Brief report

Emodin inhibits dietary induced atherosclerosis by antioxidation and regulation of the sphingomyelin pathway in rabbits

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Emodin is an important component of rhubarb, a traditional Chinese herb. Previous studies *in vitro* showed that emodin inhibited the proliferation of human vascular smooth muscle cells,[1] indicating the possible inhibitive effect of emodin on atherosclerosis. The present research evaluated the effect of emodin on the formation of atherosclerotic lesion induced by high cholesterol and fat diet in rabbits and the mechanism of this effect.

METHODS

Animal groups and diet

Five-month old male New Zealand white rabbits, weighing (2.0 ± 0.2) kg, were purchased from Medical Experimental Animal Centre of Sun Yat-Sen University in China. Animal welfare assurance: 2001A033.

Rabbits were randomly divided into 4 groups of 8: control, atherosclerosis, emodin and fenofibrate. The control rabbits were fed on a regular diet, while all other rabbits were fed on diet including 1% cholesterol and 5% fat for 10 weeks. Each rabbit was fed 135 g to150 g food per day and had free access to water.

During the last 4 weeks of diet, the rabbits in emodin and fenofibrate groups were given emodin (10 mg/kg, Guangzhou Chinese Medicine Company) or fenofibrate (25 mg/kg) orally, each of which was dissolved in 2 ml normal saline. The rabbits in control and atherosclerotic model groups were given 2 ml normal saline at the same time.

Measurements

Rabbit plasma was collected and prepared for measurement of the concentrations of total cholesterol (TC) and triglyceride (TG) by an automatic blood chemical analyser. Plasma superoxide dismutase (SOD) activity and malondialdehyde (MDA) concentration were also measured. Low density lipoprotein (LDL) was isolated by potassium bromide, stepwise density gradient, ultracentrifugation and plasma oxidized LDL (OxLDL) was detected by enzyme linked immunosorbent assay (ELISA).

The aortas were excised from the beginning of aortic arch to the abdominal crotch. Atherosclerotic lesion area and total area of the whole aorta were measured using a computer programme. Total Lipids were extracted from equal lengths of aorta after lesion analysis. Aortic ceramide (CER) content was determined by high performance, thin layer, chromatography (HPTLC) and sphingomyelinase (SMase) activity was counted with FNJ-182 radioimmunization counter. The apoptotic foam cell within aortic atherosclerotic lesions was detected by DNA transferase *in situ* nicking distal end labelling (TUNEL).

Statistical analysis

Data are presented as mean±standard deviation. Statistical analysis was carried out by analysis of variance (ANOVA) followed by contrast analysis. Significant difference was accepted at $P<0.05$.

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Table 1. Atherosclerotic lesion area, plasma levels of TC and TG, plasma activity of SOD and plasma concentration of MDA and OxLDL (mean±SD, *n* = 8)

Groups	Lesions area (%)	TC (mmol/L)	TG (mmol/L)	SOD (U/ML)	MDA (nmol/mL)	OxLDL (mg/L)
Control	0	1.66±0.88#	0.86±0.33*	197.57±28.87#	9.28±3.33#	0.132±0.046#
Model	48.87±15.5	38.23±13.82	1.31±0.37	89.78±23.73	17.29±5.13	0.465±0.057
Emodin	22.19±12.9#	33.98±11.76	1.24±0.31	78.29±18.32#	11.99±2.23*	0.317±0.065*
Fenofibrate	35.57±12.4	21.75±10.22*	0.92±0.17*	95.68±19.23	16.73±4.99	0.397±0.084

*p<0.05, #P<0.01, compared with atherosclerotic model group

Table 2. SMase activity, CER content and apoptotic foam cell index in aorta (mean±SD, *n*=8)

Groups	SMase activity (nmol • h^{-1} • mg^{-1})	CER content (μg/mg)	Apoptotic foam cell index (%)
Control	0.27±0.02#	4.03±0.73#	0
Model	1.38±0.35	10.01±2.04	58±12
Emodin	0.43±0.12#	5.19±1.22#	27±14#
Fenofibrate	1.33±0.24	9.57±2.12	40±11*

*P<0.05, #P<0.01, compared with atherosclerotic model group.

RESULTS

Effects of emodin on atherosclerotic lesions, plasma lipid and plasma oxidative activity

All rabbits fed 1% cholesterol and 5% fat diets had hyperlipidaemia. The plasma TC and plasma TG were decreased by 43% and 30% respectively in fenofibrate treated rabbits compared with atherosclerotic rabbits. The plasma SOD activity increased and the concentrations of plasma MDA and OxLDL decreased significantly in emodin treated rabbits, although their plasma TC and TG did not change compared with atherosclerotic rabbits. Atherosclerotic lesions were significantly decreased in the rabbits of emodin group contrasted to atherosclerotic model group (Table 1).

Effects of emodin on aortic SMase activity, CER content and apoptotic foam cell index

Compared with control group, the CER content and the SMase activity in rabbit aorta of atherosclerotic model group increased significantly. The CER concentration, SMase activity and apoptotic foam cell, index in rabbit aorta of emodin group were significantly lower than that of atherosclerotic group (Table 2).

DISCUSSION

SMase catalyses the hydrolysis of sphingomyelin, a constituent of plasma lipoproteins and mammalian plasma membranes, to CER and choline phosphate. Studies have revealed that anoxia, oxidative stress, ionizing radiation, cytokine and other stimuli could activate phospholipase C through the phosphatidylinositol 3-kinase pathway resulting in the formation of CER.[2] CER has been implicated in many biochemical pathways, including cell signalling events that lead to cellular differentiation, apoptosis, and inflammatory response.[3,4] In the vascular system, CER regulates both apoptosis and inflammation. CER may contribute to the development of atherosclerotic or thrombotic disease. Hyperlipaemia can damage endothelial cells and activate SMase, which induces apoptosis by elevating the amount of CER. Furthermore, reactive oxygen species are activators of SMase,[2,5] so antioxidants should inhibit SMase activity and reduce the production of CER. Therefore, adjustment of blood lipid, enhancement of antioxidative property, reduction of the amount of CER as well as impeding apoptosis can effectively prevent the formation of atherosclerotic lesions.

The results of our study showed that, in atherosclerotic rabbits, no significant alteration in plasma TC and TG were detected in emodin treated rabbits and the antioxidative activity of emodin was identified. Emodin inhibited the formation of aortic atherosclerotic lesion through reducing the plasma concentration of MDA and OxLDL as well as CER, SMase activity and apoptotic, foam cell, index in aorta. This work indicated the antiatherogenic effect of emodin was related to its antioxidative property and regulative action on sphingomyelin pathway.

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Correction

In the article entitled *Pulnomary alveolar microlithiasis: report of four familial cases* [*Chin Med J 2004; 117(6):950-952*], the address of the author should have read: Department of Respiratory Medicine, Second Affiliated Hospital of Zhejiang University Medical School.