# Effect of 20S-protopanaxadiol saponins on blood lipid metabolism and antioxidative activity in hyperlipidemia rats

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**Abstract: Object** To observe the effect of 20S-protopanaxadiol saponins from *Panax quinquefolium* (PPDS) on total cholesterol, lipoprotein cholesterol metabolism and antioxidative activity in experimental hyperlipidemia rats. **Methods** The total cholesterol (TC), lipoprotein cholesterol, and lipid peroxidation (LPO) contents, prostaglandin $I_2$ ($PGI_2$), thromboxane $A_2$ ($TXA_2$) levels, superoxide dismutase (SOD) activity and blood viscosity were measured in hyperlipidemia rats which have been given PPDS 25, 50, 100 mg/(kg·d) by ip, continuously for 12 days. In addition, fat accumulation in liver was observed. **Results** Triglyceride (TG), TC, low density lipoprotein cholesterol (LDL-c) in serum, $TXA_2$ in plasma, LPO in serum and liver, and blood viscosity were decreased significantly; and high density lipoprotein cholesterol (HDL-c) in serum, $PGI_2$ in plasma, and SOD in serum and liver were significantly increased by given PPDS [50, 100 mg/(kg·d)] in experimental hyperlipidemia rats. Moreover, PPDS can decrease TC/HDL-c and LDL-c/HDL-c ratio, increase $PGI_2/TXA_2$ ratio, and inhibit fat accumulation in liver. **Conclusion** PPDS could inhibit arteriosclerosis by improving cholesterol and lipoprotein-cholesterol metabolism, suppressing LPO, and increasing the activity of SOD.

**Key words**: 20S-protopanaxadiol saponins (PPDS); hyperlipidemia; blood lipid metabolism; free radical; $PGI_2/TXA_2$; blood viscosity

# 20S-原人参二醇皂苷对高脂血症大鼠血脂代谢的影响及其抗氧化作用

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**摘 要: 目的** 观察 20S-原人参二醇皂苷(PPDS)对实验性高脂血症大鼠血清总胆固醇 (TC)、脂蛋白-胆固醇代谢的影响及其抗氧化作用。**方法** PPDS 按 25, 50, 100 mg/(kg·d) 给大鼠连续 ip 12 d, 测血清 TC、脂蛋白-胆固醇及脂质过氧化物 (LPO) 含量, 血浆前列腺素 $I_2$ ($PGI_2$), 血栓素 $A_2$ ($TXA_2$) 水平, 血清和肝脏超氧化物歧化酶 (SOD) 活性及全血黏度, 并观察肝脏脂肪沉积情况。**结果** PPDS 50, 100 mg/kg 能明显降低甘油三酯 (TG), TC, 低密度脂蛋白胆固醇 (LDL-c), $TXA_2$, LPO 含量及全血黏度, 并能明显提高实验性高脂血症大鼠高密度脂蛋白胆固醇 (HDL-c), $PGI_2$ 含量及 SOD 活性, 亦能使 TC/HDL-c 及 LDL-c/HDL-c 比值明显降低, $PGI_2/TXA_2$ 比值明显升高。病理检查可见肝脏脂肪沉积明显减轻。**结论** PPDS 可能通过调节体内血脂代谢、提高 $PGI_2/TXA_2$ 比值及纠正自由基代谢紊乱发挥抗动脉硬化作用。

**关键词**: 20S-原人参二醇皂苷; 高脂血症; 血脂代谢; 自由基; $PGI_2/TXA_2$; 全血黏度

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Free-radical-induced lipid and biomembrane peroxidation of low density lipoprotein (LDL) and erythrocytes critically contributes to the risk of human atherosclerosis[1,2]. Therefore, inhibition of LDL and erythrocytes peroxidation by supplementation of various natural and/or synthesized antioxidants leads to a novel therapy method[3]. Vitamin C, a water soluble antioxidant which can reduce α-tocopheroxyl radical to regenerate α-tocophe-rol, may be limited in the interior of LDL

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and biomembrane because of the lipophobicity of the molecule[4,5].

20S-Protopanaxadiol saponins (PPDS) was separated from total saponins isolated from leaves of *Panax quinquefolium* L. Our previous study discovered that PPDS has a cure effect on acute myocardial infarction. The mechanism of the effect is not only the relation to blocking effect of calcium channel but also to the inhibition of sympathetic transmitter release and renin angiotensin system[6,7]. This result indicated that PPDS may influence lipoprotein cholesterol metabolism and antioxidative activity. Therefor the effect of PPDS on total cholesterol (TC), lipoprotein cholesterol metabolism, and antioxidative activity in experimental hyperlipidemia rats will be observed and compared with vitamin C in this paper.

## 1 Materials and methods

1.1 Animals Male Wistar rats weighing 200—240 g which were supplied by Experimental Animal Center of Jilin University were used in all experiments. They were housed under a controlled light/dark cycle and had free access to tap water.

1.2 Drugs and reagents PPDS was provided by Department of Natural Medicament Chemistry, College of Chemistry, Jilin University. Vitamin C was the product of Jilin Henghe Pharmaceutical Co. Ltd. lipid peroxidation (LPO) and superoxide dismutase (SOD) reagent boxes were purchased from Institute of Jiancheng Biotechnology, Nanjing, China. Prostaglandin $I_2$ RIA kit and Thromboxane $A_2$ RIA kit were purchased from Institute of Dongya Immunotechnology, Beijing, China.

1.3 Preparation of hyperlipidmia model Hyperlipidemia model was conducted according to previously described techniques[8]. The rats were fed by hyperlipidemia feed containing cholesterol (2%), propylthiouracil (0.2%), sodium cholate (0.3%), fat (7.5%) and ordinary feed (90%) at 6:00—7:00 pm for 12 days.

1.4 Experimental methods The animals were assigned to the following experimental groups: group 1, rats with the ordinary feed were treated for 12 days with normal saline (2 mL/kg, ip) (normal control group, NC); group 2, rats with hyperlipidemia feed were treated for 12 days with normal saline (2 mL/kg, ip) (model control group, MC); groups 3—5, rats with hyperlipidemia feed were treated for 12 days with PPDS (25, 50, 100 mg/kg, ip); group 6, rats with hyperlipidemia feed were treated for 12 days with vitamin C (400 mg/kg, ig). After 24 hours of the last administration the rats were anaesthetized (sodium pentobarbital 30 mg/kg ip). Blood (7 mL) was collected through aorta abdominalis and among them 3 mL was centrifuged for the serum. Triglyceride (TG), TC, high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), LPO contents and SOD activity were determined according to the method of reagent box. Blood (4 mL) was anticoagulated by heparin, among them 1 mL was put in LBY-N6A self-cleaning rotatory viscometer and blood viscosities (10/s, 40/s, and 120/s) were measured. The other was centrifuged 3 000 r/min for 10 min and the plasma was analysed for prostaglandin $I_2$ ($PGI_2$) and thromboxane $A_2$ ($TXA_2$) levels with RIA method. Half liver was put in 10% formalin and dyed in order to observe the accumulation of fat. Another half liver was measured for LPO and SOD.

1.5 Data analysis All the results were expressed as $\bar{x} \pm s$. Statistical analysis was performed in the paired and unpaired student's *t*-test. Comparison among multiple groups was made by analysis of variance (ANOVA).

## 2 Results

2.1 Effects of PPDS on the blood lipid metabolism of hyperlipidemia rats In the group of MC, the data of TC, TG, LDL-c, TC/HDL-c and LDL-c/HDL-c in the serum are significantly higher than those in the group of NC ($P < 0.05$ or $P < 0.001$); the data of HDL-c, however, decreased remarkably ($P < 0.05$), indicating that the model of hyperlipidemia is set up successfully. The value of serum TG, TC, LDL-c, TC/HDL-c, and LDL-c/HDL-c in the group given by PPDS of 25, 50, and 100 mg/kg are lower than those in the group of MC significantly ($P < 0.05$, $P < 0.01$ or $P <$

0.001), respectively, and HDL-c in serum is increased remarkably in the group given by PPDS ($P < 0.05$ or $P < 0.01$). Compared with the data of vitamin C, the effect of PPDS on preserving the blood lipid metabolism of hyperlipidemia rats is as strong as that of vitamin C (Table 1).

2.2 Effect of PPDS on the LPO and SOD of serum and liver of hyperlipidemia rats It can be seen that the increase of LPO in serum and liver ($P < 0.05$ or $P < 0.01$), with the decrease of SOD ($P < 0.05$ or $P < 0.01$), in the group of MC reveals that free-radical-induced damages occur in the hyperlipidemia rats The groups given by PPDS (50, 100 mg/kg) decrease the LPO in the serum and liver, meanwhile, increase the SOD activity significantly. This function is the same as that of vitamin C (Table 2).

2.3 Effect of PPDS on the blood viscosity of hyperlipidemia rats The blood viscosity in the group of MC is increased. This is due to the change of blood rheology in the hyperlipidemia rats But the blood viscosities (10/s, 40/s, and 120/s) in the groups given by 50 mg/kg and 100 mg/kg PPDS are decreased. Vitamin C, however, can not influence the blood viscosity significantly (Table 3). So, in the view of blood rheology, PPDS plays a protective role in hyperlipidemia rats.

**Table 1 Effect of PPDS on blood lipid metabolism in hyperlipidemia rats** ($\bar{x} \pm s$, n= **10**)

| Groups | Dosage /(mg·kg$^{-1}$) | TG /(mmol·L$^{-1}$) | TC /(mmol·L$^{-1}$) | LDL-c /(mmol·L$^{-1}$) | HDL-c /(mmol·L$^{-1}$) | TC/HDL-c | LDL-c/HDL-c |
|---|---|---|---|---|---|---|---|
| NC | - | 0.60±0.24* | 1.98±0.54*** | 4.26±1.91** | 1.10±0.38* | 1.80±0.66*** | 3.87±1.32*** |
| MC | - | 1.06±0.46 | 7.70±1.87 | 7.20±2.16 | 0.76±0.22 | 10.13±3.48 | 9.47±3.25 |
| PPDS | 25 | 0.91±0.34 | 5.87±1.48* | 5.76±2.19 | 0.89±0.27 | 6.60±1.34** | 6.47±2.06* |
| | 50 | 0.71±0.13* | 5.12±1.32** | 4.67±1.15** | 0.98±0.18* | 5.22±1.21*** | 4.76±1.89*** |
| | 100 | 0.64±0.25* | 4.53±1.21*** | 4.34±1.64** | 1.02±0.14** | 4.44±1.27*** | 4.25±1.91*** |
| Vitamin C | 400 | 0.65±0.21* | 4.64±1.36*** | 4.30±1.32** | 1.00±0.20* | 4.64±1.40*** | 4.30±1.73*** |

*$P < 0.05$ **$P < 0.01$ ***$P < 0.01$ *vs* MC

**Table 2 Effect of PPDS on LPO and SOD in hyperlipidemia rats** ($\bar{x} \pm s$, n= **10**)

| Groups | Dosage /(mg·kg$^{-1}$) | Serum | | Liver | |
|---|---|---|---|---|---|
| | | LPO /(nmol·L$^{-1}$) | SOD /(U·L$^{-1}$) | LPO /(nmol·mL$^{-1}$) | SOD /(nU·mg$^{-1}$) |
| NC | - | 6.60±1.54** | 468.3±43.2* | 5.54±1.07* | 9.86±1.93** |
| MC | - | 8.83±2.51 | 401.6±57.4 | 6.99±1.34 | 7.51±1.38 |
| PPDS | 25 | 7.87±2.49 | 428.1±46.2 | 6.59±1.74 | 8.45±2.79 |
| | 50 | 6.76±1.42* | 454.9±35.3* | 5.68±1.24** | 9.04±1.13* |
| | 100 | 6.22±1.28** | 457.8±41.0* | 5.59±1.32* | 9.18±1.35* |
| Vitamin C | 400 | 6.20±1.23** | 454.5±32.2* | 5.56±1.18* | 9.11±1.28* |

*$P < 0.05$ **$P < 0.01$ *vs* MC

**Table 3 Effect of PPDS on blood viscosity in hyperlipidemia rats** ($\bar{x} \pm s$, n= **10**)

| Groups | Dosage /(mg·kg$^{-1}$) | Blood viscosity | | |
|---|---|---|---|---|
| | | 10 s$^{-1}$ | 40 s$^{-1}$ | 120 s$^{-1}$ |
| NC | - | 11.25±1.30* | 4.11±0.87* | 3.80±0.74** |
| MC | - | 13.34±2.31 | 5.26±1.16 | 4.84±0.83 |
| PPDS | 25 | 12.26±2.45 | 4.89±1.36 | 4.25±0.67 |
| | 50 | 11.41±1.35* | 4.98±0.9 | 3.89±0.98* |
| | 100 | 11.35±1.28* | 4.29±0.67* | 3.83±0.62** |
| Vitamin C | 400 | 11.76±1.68 | 4.91±0.82 | 4.17±0.93 |

*$P < 0.05$ **$P < 0.01$ *vs* MC

2.4 Effects of PPDS on the level of $PGI_2$ and $TXA_2$ of plasma in hyperlipidemia rats The low level of $PGI_2$, 281.2 pg/mL, and high level of $TXA_2$, 438.5 pg/mL, in the group of MC, in particular, the ratio between $PGI_2$ and $TXA_2$, lower than that in the group of NC, indicates that the hyperlipidemia breaks the balance between $PGI_2$ and $TXA_2$. However, the $PGI_2$ is increased and $TXA_2$ is decreased significantly by given 50 mg/kg and 10 mg/kg PPDS, resulting that $PGI_2/TXA_2$ is increased remarkably. This function of PPDS may be better than 400 mg/kg vitamin C (Table 4).

2.5 Effect of PPDS on the fat accumulation in the liver of hyperlipidemia rats The slice of the sample in the liver is dyed in order to observe the accumulation of fat. Results show that the denaturation of fat tissue does not take place in the cell of liver in the group of NC among ten experimental

**Table 4 Effect of PPDS on plasma $PGI_2$ and $TXA_2$ in hyperlipidemia rats ($\bar{x}\pm s$, n= 10)**

| Groups | Dosage /(mg·kg$^{-1}$) | $PGI_2$ /(pg·mL$^{-1}$) | $TXA_2$ /(pg·mL$^{-1}$) | $PGI_2$/ $TXA_2$ |
|---|---|---|---|---|
| NC | - | 407.5±112.4* | 278.7± 99.5* | 1.46±1.13* |
| MC | - | 281.2± 89.7 | 438.5±156.3 | 0.64±0.32 |
| PPDS | 25 | 326.6±118.7 | 334.8±102.3 | 0.98±0.42 |
| | 50 | 384.9± 98.0* | 296.3±110.6* | 1.30±0.56** |
| | 100 | 393.8± 76.6* | 285.2± 98.8* | 1.38±0.58** |
| Vitamin C | 400 | 366.3±105.1 | 318.9± 97.2 | 1.15±0.82 |

*$P<0.05$ **$P<0.01$ *vs* MC

rats (0/10). But the denaturations of fat tissue have been found in the group of MC among all the experimental rats (10/10). To the groups given by 25, 50, 100 mg/kg PPDS and the group given by 400 mg/kg vitamin C, the ratio of denaturations of fat tissue and all the experimental rats are 5/10, 4/10, 3/10, and 4/10, respectively. This fact demonstrates that PPDS, along with vitamin C, can inhibit the fat accumulation in the liver remarkably.

## 3 Discussion

A large body of clinical evidence has accumulated in the past decade, suggesting that free-radical-induced peroxidation of lipid critically contributes to the risk of human atherosclerosis. This fatty streak is purported to the earliest lesion of the atherosclerosis characterized by the accumulation of monocyte-derived macrophages loaded with cholesteryl esters and known as foam cells just below the endothelium[9,10]. The stimulation of cell proliferation at the early stage of atherosclerosis, particularly that of smooth muscle cells which may break through the elastic lamina and form a mass of atherosclerotic plaque[10]. It has been found that the formation of atherosclerosis is related to the increase of TC, TG, and LDL; and the decrease of HDL. The obtained results in this work suggest that PPDS can decrease the value of TC, TG, and LDL-c, and increase the contents of HLD-c, demonstrating that PPDS may be good at the transfer process of cholesterol from the surrounding cells to the liver of hyperlipidemia rats, resulting in the decrease of the accumulation of cholesterol in the surrounding cells and protect arterial endothelium against the damage of cholesterol. This function of PPDS is similar to vitamin C. Moreover, the correlation between atherosclerosis and free radical induced peroxidation of lipids proposes that the increases of LPO at the beginning step of atherosclerosis generates the oxidated-LDL to form foam cell by macrophages. This leads to the accumulation and affinity of white blood cells and blood platelet on the arterial endothelium. The decrease of LPO and increase of SOD activity either in serum and liver can be given by PPDS. So, PPDS plays a protective role in hyperlipidemia rats. The formation of thrombus is always associated with the unusual balance of $TXA_2$ and $PGI_2$. The lack of $PGI_2$, related to the decrease of the synthesis of $PGI_2$ by LDL is harmful to the ischemic heart disease. On the contrary, HDL can stimulate the arterial endothelium to synthesize $PGI_2$ and be good at the ischemic heart disease. PPDS can increase $PGI_2$ and decrease $TXA_2$ remarkably, leading to increasing the ratio of $PGI_2$/ $TXA_2$. This function of PPDS is better than vitamin C. To this point, PPDS may be a novel drug to protect human against atherosclerosis.

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