rh2 was significantly increased by co-administration of piperine. The increase in Rh2 exposure also led to a significant higher serum level of IL-12. The mechanism of increase in Rh2 exposure was very likely due to the increase in absorption (by inhibition of P-gp and first pass extraction), and inhibition of elimination (by inhibition of Cyp3a) as well.

Table 2 Enzyme kinetic parameters for degradation of Rh2 in human recombinant CYP3A4 with or without of piperine

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vₐₜ₅ (pmol·min⁻¹·mg⁻¹)</th>
<th>Kₘ (pmol·mL⁻¹)</th>
<th>Kᵢ (μmol·L⁻¹) of Piperine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh2 alone</td>
<td>64.6 ± 7.2</td>
<td>1 380 ± 260</td>
<td></td>
</tr>
<tr>
<td>Rh2 + 5 μmol·L⁻¹ Piperine</td>
<td>46.4 ± 10.1</td>
<td>1 059 ± 427</td>
<td>30.5</td>
</tr>
<tr>
<td>Rh2 + 20 μmol·L⁻¹ Piperine</td>
<td>44.1 ± 9.4</td>
<td>1 710 ± 716</td>
<td></td>
</tr>
<tr>
<td>Rh2 + 50 μmol·L⁻¹ Piperine</td>
<td>23.6 ± 4.4</td>
<td>1 867 ± 653</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3 Serum IL-12 concentration in rats after treated by Rh2 (5 mg·kg⁻¹ daily for 14 days) or in combination with piperine (20 mg·kg⁻¹ daily for 14 days)

Piperine has been reported to be able to inhibit P-gp transporter and CYP3A4 activity in human subjects [19]. Our in vitro cell line and enzymatic studies showed that piperine could inhibit P-gp efflux of Rh2 and thus increase its permeability and inhibit Cyp3a activity in metabolism of Rh2, leading to a decreased clearance. The results were consistent with previous findings. As shown in the pharmacokinetic study, piperine increased the elimination half-life and decreased the clearance of Rh2 possibly by inhibiting rat Cyp3a isoforms, such as Cyp3a2 which has similar catalytic competence to human CYP3A4 [20].

CYP3A4 has an important role in drug metabolisms and is estimated to be responsible for about 50 percent of the metabolism of marketed drugs [21]. CYP3A4 is also active in the small intestine and may reduce the absorption of Rh2 [22]. However, in the present study, the recovery of Rh2 was close to 100%, indicating the intestinal metabolism of Rh2 is less essential for Rh2 bioavailability.

Piperine increased the pharmacological activity of Rh2 as indicated by the increasing level of IL-12. In previous studies, piperine could prolong sedative-hypnotic properties of midazolam (a substrate of CYP3A4) by increasing its circulating concentration. As CYP3A4 is the dominant elimination pathway of Rh2 in vivo, piperine could also enhance the immune modulating effect in human subjects by increasing Rh2 exposure.

Table 3 Pharmacokinetic parameters of Rh2 in rats receiving 10 mg·kg⁻¹ of Rh2 alone in combination with piperine (10 and 20 mg·kg⁻¹)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Rh2 (10 mg·kg⁻¹)</th>
<th>Rh2 (10 mg·kg⁻¹) + Piperine (10 mg·kg⁻¹)</th>
<th>Rh2 (10 mg·kg⁻¹) + Piperine (20 mg·kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁/₂ (h)</td>
<td>1.41 ± 0.48</td>
<td>1.63 ± 0.25</td>
<td>2.16 ± 0.32</td>
</tr>
<tr>
<td>AUC₀₋₅h (ng·mL⁻¹·h)</td>
<td>570.0 ± 237.3</td>
<td>601.1 ± 181.8</td>
<td>1 030.8 ± 356.8</td>
</tr>
<tr>
<td>AUC₀₋₁₂h (ng·mL⁻¹·h)</td>
<td>595.7 ± 241.2</td>
<td>626.7 ± 171.2</td>
<td>1 131.6 ± 368.1</td>
</tr>
<tr>
<td>Tₘₚₜ (h)</td>
<td>1.83 ± 0.75</td>
<td>1.83 ± 0.98</td>
<td>1.67 ± 0.82</td>
</tr>
<tr>
<td>Cₘₚₜ (ng·kg⁻¹)</td>
<td>156.8 ± 58.8</td>
<td>169.8 ± 37.7</td>
<td>270.8 ± 62.4</td>
</tr>
<tr>
<td>CL/F (L·h⁻¹)</td>
<td>17.5 ± 3.2</td>
<td>156.5 ± 3.6</td>
<td>9.7 ± 2.5</td>
</tr>
</tbody>
</table>

A bioenhancer is defined as a substance that can increase the bioavailability and bioactivity of a specific drug or nutraceuticals, with which it is combined ideally without any typical pharmacological activity of its own at the dose applied. Piperine is the first bioavailability enhancer identified and validated in 1979 [23]. It is found to increase bioavailability of different drugs ranging from 30% to 200% [24]. A recent search shows that it increases curcumin bioavailability by almost ten-fold [25]. As a component found in black pepper, piperine was considered to be safe, although some studies show that it also possesses pharmacological activities, such as anti-convulsant, anti-inflammation, and anti-cancer effect at relatively high doses (60 mg·kg⁻¹) [26-28]. However, piperine usually does not cause significant side effects if given at a right dose.
IL-12 is a cytokine with a broad range of biological activities, and is mainly produced by the activated inflammatory cells. IL-12 promotes T cell proliferation, enhances the activity of NK cells and T cells, and induces tumor necrosis factor and interferon [4, 15-16, 18]. In the present study, the serum level of IL-12 in the drug combination group was higher than that of the Rh2 alone group. The results suggested Rh2 may not be able to potentiate the body’s immune function when given alone. However when combined with piperine, Rh2 showed improved activity, indicating that a special formulae complex could be developed to potentiate its efficacy. However, it is still very difficult to comprehensively evaluate of immune modulation, especially the immune-boosting effect based on current techniques. In the present study, IL-12 was selected as a potential bioactive marker for Rh2. Further studies to investigate the immune response are definitely needed to verify such results. However, the present study at least proved that the combination of Rh2 and piperine could achieve better efficacy than Rh2 alone. As Rh2 is one of the active components in ginseng extract, piperine may also improve the absorption and efficacy of other active substances with similar physiochemical properties to Rh2. Thus using the bioenhancer is a potential approach to potentiating the activity of the ginseng extract when given orally.

**Conclusion**

The bioavailability of Rh2 was significantly increased when co-administered with piperine, which was believed by inhibiting P-gp and CYP3A4 activity. The increased plasma concentrations led to a potentiated immune response. Such combination can be further developed to enhance the activity of ginseng extract when given orally.

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