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Mechanism of hypocholesterolemic effect of oyster mushroom (Pleurotus ostreatus) in rats: reduction of cholesterol absorption and increase of plasma cholesterol removal

P. Bobek, L. Ozdin, and L. Kuniak[1]

Research Institute of Nutrition, Bratislava
[1] Faculty of Chemical Technology, Slovak Technical University, Bratislava

Mechanismus des Hypocholesteroleffektes des Austernpilzes (Pleurotus ostreatus) bei Ratten: Verminderte Cholesterolabsorption und erhöhte Entfernung von Cholesterol aus dem Plasma

Summary: The content of cholesterol in the serum and liver of male Wistar rats fed, for the period of 8 weeks shortly after weaning, a diet containing 0.3 % of cholesterol was reduced by 33 and 27 % by the addition of 5 % of dried oyster mushroom powder. Although the level of serum triacylglycerols was not affected by oyster mushroom, their content in liver of rats on mushroom diet was reduced by 41 %. Very-low-density lipoproteins and low-density lipoproteins participated by 55 and 38 %, respectively, in the total reduction of serum cholesterol. Cholesterol content in high-density lipoproteins was not significantly affected by oyster mushroom. Cholesterol absorption as determined by dual-isotope plasma ratio method was significantly reduced by 14 % with oyster mushroom diet. Similarly, this diet increased by 42 % the fractional catabolic rate of cholesterol determined by the analysis of decay curve of [4-^{14}C]cholesterol.

Zusammenfassung: Acht Wochen nach der Absetzung männlicher Ratten (Stamm Wistar) wurden die Tiere mit zwei Diätvarianten gefüttert: Die erste erhielt eine Zugabe von 0,3 % Cholesterol, die zweite zusätzlich noch 5 % von getrocknetem und gemahlenem Austernpilz. Die Zugabe von Austernpilz zur Cholesteroldiät verursachte eine Herabsetzung des Serumcholesterolspiegels um 33 % und des Cholesterolgehaltes in der Leber um 27 %. Der Gehalt an Serumtriazylglyzerolen wurde durch die Pilzzugabe zur Diät nicht beeinflußt, aber deren Gehalt in der Leber wurde um 41 % verringert. An der Herabsetzung des Serumcholesterolspiegels waren die Lipoproteine von sehr niedriger Dichte mit 55 % und die Lipoproteine von niedriger Dichte mit 38 % beteiligt. Der Cholesterolgehalt in Lipoproteinen hoher Dichte wurde durch den Austernpilz nicht signifikant beeinflußt. Die Austernpilzzugabe zur Diät setzte die Cholesterolabsorption signifikant um 14 % herab, was mittels der Verhältnismethode der Dualisotopenplasma-Methode bestimmt wurde. Die Austernpilze enthaltende Diät beschleunigte um 37 % den Fraktionsveränderungsgrad des Cholesterols, was mittels der Zerfallskurvenanalyse von 4-^{14}C-Cholesterol bestimmt wurde.

Key words: Oyster mushroom – cholesterol – absorption – catabolism

Schlüsselwörter: Austernpilz – Cholesterol – Absorption – Katabolismus

Abbreviations:
VLDL – ver- low-density lipoprotein;
LDL – low-density lipoprotein;
HDL – high-density lipoprotein;
FCR – fractional catabolic rate

Introduction

Based on recent knowledge, it is generally accepted that the reduction of high serum cholesterol level plays an important role in the prevention of atherosclerosis (17). In spite of the fact that fungi are almost ideally suited to the requirements of dietetic prevention of coronary heart disease due to their high content of fiber, sterols, proteins and microelements, little attention has been paid to the use of higher fungi in the investigation of natural substances with hypocholesterolemic activity. In a series of papers we have shown that the accumulation of lipids in serum or livers of experimental animals that was induced either alimentary (2), by stimulation of endogenous lipid production (3, 4) or by genetic factors (5) was effectively reduced by wood-rotting oyster mushroom (Pleurotus ostreatus) which is grown in many countries on an industrial scale. Only recently have we found that lower production of cholesterol-enriched VLDL and increased rate of their removal from circulation (7) contributed significantly to the mechanism of hypolipidemic effect of oyster mushroom. Here, we report that a diet with oyster mushrooms reduces cholesterol absorption and enhances the fractional catabolic rate of cholesterol.

Material and methods

Just weaned male rats of Wistar strain (Velaz Breeding Station, Slovakia) were used (initial body weight of 70–75 g). One group of animals (control group, n = 16) was fed semisynthetic diet (24) composed of (in %): starch (60), casein (18), pork fat (10), cellulose (6), mixture of minerals (4) and vitamins (1), Fel tauri (0.55, commercially prepared dried ox bile), cholesterol (0.3), and choline chloride (0.15). Another group of animals was fed the same diet, in which cellulose was substituted by 5 % of dried powdered oyster mushroom (mushroom group, n = 19). Powdered oyster mushroom contains (in %): polysaccharides (65–70), proteins (20–25), lipids (2.2), ash (4.8), and water (up to 5). This regime lasted for 8 weeks and during this period the animals were housed in an artificially lighted (6 a.m. to 6 p.m.) and temperature-controlled (20 °C) room, and they had a free access to the diet and water.

In two independent parallel experiments the cholesterol absorption was estimated by dual-isotope plasma ratio method (26). 78.3 kBq of [1,2-^{3}H(N)]cholesterol (Amersham) was sonicated in a mixture of 15.8 ml of H_2O, 50 mg of Na-cholate, 1.04 g of triolein and 40 mg of cholesterol. This mixture was applied by stomach tube to animals under light ether narcosis that were fasted for 18 h. Immediately after the stomach tube application 36.98 kBq of [4-^{14}C]cholesterol (Amersham) solubilized with Tween 20 in saline were injected into tail vein. The animals were food-restricted for 6 h after the treatment. Blood samples (0.3 ml) were taken from retro-orbital venous plexus of animals under light ether narcosis on days 2, 3, and 4 and the animals were killed by exsanguination on the 5th day (1st experiment). In the second experiment, cholesterol absorption was estimated by the same technique under similar conditions. The only difference was in the application of 3 ml of 10 % aqueous cellulose (Microcrystalline MKS cellulose, Slovakia) suspension to the control animals and 3 ml of 10 % aqueous suspension of mushroom powder to mushroom group immediately after the application of radioactive cholesterol. The radioactivity of serum samples was determined by liquid scintillation counting (Rackbeta, LKB-Pharmacia) after alkaline hydrolysis of serum samples and extraction to hexane (1). Cholesterol absorption was calculated according to the formula:

$$\text{absorption [\%]} = \frac{\text{serum } ^3\text{H activity x administered } ^{14}\text{C activity x 100}}{\text{serum } ^{14}\text{C activity x administered } ^3\text{H activity}}$$

Decay curve of [4-^{14}C]cholesterol was used for the calculation of the half-time ($t_{1/2}$) and fractional catabolic rate (FCR) from the relation of ln dpm-time by a least-square regression analysis (12). After killing the animals, the concentrations of serum cholesterol and triacylglycerols were determined by enzymatic kits (Bio-La-Test AM 250 and 120, Lachema, Czechia) and in chloroform-methanol (2 : 1) extracts of livers (11) by Bio-La-Test kits. VLDL, LDL and HDL (densities d <1.006, d <1.063 and d <1.21 g/ml, respectively) were isolated from the serum by sequential flotation at 36 000 rpm for 18 h (48 h for HDL) at 5 °C (13) using preparative ultracentrifuge L8–55 with rotor 50.3 Ti (Beckman). Cholesterol content in the lipoproteins was determined after alkaline hydrolysis and extraction with hexane (1, 27). The results were statistically evaluated by Student's *t*-test.

Results

The addition of oyster mushroom to the diet did not affect either the food intake during the experiment, or the final weight of animals [308 ± 12, 317 ± 7 g, mean ± SEM, control (n = 16) vs. mushroom group (n = 19)]. The levels of cholesterol both in serum and liver were significantly reduced (by 33 and 27 %, respectively) by the mushroom diet. The content of triacylglycerols in serum was not affected by oyster mushroom, while it decreased significantly (by 41 %) in livers of the mushroom group (Table 1). Oyster mushroom significantly reduced the cholesterol content in VLDL and LDL, the reduction in VLDL cholesterol being 55 % of the total reduction of serum cholesterol (Table 2). A significant reduction (by 14 %) of cholesterol absorption was observed in animals fed oyster mushroom diet. However, intragastric administration of oyster mushroom suspension after application of radiolabelled cholesterol affected the

Table 1. The content of cholesterol and triacylglycerols in serum and liver of rats fed by control and oyster mushroom diets

	Diet	
	Control (n = 16)	Mushroom (n = 19)
Serum	mmol.l^{-1}	
Cholesterol	6.07 ± 0.57$^+$	4.06 ± 0.29^c
Triacylglycerols	1.51 ± 0.24	1.24 ± 0.16
Liver	mmol.kg^{-1}	
Cholesterol	239 ± 17	174 ± 8^d
Triacylglycerols	71.4 ± 8.6	41.9 ± 4.0^c

$^+$ mean ± SEM

Superscript marks $^{a-d}$ indicated statistical significance (mushroom vs control group): $^a p < 0.05$, $^b p < 0.02$, $^c p < 0.01$, $^d p < 0.002$

absorption of cholesterol only slightly (Table 3). The mushroom diet reduced significantly the half-time of plasma cholesterol and increased its fractional catabolic rate by 42 % (Table 4).

Table 2. Cholesterol distribution in lipoproteins of rats fed by control and oyster mushroom diets

Lipoprotein	Diet	
	Control (n = 8)	Mushroom (n = 8)
VLDL (mmol.l^{-1})	3.10 ± 0.26^{+}	2.14 ± 0.15^{c}
%$^{++}$	48.2 ± 2.0	47.1 ± 1.4
LDL (mmol.l^{-1})	1.75 ± 0.23	1.09 ± 0.13^{a}
%$^{++}$	27.0 ± 2.3	23.6 ± 1.4
HDL (mmol.l^{-1})	1.37 ± 0.10	1.26 ± 0.07
%$^{++}$	24.8 ± 2.8	29.3 ± 1.9

$^{+}$ mean ± SEM (data are from pooled samples of serum from Experiments 1 and 2)
$^{++}$ % of total serum cholesterol
Statistical significance as in Table 1

Table 3. Cholesterol absorption in rats fed by control and oyster mushroom diets

Experiment	Absorption (%) Diet	
	Control (n = 8)	Mushroom (n = 8)
1	61.6 ± 2.4^{+}	52.9 ± 2.0^{b}
	+ cellulose	+mushroom
2	66.9 ± 2.6	58.6 ± 1.7^{b}

$^{+}$ mean ± SEM
Statistical significance as in Table 1

Table 4. Kinetic parameters of the decay curve of plasmatic cholesterol in rats fed by control and oyster mushroom diet

Parameter	Diet	
	Control (n = 8)	Mushroom (n = 8)
$t_{1/2}$ (day)	8.67 ± 0.98^{+}	5.44 ± 0.54^{b}
FCR (day^{-1})	0.0969 ± 0.150	0.1376 ± 0.0184^{a}

$^{+}$ mean ± SEM (data are from Experiment 1)
Statistical significance as in Table 1

Discussion

The hypocholesterolemic effect of oyster mushroom both in hamsters and in rats with alimentary induced hyperlipoproteinemia was mediated in a decisive way by the reduction of cholesterol content in primary lipoproteins (VLDL) and their final metabolite forms (LDL) (2, 7). We have found that this effect in hamsters and rats (6, 7) resulted from the reduced VLDL production. Hepatic VLDL assembly and secretion appears to be regulated by the supply of lipids (23). This suggests that the reduced absorption of cholesterol (and of other lipids) is the primary cause of decline in production of VLDL and of diminished lipid content in livers of the animals fed by mushroom diet. Decreased basal glycemia and improved glucose tolerance in rats on mushroom diet (10) could be explained in a similar way by the modification of absorption processes. This effect is probably mediated by a long-term adaptation of gastric mucosa on some component(s) of oyster mushroom, since a single-dose intragastric application of an oyster mushroom suspension did not affect either the absorption of glucose or that of cholesterol.

Oyster mushroom contains a number of substances with potential effects on the absorption of cholesterol or of other lipids. Particularly the water-soluble gel-forming components of the fiber matter (beta-1,3 D-glucan with a low degree of polymerization, forming 15–20 % of dry mass) can interact with bile acids and affect the formation of micelles. In this way such substances could interfere with the absorption of cholesterol (22). The mushroom sterols (0.2 % of dry matter) can reduce cholesterol absorption by competitive inhibition (15). Other substances present in oyster mushroom (lignin and pectin – 2 and 6 % of dry matter (18), undigested protein residues (20), chitin (5 % of dry matter) transformed in the gastrointestinal tract probably to chitosan (19, 25)) can increase the excretion of bile acids by the ability to bind them. Increased excretion can in turn reduce the pool of bile acids in liver and enhance the cholesterol catabolism to bile acids in liver (14). Reduced absorption of cholesterol together with enhanced degradation of cholesterol can significantly contribute to the decrease of cholesterol content in serum and liver. Another possibility that cannot be excluded is that the decrease of liver cholesterol releases the block of ApoB/E receptors leading to a stimulation of plasma cholesterol removal demonstrated in hamsters (6) and in this study.

Reduced absorption of cholesterol is a significant mechanism of hypocholesterolemic (or generally hypolipidemic) effect of oyster mushrooms, although, with respect to the results of other studies, it is not the exclusive one. Hypolipidemic effect of oyster mushroom by the ethanol-stimulated (3) or streptozotocine diabetes-stimulated overproduction of VLDL (4) is probably mediated by the enhanced plasma VLDL removal (7). Recently we found that oyster mushroom decreases the cholesterol concentration in serum and in liver, not only in highly hypercholesterolemic rats (high cholesterol intake), but also in rats with mild hypercholesterolemia fed a diet containing 0.009 % of cholesterol (unpublished data). In case of low cholesterol diet the accelerated plasma removal of VLDL (7), LDL, and HDL (8) in the hypocholesterolemic effect of oyster mushroom can play a more important role than the affected absorption. In oyster mushroom we were not able, so far, either to confirm or to exclude the presence of eritadenin, a substance with high hypocholesterolemic activity from a related fungus Lentinus edodes. The effect of this substance is explained by the inhibition of the formation of primary lipoproteins with no effect on lipid absorption (16) and by acceleration of the transfer of plasma cholesterol to the peripheral tissues (21). The results obtained in this study support the significance of the use of oyster mushroom in the prevention

and dietetic therapy of hypercholesterolemias, especially in relation to the potential use of aqueous or ethanolic extracts of this fungus with hypocholesterolemic activity (9).

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Authors' address:
RNDr. Pavel Bobek, CSc, Research Institute of Nutrition, Limbová 14, Bratislava 833 37, Slovakia