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Effect of oyster mushroom and isolated β-glucan on lipid peroxidation and on the activities of antioxidative enzymes in rats fed the cholesterol diet

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The effect of 5% dried oyster mushroom (Pleurotus ostreatus) *or β-glucan isolated from oyster mushroom on cholesterol levels in serum and liver, on lipid peroxidation, and on activities of antioxidative enzymes was studied in male Wistar rats. Animals were fed a diet with 0.3% cholesterol for 10 weeks after weaning. The diet containing whole oyster mushroom strikingly reduced cholesterol content in serum and in liver (by 27 and 46%, respectively). In addition, a shift in cholesterol distribution in lipoproteins (70% decrease in very low-density lipoprotein cholesterol and 50% increase in high-density lipoprotein cholesterol) was observed. β-glucan in the diet did not affect cholesterol levels in serum and liver. Whole oyster mushroom reduced the levels of conjugated dienes in erythrocytes and in liver (by 40% and 36%, respectively), reduced the activity of catalase in erythrocytes and stimulated the activities of superoxide dismutase, catalase and glutathione peroxidase in liver by 30% to 70%. The diet supplemented with β-glucan did not affect neither lipid peroxidation nor activities of antioxidative enzymes.* (J. Nutr. Biochem. 8:469–471, 1997) © *Elsevier Science Inc. 1997*

Keywords: oyster mushroom; β-glucane; cholesterol; lipid peroxidation

Introduction

Oyster mushroom (*Pleurotus ostreatus*) is a wood-rotting mushroom produced on large scale for the use in food industry. We reported earlier that supplementation of the diet by oyster mushroom effectively retarded the progression of hypercholesterolemia and accumulation of cholesterol in liver induced by cholesterol diet (0.3%) in rats.[1] The mechanisms of hypocholesterolemic effect of oyster mushroom include delayed absorption of cholesterol followed by reduced production of cholesterol-rich very-low-density lipoproteins (VLDL).[1,2] This effect could be mediated by oyster mushroom fibrous matter that is able to sequester bile acids thus inducing acceleration of cholesterol catabolism (analogously to the effect of dietetic fibrous matter from other sources).[3] It was found that oyster mushroom inhibited in vitro peroxidation of lipids.[4] Because β-1, 3-D-glucan is the exclusive representative of fibrous matter in oyster mushroom, we tested the effect of diet supplemented with this polysaccharide isolated from oyster mushroom on the levels of cholesterol in serum and liver, on lipid peroxidation and on antioxidative enzyme activities in rats fed the cholesterol diet.

Methods and materials

Male rats (strain Wistar, Velaz, Czech Republic; n = 30) with initial body weight of 65 gm were used in the experiment. Animals were bred in standard conditions without modification of light regime. They had unrestricted access to drinking water and to feed of following composition[5] (in %): starch 60, casein 18, pork fat 10, cellulose 6, mineral mixture 4, vitamin mixture 1, Fel tauri (commercially available ox bile) 0.55, cholesterol 0.30, and choline chloride 0.15. Composition of vitamin and mineral mixtures was as described.[5] Animals were splitted into three equally sized groups. One-third (control group) was fed this standard diet without any further modification. Animals in experimental groups received diet containing 1% cellulose, whereas the rest of cellulose was substituted either by 5% of powdered dried fruiting bodies of oyster mushroom (mushroom group) or by 5% of β-1, 3-D-glucan

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Table 1 Cholesterol concentration in serum, lipoproteins, and in liver of rats fed a diet containing whole oyster mushroom or β-glucan

	Control	Group oyster mushroom	β-glucan
Body weight (gm)	300 ± 14	298 ± 11	305 ± 12
Cholesterol			
Serum (mmol.l^{-1})	4.00 ± 0.32	2.93 ± 0.11^{b}	4.36 ± 0.58
VLDL (mmol.l^{-1})	1.81 ± 0.26	0.55 ± 0.05^{d}	2.10 ± 0.45
LDL (mmol.l^{-1})	1.20 ± 0.24	0.67 ± 0.07^{a}	1.11 ± 0.17
HDL (mmol.l^{-1})	1.08 ± 0.11	1.63 ± 0.09^{c}	0.95 ± 0.17
Liver (mmol.kg^{-1})	226 ± 13	123 ± 11^{d}	204 ± 12

Values are means ± SEM (for n = 10 in all groups).
$^{a-d}$Statistical significance (Student's t-test) compared to control group: $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.002$, $^{d}P < 0.001$.
VLDL: very-low-density lipoproteins; LDL: low-density lipoproteins; HDL: high-density lipoproteins.

(glucan group). Powdered oyster mushroom contained (in %): polysaccharides 65 to 70, proteins 20 to 25, lipids 2.2, ashes 4.8, and water up to 5.

β-glucan was isolated from fresh fruiting bodies of oyster mushroom by following procedure.[6] Oyster mushroom fruiting bodies were homogenized in blender and extracted in 0.15 aqueous sodium hydroxide solution under stirring at 5°C for 2 hr. The mixture was centrifuged (1500 × gm/10 min), the sediment was washed with water until neutral and suspended in 0.06% aqueous sodium chloride solution. pH was adjusted to 4.5 with acetic acid, the suspension was stirred for 6 hr. at 50°C and centrifuged again. The residue was washed successively with water and acetone and then dried in vacuo at 60°C.

After 10 weeks of feeding, animals (fasted for 18 hr.) were killed by decapitation in light ether narcosis. Cholesterol in serum and in lipoproteins was estimated with Oxochrom-Biolatest kit (Czech Republic). Lipoproteins were isolated by sequential flotation on ultracentrifuge as described earlier.[1] Cholesterol in chloroform-methanol (2:1) extract from liver was estimated by Biolatest (Czech Republic).

Concentration of conjugated dienes of fatty acids in erythrocytes and liver was measured by spectrophotometry at 233 nm in heptane lipid extracts.[7] The results were expressed as optical density (D) per 1 mL of erythrocytes (or 1 gm of liver). Activity of superoxide dismutase was estimated by commercial test kit (Randox Lab. Ltd). Previously published procedures were used for measurement of activities of catalase and glutathione peroxidase in blood and liver, as well as protein concentration in liver.[8] The results were statistically evaluated by Student's t-test.

Results

Feeding the diet containing whole oyster mushroom or β-glucan did not affect the final body weight of experimental animals. Oyster mushroom diet reduced cholesterol level in serum and liver (by 27% and 45%, respectively). The distribution of cholesterol in lipoproteins was significantly shifted toward physiological pattern: cholesterol content in VLDL decreased by 70%, whereas it increased in high-density lipoproteins (HDL) by 50%. β-glucan in the diet did not affect neither cholesterol levels in serum and liver nor its distribution in lipoproteins (*Table 1*).

Oyster mushroom diet reduced significantly concentration of conjugated dienes in erythrocytes and liver (by 43% and 35%, respectively), whereas β-glucan did not affect lipid peroxidation. Oyster mushroom in the diet reduced the activity of catalase in erythrocytes and strikingly stimulated activities of superoxide dismutase, catalase, and glutathione peroxidase in liver. Glucan in the diet did not alter the activities of antioxidant enzymes in liver or in erythrocytes (*Table 2*).

Table 2 Lipid peroxidation and antioxidant enzyme activities in erythrocytes and in liver of rats fed a diet containing whole oyster mushroom or β-glucan

	Control	Group oyster mushroom	β-glucan
Conjugated dienes			
Erythrocytes (D · ml^{-1})	9.50 ± 1.1	5.41 ± 0.86^{b}	8.32 ± 0.95
Liver (D · g^{-1})	7.70 ± 0.53	4.97 ± 0.21^{e}	6.95 ± 0.42
Erythrocytes (μmol · ml^{-1} blood)			
SOD	197 ± 10	181 ± 6	188 ± 9
CAT	664 ± 53	435 ± 53^{b}	763 ± 48
GSH-Px	3.26 ± 0.31	4.11 ± 0.38	2.98 ± 0.31
Liver (U · mg^{-1} protein)			
SOD	9.54 ± 0.75	16.97 ± 1.02^{d}	11.26 ± 0.88
CAT	6.58 ± 0.37	8.65 ± 0.51^{b}	6.51 ± 0.63
GSH-Px	0.0805 ± 0.0032	0.1412 ± 0.0095^{d}	0.089 ± 0.003

Values are means ± SEM (for n = 10 in all groups).
SOD, superoxid dismutase; CAT, catalase; GSH-Px, glutathioneperoxidase.
$^{a-d}$Statistical significance (see *Table 1*).

Discussion

Oyster mushroom-derived glucan used in this study is water-insoluble β-1, 3-D-homeopolyglucan (trivial name pleuran) containing a small proportion (7%) of (1, 6) and (1, 4)-linked internal residues and about 5% of chitin in the dry matter.[6] Oyster mushroom contains approximately 55% of insoluble and 10 to 15% of soluble pleuran fraction. In spite of almost doubled dose of this polysaccharide in the glucan diet (compared with corresponding glucan content in the diet supplemented with 5% of whole oyster mushroom) we did not find any change in cholesterol level in serum or liver in animals fed this diet. Our observation is actually in agreement with the conclusion of several authors that hypocholesterolemic effect is primarily linked to soluble components of dietetic fiber.[9] This implies that β-glucan does not interfere so significantly with cholesterol metabolism as originally assumed. Hypocholesterolemic effect of oyster mushroom is mediated in the first place by repressed formation of cholesterol-rich VLDL induced by cholesterol diet. Cholesterol distribution in lipoproteins was shifted to more physiological pattern with 60 to 70% of plasma cholesterol carried by HDL. Reduction of cholesterol absorption and acceleration of cholesterol catabolism by oyster mushroom in the diet is probably a cumulative effect of several compounds from oyster mushroom having the potential to affect these processes, such as sterols, chitin-chitosan, proteins, and probably also soluble components of β-glucans (as we mentioned previously).[2] The role of chitin in the hypocholesterolemic effect is not clear because the degree of metabolic conversion (deacetylation) of chitin to active chitosan in the gastrointestinal tract of rats is not known yet.[10] Hypocholesterolemic effect of 5% chitosan in the diet was described in rats.[11] Furthermore, hypocholesterolemic effect of oyster mushroom definitely includes the effect of mevinolin (an inhibitor of the key enzyme of cholesterol biosynthesis HMG-CoA reductase[12]—isolated recently from oyster mushroom), as well as the effects of several unidentified compounds that accelerate catabolism of all cholesterol-carrying lipoproteins.[1,13]

Contrary to the effect of whole oyster mushroom, β-glucan did not affect lipid peroxidation indicated by concentration of conjugated dienes as the predominant initial peroxidation products. Similarly β-glucan did not alter the activities of antioxidant enzymes catalyzing the removal of deleterious oxygen intermediates. Filipek[4] explained antilipoperoxidative activity of oyster mushroom extracts in vitro by the presence of chitin in these extracts because some carboxymethylglucans are able to quench free radicals. There is no additional information explaining the ability of oyster mushroom to affect lipid peroxidation and the activity of the protective enzymatic system. However, we think that it is not a mere coincidence that hypocholesterolemic and antilipoperoxidative effect of oyster mushroom correspond to the effect of some commonly used hypocholesterolemic drugs with similar mechanisms of activity as we have experimentally demonstrated for oyster mushroom. The examples of such drugs are cholestyramin (active via sequestration of bile acids that stimulates their excretion and reduces their recovery in liver with subsequent acceleration of cholesterol catabolism) and pravastatin (inhibitor of HMG-CoA reductase), both reducing simultaneously the susceptibility of lipoproteins to lipid peroxidation.[14] Our original idea was that β-glucan could be the common denominator of hypocholesterolemic and antilipoperoxidative effect of oyster mushroom. Reactive oxygen species are thought to be the causative agent in pathogenesis of atherosclerosis, carcinomas and of inflammatory or degenerative processes. Immunostimulatory and antimutagenic[15] activities of parenterally administered β-glucan isolated from oyster mushroom and from the related species Lentinus edodes have been already demonstrated and could be explained by their ability to quench free radicals. However, we have found recently that the diet containing whole oyster mushroom or β-glucan isolated from this mushroom did not show any immunostimulatory effect (unpublished results). It is evident from these results and from the general context that insoluble β-glucan from oyster mushroom (at least at peroral administration) does not participate in hypocholesterolemic and antilipoperoxidative effect of oyster mushroom.

References

1 Bobek, P., Kuniak, Ľ., and Ozdín, Ľ. (1993). The mushroom pleurotus ostreatus reduces secretion and accelerates the fractional turnover rate of very-low-density lipoproteins in the rat. *Ann. Nutr. Metab.* **37,** 142–145

2 Bobek, P., Ozdín, Ľ., and Kuniak, L. (1994). Mechanism of hypocholesterolemic effect of oyster mushroom (Pleurotus ostreatus) in rats: Reduction of cholesterol absorption and increase of plasma cholesterol removal. *Z. Ernährungswiss.* **33,** 44–50

3 Vahouny, G.V., Tombes, R., Cassidy, M.M., Kritchevsky, D., and Gallo, L.L. (1980). Dietary fibers. V. Binding of bile salts, phospholipids and cholesterol from mixed micels by bile acid sequestrans and dietry fibers. Lipids **15,** 1012–1018

4 Filipek, J. (1992). The effect of the mushroom pleurotus ostreatus on the lipid peroxidation of phosphatidylcholine liposomes. Pharmazie **47,** 393

5 Yamashita, S., Yamashita, K., and Yasuda, H. (1980). High-fibre diet in the control of diabetes in rats. Endocrinol Jap. **27,** 169–173

6 Karácsonyi, Š. and Kuniak, Ľ. (1994). Polysaccharides of pleurotus ostreatus: Isolation and structure of pleuran, an alkali-insoluble β-D-glucan. Caarbohydrate Polymers **24,** 107–111

7 Recknagel, R. and Glende, E.A. (1984). Spectrophotometric detection of lipid conjugated dienes. In: *Methods in Enzymology* (S.R. Colowick and N.O. Kaplan, eds.), pp. 331–337 Academic Press, San Diego, CA USA

8 Volkovová, K., Chorváthová, V., Jurčovičová, M., Köseghyová, L. and Bobek, P. (1993). Antioxiadative status of the myocardium and kidneys in acute diabetic rats. *Physiol. Res.* **42,** 251–255

9 Story, J.A. (1985). Dietary fiber and lipid metabolism. Proc. Soc. *Exper. Biol. Med.* **180,** 447–452

10 Zemek, J., Kučár, Š., and Anderle, D. (1987). The enzyme partial deacetylation of 1,6-anhydro-2,3,4-tri-O-acetyl-β-D-glucopyranose. *Coll. Czechoslovak Chem. Commun.* **52,** 2347–2352

11 Jennings, C.D., Boleyn, K., Bridges, S.R., Wood, P.J., and Anderson, J.W. (1988). A comparison of the lipid-lowering and intestinal morphological effects of cholestyramine, chitosan, and oat gum in rats. *Proc. Soc. Exp. Biol. Med.* **189,** 13–20

12 Gunde-Cimerman, N., Friedrich, J., Cmerman, A., and Benički, N. (1993). Screening fungi for the production of an inhibitor of HMG CoA reductase: Production of mevinolin by the fungi of the genus pleurotus. *FEMS Microbiol. Lett.* **111,** 203–206

13 Bobek, P., Ginter, E., and Ozdín, L. (1993). Oyster mushroom (Pleureotus ostreatus) accelerates the plasma clearance of low-density and high-density lipoproteins in rats. *Nutr. Res.* **13,** 885–890

14 Hoffman, R., Brock, G.J., and Aviram, M. (1992). Hypolipidemic drugs reduce lipoprotein susceptibility to undergo lipid peroxidation: In vitro and in vivo studies. *Atherosclerosis* **93,** 105–113

15 Yoshioka, Y., Tabeta, R., Saito, H., Uchara, N., and Fukuoka, F. (1985). Antitumor polysaccharides from P. ostreatus (fr.) quel: Isolation and structure of a β-glucan. *F. Carbohydr. Res.* **140,** 93–100