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Effects of Peroxidase on Hyperlipidemia in Mice

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To observe the effects of peroxidase on hyperlipidemia, mice were fed a diet high in cholesterol and fat. At the same time, the mice were given different-purity peroxidase (radish juice, crude radish peroxidase, and horseradish peroxidase), and their serum cholesterol, triglyceride, blood glucose, amylase, and esterase activities, and malondialdehyde in the mouse small intestines and livers, were tested after 15 days on the test diets. The results showed that peroxidase decreased the levels of total serum cholesterol, triglyceride, blood glucose, and lipid peroxidation in the small intestines and livers of hyperlipidemic mice. This suggests that peroxidase may be a contributing factor in the prevention of hyperlipidemia.

KEYWORDS: Radish juice; crude radish peroxidase; horseradish peroxidase; cholesterol; triglyceride; blood glucose; malondialdehyde; lipid peroxidation

INTRODUCTION

Modern medical studies show that about 70% of adults over 50 years old suffer atherosclerosis. And hyperlipidemia is a well-known risk factor for atherosclerosis (*1*, *2*). To prevent this disease, a number of hypolipidemic drugs have been administered in-clinic. However, they all have obvious or potential side effects to some extent (*3*). The search continues for safe, dependable, and effective drugs. Epidemiological studies suggest that populations that have high dietary intakes of fruits and vegetables containing natural antioxidants show a reduced incidence of coronary artery disease (*4*). As a result, the potential of antioxidants in the prevention or management of atherosclerosis is being investigated in several large prospective studies. It has been reported that small fruits have high contents of antioxidant compounds including ascorbate, β-carotene, glutathione, anthocyanins, and phenolics. These types of compounds are involved in the oxidative protective process and seem to play an important role in scavenging harmful free radicals and preventing various human diseases (*5*). It is well-known that peroxidase (EC 1.11.1.7, POD), is an oxido-reductase and plays an important role in scavenging oxygen free radical and delaying senescence (*6*, *7*). It has also been used in some "health foods". POD is a protein containing Fe^{2+} heme. Is it degraded by digestive enzymes? What are the effects, if any, when it is in the digestive tract? We have seen no reports addressing these questions. In this study, we look at the actions of different-purity POD in experimental hyperlipidemia in mice to explore a possibly valuable and safe material that lowers serum lipids.

MATERIALS AND METHODS

Animals and Diets. Ten-week-old Kuiming female mice (50) weighing 40 ± 2 g were supplied by the Laboratory Center of Animal, Henan Medical University. The animals were kept in an environmentally controlled animal facility operating on a 12-h dark/light cycle at 25 °C and 55% humidity for 6∼7 days before the experiment, with free access to tap water and a standard diet (Laboratory Center of Animal, Henan Medical University). Mice were randomly divided into five groups with 10 mice in each group. One group of mice was the control; they were orally administered 1 mL of water every day (1 mL/d). A second group of mice was placed on a hyperlipidemic diet, and also orally administered 1 mL/d of water. A third group of mice was designated "radish juice group", because they were orally administered 1 mL/d of Xinlimei radish (*Raphunus sativas L.*) juice (containing POD activity 57.6×10^3 U). A fourth group of mice was designated "radish crude POD group", and they were orally administered 57.6×10^3 U/d of radish crude POD ($RZ = A_{403}/A_{280} = 1$, which is a expression of purity of POD) in 1 mL of distilled water, and a fifth group of mice was given orally 57.6×10^3 U/d horseradish peroxidase (HRP, $RZ = 3$) in 1 mL of distilled water. All administrations were given each afternoon by direct stomach intubation for a 15-day period. Except for the control group, which was allowed free access to tap water and a standard diet, the other groups (although allowed free access to tap water) were offered a high cholesterol and fat diet (88.25% standard diet, 1.5% cholesterol, 10% lard, and 0.25% sodium cholic acid). After the last treatment, all the mice were fasted for 12 h. Then blood samples were collected from the vena ophthalmica. The serum was immediately prepared by centrifugation for 20 min at 4 °C and 1000*g*, and stored at −20 °C until determination. The mice were then sacrificed with intestine and liver tissue removed. These tissues were washed with physiological saline, and then stored at −20 °C until malondialdehyde (MDA) assays were run.

Analysis. The reagent kits for measurement of total blood cholesterol, triglyceride, amylase, and glucose were purchased from Zhongsheng Biotechnology Co., Beijing, and their published procedure was followed. The contents of MDA were determined by the method of improved thiobarbituric acid spectrophotometry (*8*).

Polyacrylamide Gel Electrophoresis. Polyacrylamide gels (7%) were prepared according to Laemmli (*9*), but without SDS and thioethanol (native conditions). Runs were performed at a constant voltage intensity of 200 V per plate, and at a temperature of 4 °C. After the run was completed, the detection solution of esterase

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Table 1. Effects of Peroxidase on Blood Lipid and Blood Sugar Levels

treatment	cholesterol (mmol/L)	triglyceride (mmol/L)	glucose (mmol/L)	amylase (μ/dL)
control group (normal diet)	2.760 ± 0.500	1.028 ± 0.374	4.238 ± 0.768	755.577 ± 26.25
hyperlipidemic diet (HD)	6.646 ± 0.715[a]	1.087 ± 0.132	6.966 ± 0.606[a]	790.731 ± 27.22[b]
HD + radish juice	4.173 ± 0.421[d]	0.617 ± 0.113[d]	5.772 ± 0.843[c]	795.503 ± 43.9
HD + crude radish POD	4.510 ± 0.529[c]	0.558 ± 0.146[d]	5.396 ± 1.445[c]	772.711 ± 40.211
HD + HRP	4.540 ± 1.107[c]	0.686 ± 0.136[c]	5.370 ± 1.245[c]	772.285 ± 102.08

[a] Compared with the control group $p < 0.01$. [b] Compared with the control group $p < 0.05$. [c] Compared with the hyperlipide mice $p < 0.05$. [d] Compared with the hyperlipide mice $p < 0.01$ in the same column.

Table 2. Effects of POD on Contents of Malondialdehyde (MDA) in Tissue (μmol/g Tissue Weight)

treatment	intestinal MDA	liver MDA
control group (normal diet)	75.09 ± 3.70	6.01 ± 1.19
hyperlipidemic diet (HD)	89.52 ± 15.53[a]	18.60 ± 7.07[b]
HD + radish juice	58.58 ± 7.64[c]	9.77 ± 3.75[c]
HD + crude radish POD	47.79 ± 4.15[d]	10.26 ± 3.97[c]
HD + HRP	57.44 ± 6.48[d]	11.11 ± 5.89[c]

[a] Compared with the control group, $p < 0.05$. [b] Compared with the control group, $p < 0.01$. [c] Compared with the hyperlipidemic control group, $p < 0.05$. [d] Compared with the hyperlipidemic control group, $p < 0.01$ in the same column.

isoenzyme was prepared according to the method of Hu and Wan (*10*). The enzyme substrates were 1-naphthyl and 2-naphthyl acetate. After staining, the gel was scanned by Tanon GIS-1000 gel documentation and digital system.

Statistical Analysis. Data are presented as individual group mean ± SD, and the differences between the control and dosing groups were statistically analyzed with a significant level of less than 5%. Student's *t*-test was used for all pairwise comparisons. The statistical analysis was performed with the computer program SPSS 9.0 for windows (SPSS Inc).

RESULTS

Effects of POD on Blood Lipid and Glucose. After the mice were fed on a high cholesterol and fat diet for 15 days, the contents of serum cholesterol, amylase, and glucose distinctly increased compared with those of the control group, with the serum triglyceride levels increasing. **Table 1** shows that radish juice, crude radish POD, and HRP all can decrease the contents of serum lipid and glucose compared with the hyperlipidemia group. They had the trend of lowering amylase activities compared with those of the hyperlipidemia model group.

Effects of POD on Lipid Peroxidation. Table 2 shows that the high cholesterol and high fat feed enhanced the lipid peroxidation levels. That is, the levels of small intestine and liver MDA of the hyperlipidemia group were increased evidently compared with those of the control group. Radish juice, crude radish POD, and HRP all decreased the contents of small intestine and liver MDA compared with those of the hyperlipidemia group in our experiment.

Effects of POD on PAGE Pattern of Esterase Isoenzyme. PAGE separated the serum esterase into five type regions: A, B, C, D, and E (**Figure 1**). The control group had no E_3 band. The radish juice, crude radish POD, and HRP all decreased the esterase activities contrasting with those of the hyperlipidemia group. On the basis of our assays given in **Table 2** it is noted that the radish juice shows A, B_1 band but no band at E_2.

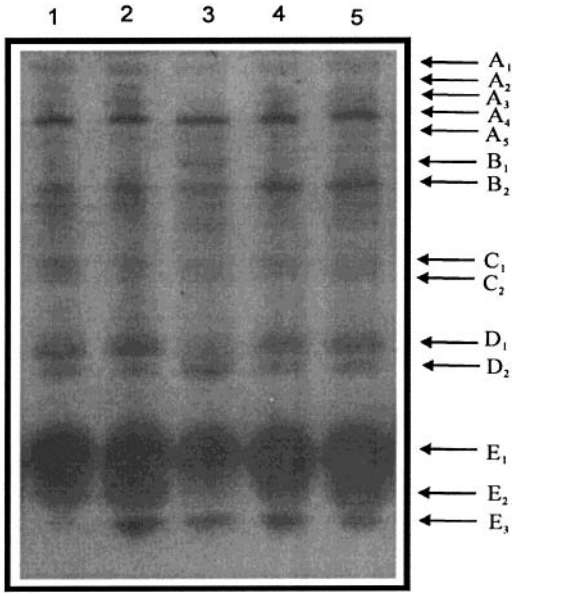

Figure 1. PAGE of esterase isoenzymes in mice. Lane 1 is the control group, lane 2 is the hyperlipidemic group, lane 3 is the radish juice group, lane 4 is the crude radish POD, and lane 5 is the HRP group. The positions of esterase isoenzymes are shown on the right.

DISCUSSION

Total cholesterol is believed to be the main factor causing hyperlipidemia (*1*, *2*), and is one of the risk factors causing atherosclerosis and coronary heart diseases (*11*, *12*). It is known that a diet high in cholesterol and fat will cause hyperlipidemia in animals (*13*). In this study we find that the total serum cholesterol of the hyperlipidemic group is significantly higher than that of the control group. **Table 1** shows that a high cholesterol and fat diet made serum total cholesterol increase along with blood glucose in hyperlipidemic mice. **Figure 1** shows that serum esterase isoenzyme bands are only slightly different among treatments. Compared with the control group, the high cholesterol and fat diet increased the expression of esterase isoenzyme (E_3 bands). And there were lighter stains in radish juice, crude radish POD, and HRP than in the hyperlipidmic group. These indicated that POD decreased the expression or activity of esterase isoenzyme and resulted in change of lipid metabolism. There were obvious differences in esterase isoenzyme between the radish juice group and others (no E_2 and addition B_1 band). It may be that other materials also effected esterase expression. In the experiment the substrates of esterase were 1-naphthyl and 2-naphthyl acetate. Their molecular structures are similar to that of cholesterol ester. **Table 1** shows that the total serum cholesterol has a change similar with that of esterase isoenzyme. It may be the result of a relation between them. On the other hand, POD can decrease the blood glucose and have no effect on serum amylase (statistically). Although POD and amylase coexist in the submandibular gland (*14*), POD had no influence on amylase secretion while POD was given orally. Inhibition of the amylase activity lowered the

serum glucose (*15*). High blood glucose may partially result from rising amylase in hyperlipidemic mice.

MDA is the indicator of lipid peroxidation, and the levels of MDA reflect indirectly the levels of lipid peroxidation in an organ. The contents of MDA in tissue reflect the degree of lipid peroxidation in tissue (*16*). The liver is the main organ that synthesizes and transfers cholesterol. At the same time, the intestine is the main organ that digests lipid from animal diet. The lipid peroxidation of hyperlipidemic mice in the small intestine and liver were enhanced compared with those of the control group, so the lipid peroxidation may also be a tissue change and may affect lipid metabolism in hyperlipidemia. POD decreased the contents of MDA of liver and intestine, resulting in lowering the lipid peroxidation in tissue.

It has been reported that antioxidants can help prevent atherosclerosis (*16*, *17*). Do the antioxidants with high molecular weight have preventive action? Our results showed that the radish juice, crude radish POD, and HRP all lowered the serum cholesterol and triglyceride. Although the difference in POD sources is substantial, there is no difference in their action on the levels of cholesterol and triglyceride. This finding suggests that POD might prevent forming hyperlipidemia and may be related to its function of scavenging free radicals. The way of action needs to be investigated further.

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