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Effect of the Chinese Drugs Nao Xintong and Dan Hong on Markers of Inflammation and the Lipid Profile in a Hypercholesterolemic Rabbit Model

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Abstract

Objectives: This study assessed the effect of two commonly prescribed Chinese traditional medications, Nao Xintong and Dan Hong, on aortic intimal thickness, lipids and markers of inflammation in a hypercholesterolemic rabbit model.

Study design: Female New Zealand White rabbits (N=50) were divided into 4 groups and treated for 90 days: Group 1 (N=12); control group; common rabbit chow; no treatment. Group 2 (N=12); 2% cholesterol enriched rabbit chow (fat chow); no treatment. Group 3 (N=13); fat chow; daily treatments of oral Nao Xintong (0.3 g/kg). Group 4 (N=13); fat chow; daily treatments of oral Nao Xintong (0.3 g/kg) combined with regular injections of Dan Hong (0.3 ml/kg, IV or IP, every third day). Total cholesterol, triglycerides and CRP were measured at baseline, 60 days and 90 days when each animal was euthanized and thoracic aortic intimal thickness was quantified by measuring the percent area stenosis (PAS).

Results: Group 1 showed no aortic intimal growth. PAS averaged 36.410.8% for Groups 2, 3 and 4 with no significant differences between each group. The high cholesterol diets of Groups 2, 3 and 4 produced marked increases in total cholesterol levels that trended down between 60 and 90 days. Peak elevations of total cholesterol at 60 days were blunted by both Nao Xintong and the combination of Nao Xintong and Dan Hong, but these differences were not statistically significant. A statistically significant 20% reduction in triglycerides was found in rabbits receiving both Nao Xintong and Dan Hong. Likewise, the combination therapy also significantly reduced CRP (change score [baseline - 60 days = 14.2 mcg/ml; change score [baseline - 90 days] = 8.5 mcg/ml).

Conclusions: In this very hyperlipidemic rabbit model, the combination of Nao Xintong and Dan Hong produced no affect on aortic intimal thickness, but did significantly reduce both triglycerides and CRP. These positive effects both on inflammatory markers and triglycerides may well translate into significant human clinical benefit.

Introduction

Atherosclerosis is the primary underlying cause of coronary heart disease (CHD), the most common source of mortality in the industrial world [1]. Its natural history and disease associations have been extensively studied, but the actual etiology remains unknown. Hypertension, smoking, diabetes, hyperlipidemia, and a positive family history are identified disease associations, but cannot account for all cases of CHD.

Progressive plaque development in atherosclerosis leads to arterial stenosis, which is characterized clinically by angina and may eventually lead to unstable angina, myocardial infarction, and cardiac death. The presence of lipid particles (especially cholesterol) in the blood and chronic inflammation at the site of endothelial injury contributes to the initiation, growth and disruption of atherosclerotic plaque. A variety of therapies for the prevention of atherosclerosis have been developed that are moderately effective. These include lipid lowering agents, antiplatelet agents, Renin Angiotensin Aldosterone System (RAAS) inhibitors, and anti-inflammatory agents. However, many of these agents result in intolerable side effects and despite their widespread use, atherosclerosis remains a major cause of death in most of the world. Other approaches for therapy or prevention are needed.

Nao Xintong [2] and Dan Hong [3] (both produced by BuChang Corporation, Xian, China), traditional Chinese herbal formulations, have been proposed to contain a variety of potential antiatherogenic properties that may be beneficial in the primary and secondary prevention of atherosclerosis. These include lipid lowering properties, [4] anti-inflammatory effects, [5,6] and anti-oxidant potential [7]. Although they have been used successfully for many thousands of years in traditional Chinese medicine, they have not been rigorously tested in the modern scientific sense of the word.

The cholesterol-fed New Zealand White rabbit model has been successfully used in many circumstances to evaluate the underlying pathophysiology of atherosclerosis [8]. It has also been used to assess the effect of various proposed antiatherogenic therapies [9]. In addition to acutely raising a variety of blood lipid levels, feeding the New Zealand White rabbit with 1-2% cholesterol enriched chow also results in rapid development of arterial atherosclerotic intimal thickening and increased levels of inflammatory markers such as C-reactive protein (CRP) [9].

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The objective of this study was to determine if administration of either Nao Xintong alone, or in combination with Dan Hong, has a positive antiatherogenic effect on either the blood lipid levels of total cholesterol or triglycerides, CRP concentration or intimal thickening in the cholesterol fed rabbit.

Materials and Methods

Animal experiments

A total of 50 young female New Zealand White rabbits were used in the study. The rabbits were cared for under the approval and supervision of an accredited institutional animal care and use committee. They were divided into 4 groups: Group 1 (N=12) was the control group and was fed common rabbit chow with no additives and received no specific other therapy. Group 2 (N=12) was fed 2% cholesterol enriched rabbit chow and no received specific other therapy. Group 3 (N=13) was fed 2% cholesterol enriched rabbit chow and also received daily treatments of oral Nao Xintong (0.3 g/kg). Group 4 (N=13) was fed 2% cholesterol enriched rabbit chow and also received daily treatments of oral Nao Xintong (0.3 g/kg) combined with regular injections of Dan Hong (0.3 ml/kg, IV or IP, every third day). Granulated Nao Xintong was mixed with sweet corn syrup and fed directly to the rabbits to verify accurate dosing. Dan Hong was injected via the rabbit ear vein whenever possible. In the event that both ear veins became sclerosed to the point of non-patency, it was injected intraperitoneally. The animals were treated for a total of 90 days.

Baseline blood samples (5cc) were drawn from each rabbit and frozen for future analysis. Blood samples were also obtained after 60 days and at the end of the study.

Pathologic investigations

Upon completion of the study, each animal was euthanized and the heart, including ascending and descending aorta to include iliac bifurcation, were dissected and placed in a sterile specimen cup containing sterile water. Thoracic aortic cross-sections were cut measuring approximately 3mm in length and placed in a tissue cassette and treated with formalin. The cassette was sent for histological examination and temporary embedment in paraffin. The prepared slides were viewed using an Olympus photographic microscope at 100x power for complete cross-section view to determine the degree of intimal atheromatous involvement and 300x power for an area-specific view of atherosclerosis composition. Histological specimens were fixed in 10% buffered formalin, paraffin imbedded, and stained with hematoxylin and eosin (H&E). Quantitative evaluation was performed using an Olympus BH-2 microscope equipped with an eyepiece micrometer. The degree of intimal atheromatous involvement was determined by calculating the percent area stenosis (PAS) for each cross sectional specimen. This value was obtained by computerized cross-sectional measurement of the intimal plaque area (IPA) and the total luminal area (TLA) defined as the cross-sectional area inside the aortic internal elastic lamina. PAS was calculated by the following formula: PAS = 100 x (IPA/TLA). The remaining tissue specimens were stored in formalin for potential future specialized pathological evaluation.

Blood plasma analysis

The three specimens for each rabbit in both treatment and control groups were frozen until the end of the entire study and then sent for specific blood analyses. These included a fasting lipid panel and C-reactive protein, a marker of vascular inflammation. It should be noted that due to the tremendously high levels of triglycerides produced by the high cholesterol diet, HDL-cholesterol levels could not be measured and LDL-cholesterol levels could not be calculated. Therefore total cholesterol and triglyceride levels are reported. The remaining plasma was frozen at -90 degrees C for possible future further study.

Statistical analysis

PAS and levels of total cholesterol, triglyceride and CRPs are expressed as mean ∀SD for the different treatment groups. PAS was prospectively selected as the primary end point, with total cholesterol, triglyceride and CRP levels as secondary end points. Differences among the three treatment groups were evaluated by a two-tailed Student's t-test. (GB-Stat, version 9.0). For the secondary endpoints, primary statistical analysis was considered to be the results of comparisons between the test results between treatment groups at 90 days, just before autopsy. To further analyze the effect of various treatments on CRP, we also calculated the mean CRP change score based on treatment group.

Results

The project was completed as planned. One rabbit in group 4 died prematurely, presumably secondary to the toxic effects of the very high cholesterol diet. The remaining rabbits all survived to the end of the study.

Pathology results

The primary endpoint of this project was the effect of treatment with NaoXintong alone (Group 3) or the combination of NaoXintong and Dan Hong (Group 4) on the PAS of the thoracic aorta of rabbits fed a high cholesterol diet for 90 days. Figure 1 shows representative examples of H&E cross sections from the thoracic aortas of each of the four study groups. Combined results of all animals are shown in Table 1. No atherosclerosis intimal growth was found in any of the rabbits fed common rabbit chow. Among groups 2, 3 and 4, who all received rabbit chow enriched with 2% cholesterol, atherosclerotic intimal hyperplasia resulted in an average PAS of 36.4 ∀10.8%. No significant differences were noted in average PAS between groups 2, 3

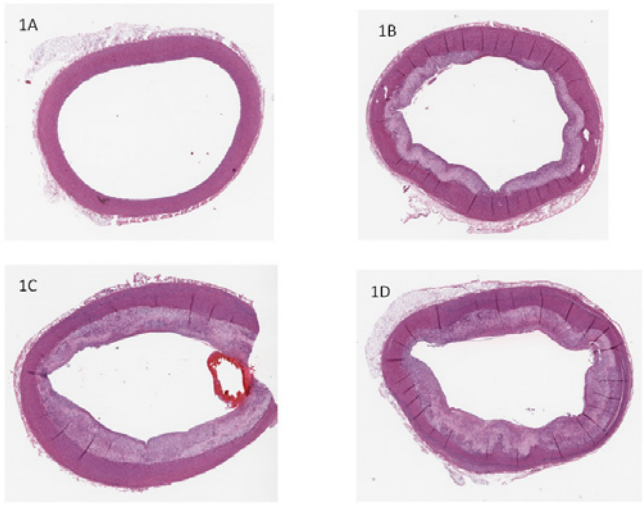

Figure 1: Representative H&E cross-sections of the thoracic aortas from each of the treatment groups. 1A = Group 1 (control); 1B = Group 2 (fat chow, no therapy); 1C = Group 3 (fat chow, Nao Xintong); 1D = Group 4 (fat chow, Nao Xintong +Dan Hong).

or 4. Qualitative analysis of the neointimal lesions of Groups 2, 3 and 4 demonstrated the accumulation of lipid-loaded foam cells and a low collagen content, independent of the treatment group. In summary, pathologic analysis revealed no differences between the atherosclerotic lesions produced by the high cholesterol diet, regardless of treatment by either study agent.

Blood plasma results

The results of plasma total cholesterol, triglycerides and CRP are shown in Table 2. The high cholesterol diet produced marked nearly 20-fold increases in plasma total cholesterol levels that trended down between 60 and 90 days. Although the peak elevations of total cholesterol at 60 days were blunted by both Nao Xintong and the combination of Nao Xintong and Dan Hong, these differences were not statistically significant. By 90 days, there were no differences in total cholesterol concentrations between groups 2, 3 and 4.

The high cholesterol diet also produced just over a doubling of the plasma triglyceride levels. Although there was a modest non-significant reduction in plasma triglycerides by treatment with Nao Xintong alone, a statistically significant 20% reduction in triglycerides was found in the patients treated with the combination of NaoXintong and Dan Hong both at 60 and 90 days.

As can be seen from the wide standard deviations associated with baseline CRP concentrations for each group, baseline CRP varied greatly from rabbit to rabbit. Additionally, Group 2 demonstrated a significant rise in CRP at 60 days, but this returned to baseline by 90 days. No significant rise, over time, in CRP was noted in Group 3. Group 4, likely by chance, had a higher mean CRP at baseline than groups 1, 2 or 3. However, no rise occurred over time, and a statistically significant reduction in mean CRP from baseline was found at both 60 and 90 days. Mean CRP change scores from baseline to 60 and 90 days are shown in Figure 2. These showed no change over time in CRP in Group 1. In Group 2, there was a significant rise in CRP by 60 days that resolved by 90 days. In group 3, there was a non-significant rise in CRP at both 60 and 90 days. In group 4, there was a significant reduction in CRP from baseline both at 60 and 90 days.

Rabbit Treatment Group	PAS±SD
Group 1 (N=12, Normal Diet / No Medication):	0% - No plaque
Group 2 (N=12, High Cholesterol Diet / No Medication):	31.4±10.8%
Group 3 (N=13, High Cholesterol Diet / NaoXintong):	37.7±10.6%
Group 4 (N=13, High Cholesterol Diet / NaoXintong + Dan Hong)	0.1±11.1%

PAS = Percent Area stenosis; SD = Standard Deviation

Table 1: Atherosclerosis Percent Area Stenosis of Rabbit Thoracic Aortas By Treatment Group.

Treatment Group	Baseline	60 Days	90 Days
	Total Cholesterol Levels ± SD (mg/dL)		
Group 1	102.4±37.4	63.2±11.4	53.8±16.3
Group 2	102.1±28.4	2107±343*	1483±254*
Group 3	106.2±38.4	1928±584*	1550±436*
Group 4	108.5±33.0	1808±236*	1486±326*
	Triglyceride Levels ± SD (mg/dL)		
Group 1	88.9±18.8	39.2±6.4	47.4±25.5
Group 2	83.5±40.1	237.2±54.2*	228.8±68.7*
Group 3	112.6±58.7	223.5±71.7*	193.8±56.3*
Group 4	78.3±43.1	177.9±46.5*#	170.8±36.2*#
	CRP Concentrations ± SD (mcg/mL)		
Group 1	16.9±9.8	16.8±19.8	15.2±31.1
Group 2	13.7±5.6	26.3±20.1^	13.0±9.0
Group 3	12.4±13.1	16.7±15.0	17.8±11.9
Group 4	26.0±28.1	11.8±7.1^	19.2±24.7^

* = P<0.001 versus Group 1; # = P=0.04 versus Group 2; ^ = P<0.05 versus baseline for the same group

Table 2: Results of mean plasma total cholesterol, triglyceride and CRP levels, based on treatment group, and reported at baseline, 60 days and 90 days.

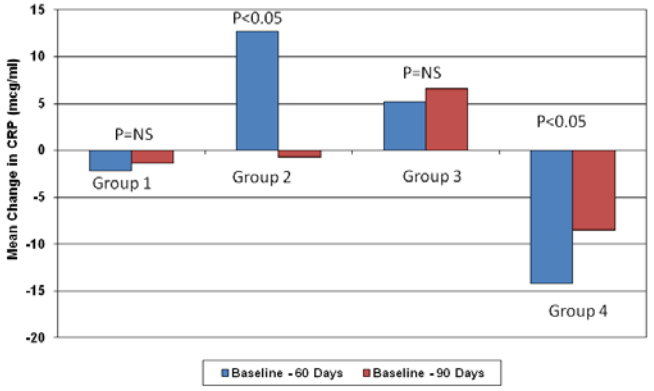

Figure 2: Mean change score of CRP concentration from baseline to 60 and 90 days for each treatment group. Group 1 = control; Group 2 = fat chow, no therapy; Group 3 = fat chow, Nao Xintong; Group 4 = fat chow, Nao Xintong +Dan Hong.

Discussion

This paper presents the results of the first randomized trial performed outside of China that has objectively evaluated the effects of two commonly used traditional Chinese herbal medicines, Nao Xintong and Dan Hong, on a rabbit model fed a very high cholesterol diet. In summary, no effect on aortic atherosclerosis progression was seen from either Nao Xintong or Dan Hong. However, treatment with the combination of Nao Xintong and Dan Hong did result in a significant reduction in both triglyceride levels and plasma CRP concentrations.

Nao Xintong consists of a proprietary pure herbal oral preparation with ingredients from sixteen varieties of Chinese herbs including Huang Qi, Dan Shen,Chishao, Danggui, Chuangxiong, Hong Hua, Ru Xiang, Mo Yao, Jixueteng, Niuqi, Guizhi, Sangzhi, Quan Xie, Di Long, Shuizhi and Tao Ren [10]. This traditional Chinese preparation is thought to have properties capable of inhibiting platelet aggregation in a manner similar to the Chinese traditional medicine named Huoxuehuayu. It is also felt to play a role in the regulation of lipids [11,12] and inflammation [13,14] in such a manner as to be protective of endothelial cells [2,4]. Chinese studies have demonstrated that it may have a role in the prevention of atherosclerosis [15,16]. Dan Hong consists of an injectable extract from safflower and is felt to have anti-atherogenic effects including inhibition of platelet aggregation, anti-thrombosis, anti-oxidation and the protection of cardiac microvascular endothelial cells [17]. Both are extensively used clinically in China, but as of yet have undergone little evaluation elsewhere.

In our study, neither Nao Xintong nor the combination of Nao Xintong and Dan Hong, had any effect on the development of atherosclerotic intimal plaque in the thoracic aorta of a rabbit fed a very high cholesterol diet and followed for three months. This may represent that the tested agents have no direct effect on atherosclerotic

plaque. However, it may also be explained by our use of an extremely hypercholesterolemic rabbit model that received a 2% cholesterol enriched diet. The advantage of this model is that it results in a large accumulation of atherosclerotic plaque in a short time; thus providing the opportunity to make precise comparisons between study groups [18]. However a potentially significant disadvantage to this model is that the unnaturally high induced levels of cholesterol may produce such a malignant form of atherosclerosis progression that the effects of a modestly active agent may be completely overshadowed. It is useful as a study model, but certainly does not perfectly mimic atherosclerosis in humans. The use of a longer (6-12 months) term administration of a lower (0.2 - 0.6%) amount of cholesterol [19] perhaps should be considered in future studies.

But despite these disadvantages, this model did demonstrate significant biological activity for especially the combination of both agents. Specifically, the combination of NaoXintong and Dan Hong reduced levels of both CRP and triglycerides. Because of the known atherogenic effects of both systemic inflammation and hypertriglyceridemia, the potential for positive, clinically relevant effects on human atherosclerosis progression from the use of these herbal supplements remains high. These findings certainly justify further clinical trials involving these agents.

Conclusions

Although this single study does not completely elucidate all the potentially beneficial effects of Nao Xintong and Dan Hong, it does demonstrate, in an objective unbiased way, that they both have real physiologic effects above and beyond placebo and therefore justify further study. They are both extensively used in China and have been found to have very few side effects. Their positive effects both on inflammatory markers and triglycerides in this high cholesterol rabbit model may well translate into significant human clinical benefit.

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Author Disclosure Statement

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Junhui Xiao, MD:	Nothing to disclose
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