

The hypolipidemic effect of artesunate and ursolic acid in rats

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Abstract: To find the novel hypolipidemic agents, the effects of ursolic acid and artesunate on hyperlipidemia were determined in rats fed a Western-type diet developed a hyperlipidemia. Rats received ursolic acid (50 mg/kg) or artesunate (50 mg/kg) alone, or in combination (25+25 mg/kg and 50+50 mg/kg), to prevent hyperlipidemia. Ursolic acid or artesunate alone significantly decreased the plasma triglyceride, but had no effect on the levels of cholesterol. The combination of ursolic acid and artesunate can reduce both triglyceride and cholesterol, and the effects were more potent than either agent alone, which indicates a strong synergistic effect. The hypolipidemic effect of artesunate is firstly reported. Its combination with ursolic acid might have the potential to further develop for the treatment of hyperlipidemia.

Keywords: Artesunate, ursolic acid, combination, hyperlipidemia.

INTRODUCTION

Hyperlipidemia is an important induced factor for cardiovascular diseases, because high concentration of cholesterol and triglyceride are strongly associated with cardiovascular disease. In order to reduce the toxic effects of lipid-lowering drugs available, more and more of the natural ingredient were used to develop new lipid-lowering drugs. The lipid-lowering effects of berberine, phytosterols, and Omega-3 have been observed in human or animal studies (Gupta *et al.*, 2011, Kong *et al.*, 2004, Parker *et al.*, 2012).

Ursolic acid is a pentacyclic triterpenoids presented in many plants, and its anti-oxidative, anti-inflammatory and other potentially cardio-protective properties were considered beneficial cardiovascular disease prevention (Somova *et al.*, 2003). Artesunate is one of derivatives of artemisinin extracted from *Artemisia annua*. In Chinese traditional medicine, *Artemisia annua* is used to disperse stagnated liver qi for promoting bile flow. In addition to the effectiveness against malaria parasite, artesunate have also been shown to affect immune responses that were relevant to atherosclerosis development (Li *et al.*, 2010, Li *et al.*, 2008, Xu *et al.*, 2007). Therefore, we hypothesized that artesunate has a lipid-lowering effect. In the current study, we accessed the effects of ursolic acid or artesunate alone and in combination on plasma lipid levels and hepatic biochemical parameters in rats of diet-induced hyperlipidemia.

MATERIALS AND METHODS

Drugs and chemicals

Artesunate (Guangzhou Hanfang Pharmaceutical Co., Ltd., China, 000129); Ursolic acid (Xian Xiaocao

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Botanical Development Co., Ltd., China; XC091225); Sodium salt of Caboxy Methyl Cellulose (Sinopharm Chemical Regent Co., Ltd., China, F20121012)

Methods

Guide for The Care and Use of Laboratory Animals was complied in all animal procedures and experiments. The Bioethics Committee of Shanghai Jiao Tong University approved the related study.

Seventy male Sprague-Dawley rats weighing 150-170g were purchased from Shanghai Laboratory Animal Center of Chinese Academy of Sciences, and were housed in cages with a 12 h light cycle and free access to food and water. After 1 wk of adaptation, rats were randomly divided into seven groups based on body weight (n=10): control group, regular rodent chow, treatment with 0.9% saline; model group, Western-type diet (2% cholesterol), treatment with 0.9% saline; atorvastatin group, Western-type diet (2% cholesterol), treatment with 10 mg/kg/day atorvastatin calcium; artesunate group, Western-type diet (2% cholesterol), treatment with 50 mg/kg/day artesunate; ursolic acid group, Western-type diet (2% cholesterol), treatment with 50mg/kg/day ursolic acid; low-dose combination group, Western-type diet (2% cholesterol), treatment with 25mg/kg/day artesunate plus 25mg/kg/day ursolic acid; high-dose combination group, Western-type diet (2% cholesterol), treatment with 50mg/kg/day artesunate plus 50mg/kg/day ursolic acid. The mode of administration is by oral gavage once a day. The volume was administered in different groups were calculated according to the same standard (10ml/kg body weight).

After 4 weeks of treatment, rats were sacrificed and blood was collected from abdominal aorta. Plasma was separated by centrifugation and livers were stripped and weighed. Plasma and livers were all stored in -80℃ for next analysis.

Table 1 The weight values of rats (n=10, X±s)

Groups	Weight (g)				
	Before treatment	After 1wk	After 2wk	After 3wk	After 4wk
Control group	253±8.6	287±15.4	311±25.4	331±19.9	341±22.9
Model group	252±8.0	277±14.3	299±17.2	318±15.7	330±17.3
atorvastatin group	253±9.7	273±12.2	296±14.7	310±18.1	317±17.1
artesunate group	253±10.0	277±9.3	299±12.9	313±12.4	322±18.0
ursolic acid group	253±9.3	269±9.7 [#]	296±11.4	317±12.4	323±14.4
low-dose combination group	253±9.6	285±11.4	301±15.4	321±13.9	331±20.9
high-dose combination group	252±9.1	276±9.0	296±12.1	316±11.7	325±12.3

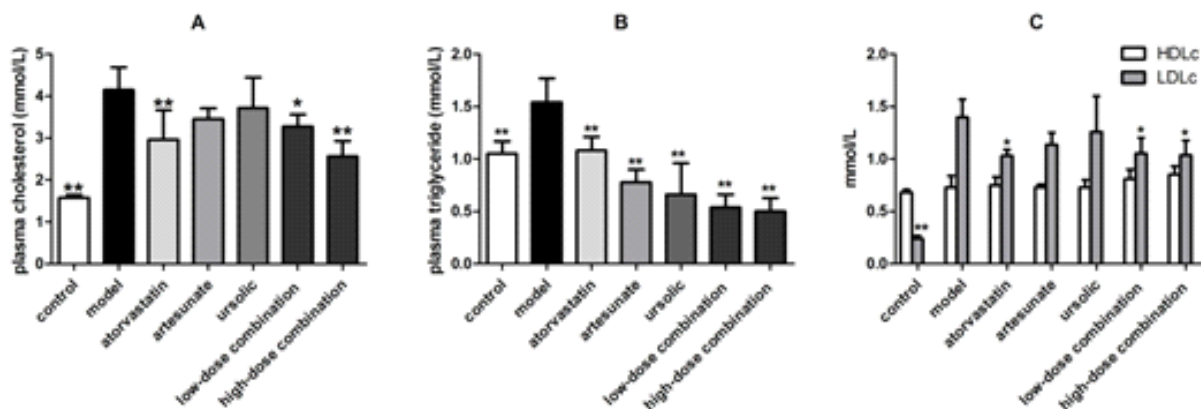


Fig. 1: Comparative plasmalipid profiles of control, model and experimental groups of rats is shown by labeling in the Figure after 4 weeks of experimental periods.

Control group: regular diet, Model group and the experimental group: fed western-type diet to induce hyperliprdemia. *: p<0.05 versus model; **: p<0.01 versus model

Commercial assay kits (Shanghai Fosun Long March Medical Science Co., Ltd) were used to determine the contents of plasma total cholesterol (TC), triglycerides (TG), LDL cholesterol (LDLc), and HDL cholesterol (HDLc). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) of plasma were analyzed using the assay kits produced by Nanjing Jiancheng bioengineering institute.

500 mg of liver was homogenated in 4 ml of lipid extract (heptane: isopropyl alcohol =2:3.5, v/v). After 15 minutes of shaking, the supernatant after centrifugation were collected for the measurement of TC and TG.

STATISTICAL ANALYSIS

We presented the results as mean ± SD from 10 animals. Statistical difference between the treatments and the controls were analyzed using One-Way Analysis of Variance (ANOVA) with Dunnett post-hoc test. P<0.05 was considered to be statistically significant.

RESULTS

During the 4 weeks study period, there were no differences in food intake and body weight (table 1)

between model group and treatment groups. The model group fed Western-type diet developed an effective hyperlipidemia compared with control group (total cholesterol: 4.15±0.54 vs. 1.58±0.07 mmol/L; triglycerides: 1.54±0.23 vs. 1.05±0.12 mmol/L, p<0.01). All agents significantly decreased plasma triglyceride compared to model group, but only atorvastatin and combination of artesunate and ursolic acid significantly reduced plasma cholesterol and LDLc compared to model group. The lipid-lowering effect of combination is dose dependent. Plasma HDLc levels were unchanged by all agents. (fig. 1A, B, C)

The effects of different treatments on liver cholesterol and triglyceride were presented in fig. 2. Compared to the model group, cholesterol and triglyceride content in the liver were lowered significantly only by high-dose combination. High-dose combination lowered liver cholesterol by 34.0% and triglyceride by 33.9% (p<0.01). Similarly, plasma ALT and AST were not affected by atorvastatin, artesunate, ursolic acid, and low-dose combination, and only significantly decreased by high-dose combination (AST, 99±12 U/L; ALT, 66±16U/L) compared to model group (AST, 99±12; ALT, 82±6) (fig 3).

DISCUSSION

This is the first study investigating the effect of artesunate on plasma lipid in rats fed Western-type diet. Our current studies reveal a significant triglyceride-lowering effect of artesunate or ursolic acid in rats. However, their decreasing effects on plasma cholesterol level were not observed. Further reduction in plasma lipid were observed in the combination of artesunate and ursolic acid. Especially, the combination had a significant lowering effect on plasma cholesterol, LDL and liver cholesterol and triglyceride in rats. These effects were not observed as artesunate or ursolic acid alone administration.

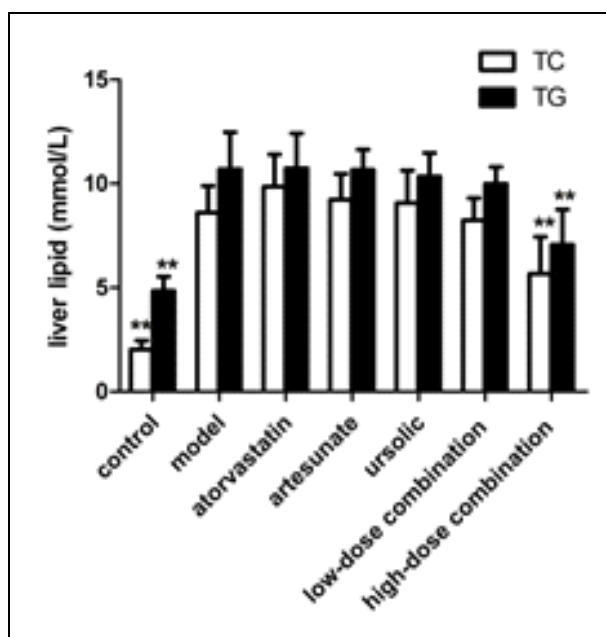


Fig. 2: Total cholesterol (TC) and triglyceride (TG) of liver in different groups of rats as given in fig.

*: $p < 0.05$ versus model; **: $p < 0.01$ versus model.

In previous study, ursolic acid showed a significant reducing effect on cholesterol and triglyceride in HepG2 cells (Jia *et al.*, 2011), and plasma triglyceride in rats (Kim *et al.*, 2009, Somova *et al.*, 2003), which are in agreement with our current study. These effects of ursolic acid might be attributable to its anti-lipase, lipolytic, and PPAR- α agonist activity (Jia *et al.*, 2011, Kim *et al.*, 2009). The hypolipidemic effect of artesunate is observed for the first time, to our knowledge. Artesunate is one of derivatives of artemisinin extracted from *Artemisia annua*. In Chinese traditional medicine, *Artemisia annua* is used to disperse stagnated liver qi for promoting bile flow that might explain the hypolipidemic effect of artesunate. The different mechanisms of artesunate and ursolic acid contribute to the synergistic lowering effect of the combination on the lipid levels in plasma and liver.

Hepatic biochemical parameters in plasma are important indicator in basic toxicological research and clinical

toxicity testing. ALT and AST are the most commonly used to evaluate liver damage. Ursolic acid or artesunate administration alone have not significant effect on plasma ALT and AST compared with the model group, which indicates that they do not induce the extra liver damage at given dose. The combination of ursolic acid and artesunate significantly reduced the plasma ALT and AST level compared with the model group. The results suggest that supplementation of ursolic acid or artesunate is not toxic in rats under the Western-type diet.

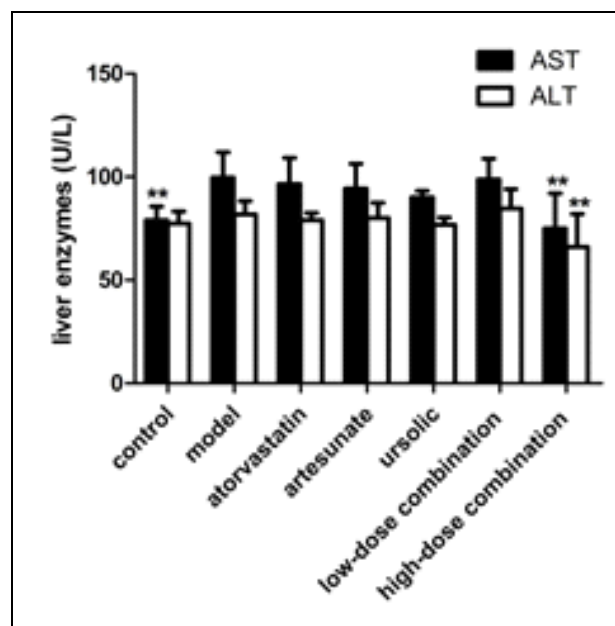


Fig. 3: Plasma AST and ALT levels in different groups of rats as mentioned above.

*: $p < 0.05$ versus model; **: $p < 0.01$ versus model.

CONCLUSION

In conclusion, the hypolipidemic effect of artesunate is firstly reported. The most interesting observation is that the combination of artesunate and ursolic acid markedly improve the hypolipidemic efficacy through synergistic mechanism and produced an additive effect on liver cholesterol and triglyceride reduction. Toxic effects were not observed after artesunate and ursolic acid alone or combination treatments. Results of current study demonstrate that ursolic acid and artesunate, especially their combination, hold a promising potential for developing new natural products to lower lipid level and prevent and/or treat atherosclerosis.

ACKNOWLEDGEMENT

This research was supported by Science and Technology Commission of Shanghai municipality (No.10DZ1971500) and Shanghai Leading Academic Discipline Project (Project Number:B209).

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