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Dietary Oyster Mushroom (Pleurotus ostreatus) Accelerates Plasma Cholesterol Turnover in Hypercholesterolaemic Rat

P. BOBEK, O. OZDÍN, M. MIKUŠ

Research Institute of Nutrition, Bratislava, Slovak Republic

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Summary

The effect of adding 5 % powdered oyster mushroom *(Pleurotus ostreatus)* during 12 weeks on kinetic parameters of cholesterol metabolism was studied in male rats (Wistar, initial body weight 85 g) fed a semisynthetic diet containing 0.3 % of cholesterol. The plasma cholesterol decay curve (examined for the final 29 days of the experiment after a single dose of cholesterol-4-^{14}C) was evaluated by mathematical analysis using a two-pool model of plasma cholesterol metabolism. The oyster mushroom in the diet reduced the half-times of both exponentials resulting in lower calculated values (by 28 %) of total entry of cholesterol into the body cholesterol pool (absorption + endogenous synthesis) and lower sizes of both pools (with slower and faster cholesterol exchange). The rate of cholesterol exchange between the pools was enhanced and the rate of total clearance of cholesterol from the system (metabolic turnover rate of cholesterol, i.e. the rate of degradation and excretion of cholesterol from the organism) was enhanced by 50 %. The oyster mushroom diet effectively prevented the progress of hypercholesterolaemia (decrease by 38 %) and cholesterol accumulation in liver (decrease by 25 %) that were induced by the cholesterol diet.

Key words

Pleurotus ostreatus – Cholesterol – Serum – Liver – Turnover

Introduction

An unfavourable development in the incidence of hypercholesterolaemia and clinical complications related to atherosclerosis in our country (Babinská *et al.* 1994) makes the search for natural substances with a hypocholesterolaemic effect and their investigation highly actual. Despite their valuable composition in this respect (high content of fibrous matter, proteins, sterols, microelements, low energy content), fruiting bodies of higher fungi are only sporadically applied in the prevention and dietotherapy of cardiovascular diseases. We have found in a series of experiments that addition of low amounts of dried fruiting bodies of oyster mushroom (*Pleurotus ostreatus*, a wood-rotting fungus produced in our country on an industrial scale in ligno-cellulose substrates) into the diet of experimental animals effectively prevented the development of hyper-cholesterolaemia and accumulation of cholesterol in the liver induced either nutritionally, by stimulation of cholesterol biosynthesis or by genetic factors (Bobek *et al.* 1991a,b,c, 1993b). Our recent results revealed that the presence of the oyster mushroom in the diet affects crucial steps in the regulation of cholesterol homeostasis: it reduces the rate of cholesterol absorption (Bobek and Ozdín 1994) and accelerates the turnover of all cholesterol-carrying lipoproteins (Bobek *et al.* 1993a,b). In an attempt to obtain additional information about the dynamics of cholesterol transformation in rats fed the oyster mushroom diet, we performed a kinetic analysis of the plasma cholesterol decay curve (after a single administration of cholesterol-4-^{14}C) in a two-pool model of cholesterol metabolism (Goodman and Noble 1968).

Material and Methods

Male rats of the Wistar strain (Velaz, Prague, Czech Republic), with initial body weight about 85 g (n=21), were used in the experiments. Animals were bred under standard conditions without modifications

of the light regime and with unrestricted access to food of the following composition (Yamashita *et al.* 1980) (in %): starch 60, casein 18, pork fat 10, cellulose 6, mineral and vitamin mixtures 4 and 1, respectively, Fel tauri (a commercially produced ox bile) 0.55, cholesterol 0.3, and choline chloride 0.15 (control diet). Animals in the experimental group received 5 % of dried oyster mushroom fruiting bodies at the expense of cellulose in their diet (mushroom diet). Dried powder of oyster mushroom contained (in %): polysaccharides 65–70, proteins 20–25, lipids 2.2, ash 4.8 and water up to 5.0. After 8 weeks, an emulsion of cholesterol-4-^{14}C (specific activity 1887 MBq/mmol, Amersham) in saline and Tween 20 was administered into the tail vein of animals under light ether narcosis in amounts of 323 kBq per animal. Blood samples were taken from the retroorbital venous plexus of animals under light ether anaesthesia after 9 h, 1, 3, 5, 7, 15 and 22 days. Animals were decapitated on day 29 after 18 hours' of fasting. The cholesterol concentration was estimated in the serum (for calculating plasma cholesterol specific activity and lipoproteins) (Oxochrom Chol 250E, Czech Republic) and in chloroform-methanol (2:1; Folch *et al.* 1957) extracts of the liver, intestine, heart muscle, adrenal gland and in the aorta (Bio-La-Test, Lachema, Brno, Czech Republic). Radioactivity in the serum, lipoproteins and in organ extracts was measured by liquid scintillation spectrometry using Rackbeta instrument (LKB-Pharmacia).

Mathematical analysis of serum cholesterol specific activity-time curve in a two-pool model

In all the species of animals studied previously, the log of plasma cholesterol specific activity during the first weeks after injecting labelled cholesterol, was found to be a nonlinear function of time, while it had a linear function at later intervals. Goodman and Noble (1968) put forward the hypothesis that radioactive serum cholesterol was not exchanged uniformly for a single cholesterol pool. It was exchanged with pool A, which is characterized by a rapid exchange of cholesterol (in the order of days; this includes cholesterol in the blood, liver and majority of inner organs) and with pool B, the exchange with which is significantly lower (weeks, including the skin, peripheral tissues, vascular system, etc.). The change in the slope of the specific activity-time curve is caused by a decrease in the rate of equilibration between the cholesterol pool in the plasma and in various tissues. For a kinetic analysis of the two-pool system Goodman and Noble (1968) used, a mathematical apparatus originally designed for studying steroid hormone metabolism (Gurpide *et al.* 1964) and applied later to the calculation of kinetic parameters of cholesterol metabolism in man and rat (Goodman and Noble 1968, Nilsson and Zilversmit 1972). The two-pool system (which is only an approximation of the real situation) can by characterized in general by the scheme in Fig. 1.

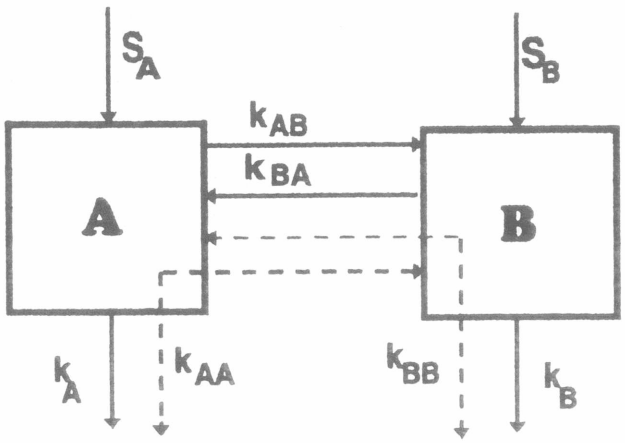

Fig. 1
General scheme of the two-pool (A and B) model. Rate constants are denoted by the k-values, S_A and S_B are the rates of entry of material into the pools from outside the system. After Gurpide *et al.* (1964).

The specific activity of a labelled substance in pool A is determined by the equation:

$$a = C_A.e^{-\alpha t} + C_B.e^{-\beta t}$$

in which a is specific activity of blood serum or plasma, C_A, C_B, α and β are constants, e is base of the natural logarithm and t is time). The values of the four constants can be obtained by analysis of the specific activity-time curve. Extrapolation of the linear part of the curve to zero time gives the value of C_B. Deduction of the values for the extrapolated part of this line from the experimental values obtained during the time, when the correlation of the log of specific activity to time is nonlinear, furnished data for the construction of the second curve. Extrapolation of this line to zero time gives the value of C_A. The size of the constants α and β is determined by the slope (or half-time) of the two exponentials: $\alpha = \ln 2 / t_{1/2}$ of the first exponential; $\beta = \ln 2 / t_{1/2}$ of the second exponential. Using the above data and the known amount of the isotope injected into pool A (R_A), we can compute a whole series of pool and kinetic parameters, the characteristics of which, together with the computation methods, are shown in Table 3.

Assuming that irreversible removal of cholesterol is possible from pool A only (the liver is practically the only organ of cholesterol catabolism and of excretion of cholesterol or its degradation products – bile acids), the values of rate constants characterizing the rate of cholesterol transfer between the pools and the rate of irreversible cholesterol

removal from the system (i.e. metabolic turnover rate) can be calculated. The rate constants have the dimension of days and they actually indicate the percentage of cholesterol transferred from a given pool in a specific direction. Another approximation – neglecting endogenous cholesterol synthesis in the tissues containing pool B (biosynthesis of cholesterol in these tissues is insignificant compared to pool A tissues) – also enables the calculation of the minimal size of pool B. The results were statistically evaluated by Student's t-test.

Table 1
Effect of oyster mushroom diet on cholesterol content in the serum, lipoproteins and inner organs of the rat

Parameter	Diet	
	Control	Mushroom
n	15	13
Body weight (g)	387±18	413±18
Serum cholesterol (mmol.l^{-1})	6.70±9.80	4.09±0.26^{c}
Lipoproteins		
n	9	9
VLDL	3.42±0.67	1.41±0.20^{b}
%$^{+}$	48.1±3.0	32.1±3.3^{c}
LDL	2.37±0.27	1.8±0.24
%$^{+}$	37.0±1.5	41.1±3.0
HDL	0.88±0.07	1.15±0.11
%$^{+}$	14.9±1.8	26.9±1.8^{d}
Cholesterol (mmol.kg^{-1})		
n	15	13
Liver	294±16	221±3^{d}
Small intestine	4.71±0.22	4.32±0.19
Adrenals	211±19	219±16
Heart	3.70±0.15	3.49±0.28
Aorta	5.44±0.20	4.84±0.11^{a}

Values are means ± S.E.M. n is number of animals. $^{+}$Contribution to total serum cholesterol. VLDL, LDL, HDL: very-low-density, low-density and high-density lipoproteins separated at $d<1.006$, $d<1.063$ and $d<1.21$ g.ml^{-1}, respectively, by sequential flotation (Havel et al. 1955) on preparative ultracentrifuge (L8-50, rotor 50.3 Ti, Beckman; 36 000 rpm/18 h/6 °C, HDL at 40 000 rpm/42 h). Superscripts indicate statistical significance (mushroom vs control group) $^{a}p<0.05$, $^{b}p<0.02$, $^{c}p<0.01$, $^{d}p<0.001$

Results

The described cholesterol diet, given to rats shortly after weaning, markedly increased serum and liver cholesterol levels. The presence of oyster mushroom in this diet did not significantly affect the final body weight of animals. Serum cholesterol concentrations were reduced highly significantly (by 39 %). Major part of this reduction (78 %) could be attributed to a decrease of cholesterol in very-low-density lipoproteins (VLDL) and 22 % to a decrease in low-density lipoproteins (LDL). The fraction of total cholesterol carried by VLDL was reduced by 16 % while the fraction carried by high-density lipoproteins (HDL) was almost doubled. The absolute concentration of HDL-cholesterol increased by 31 %. In addition, there was a significant decrease of cholesterol content in the liver (by 25 %) and the aorta (by 12 %) while no changes were observed in the intestine, adrenal glands and heart muscle (Table 1).

Feeding with oyster mushroom reduced the fraction of administered radioactivity found in the serum by almost 50 %, the fraction found in VLDL by more than 60 %, in HDL almost by 30 % and in the liver by 20 %. No changes in radioactivity were detected in tissues from other analyzed organs (Table 2).

Table 2
Effect of oyster mushroom diet on the distribution of radioactivity in lipoproteins and organs of the rat

Parameter	Diet	
	Control	Mushroom
n	9	9
	% of radioactivity administered.l^{-1}	
Serum	0.39±0.08	0.21±0.02^{b}
VLDL	0.19±0.04	0.06±0.01^{b}
LDL	0.12±0.02	0.09±001
HDL	0.044±0.004	0.053±0.002
	% of radioactivity administered.g^{-1}	
Liver	2.36±0.19	1.88±0.13^{a}
Adrenals	0.75±0.12	0.82±0.09
Small intestine	0.031±0.0034	0.032±0.0017
Heart	0.029±0.0027	0.030±0.0032
Aorta	0.029±0.0030	0.030±0.0019

For other legend see Table 1

The oyster mushroom diet significantly reduced the half-times of both exponentials which was reflected in higher values of rate constants for total removal of cholesterol from pool A (i.e. excretion and transfer to pool B), for cholesterol transfer from pool A to pool B and for irreversible removal of cholesterol from pool A (k_A representing catabolism of cholesterol to bile acids and excretion). Furthermore the oyster mushroom diet significantly reduced the rate of entry of new cholesterol (i.e. cholesterol from absorption and from endogenous synthesis) into pool A. Restricted entry of new cholesterol together with accelerated removal of cholesterol from the system explain significantly lower sizes of pools A and B in animals fed the oyster mushroom diet (Table 3).

Table 3
Effect of oyster mushroom diet on kinetic parameters of cholesterol metabolism in the rat

Parameter	Diet	
	Control	Mushroom
n	10	9
$t_{1/2}\alpha$ (day)	0.63±0.15	0.29±0.019[a]
$t_{1/2}\beta$ (day)	31.21±6.05	15.45±0.75[b]
M_A (mg)	299±40	147±15[c]
PR_A (mg.day^{-1})	75.93±7.91	54.31±4.96[a]
k_{AA} (day^{-1})	−1.470±0.247	−2.333±0.145[c]
k_A (day^{-1})	0.239±0.041	0.365±0.033
k_{AB} (day^{-1})	1.231±0.211	1.871±0.150[c]
k_{BA} (day^{-1})	0.189±0.026	0.316±0.046[a]
$M_{B\ min}$ (mg)	1753±107	957±149[d]

$t_{1/2}\alpha$ and $t_{1/2}\beta$: half-times of first and second exponential; M_A: size of pool A; $M_A = R_A/(C_A + C_B)$. $M_{B\ min}$: minimum size of pool B; $M_{B\ min} = k_{AB}.M_A/k_{BA}$. PR_A: production rate in pool A – cholesterol flow to pool A (exogenous cholesterol + endogenous synthesis); $PR_A = R_A\alpha\beta/(\alpha C_B + \beta C_A)$. Rate constants: k_{AA} and k_{BB} – for total cholesterol removal from pool A and B [$k_{AA} = (-\alpha M_A C_A - \beta M_A C_B)/R_A$; $k_{BB} = -(\alpha + \beta + k_{AA})$]; k_A – for irreversible removal of cholesterol from pool A and from the whole system ($k_A = \alpha\beta/k_{BA}$); k_{AB} and k_{BA} – for inter-pool cholesterol shifts: $k_{AB} = -k_{AA} - k_A$; $k_{BA} = -k_{BB}$ (if $k_B = 0$). For other legend see Table 1.

Discussion

The results of kinetic analysis of the decay curve of plasma cholesterol revealed that the mechanism of hypocholesterolaemic effect of oyster mushroom present in the diet influences both fundamental phases of the regulation of cholesterol metabolism – its entry into and its removal from the system. A decrease of PR_A reflects a decrease of the rate of entry of "new" cholesterol into pool A. This is in agreement with our previous finding that dietary oyster mushroom significantly decreases cholesterol absorption (Bobek *et al.* 1994) as well as its biosynthesis in the liver (Bobek 1995). On the other hand, increased values of the rate constant k_A indicate increased cholesterol catabolism in pool A, i.e. in the liver. This finding is indirectly supported by a higher fractional catabolic rate of all cholesterol-carrying lipoproteins (Bobek *et al.* 1993a) and by the results of short-term studies of fractional turnover of 4-^{14}C-cholesterol in the rat (Bobek *et al.* 1994a). A decrease in the rate of cholesterol entry into pool A (i.e. predominantly into the liver) and an increase of its removal from this pool are a prerequisite for a decreased production of cholesterol-rich VLDL (Bobek *et al.* 1993b, Bobek and Ozdín 1995) and a decrease of cholesterol content in the liver, which was repeatedly observed. The decrease of VLDL-cholesterol concentration is important for the decrease of total cholesterol levels in the serum. It is interesting that oyster mushroom similarly increases values of the rate constants of inter-pool cholesterol shifts (by 50–60 %), but the minimal size of pool B was almost reduced to one half. Unfortunately, we had no opportunity to compare these theoretical data with chemical analyses.

Oyster mushroom contains several compounds that are known from other food sources to affect both absorption and catabolism of cholesterol. Particularly water-soluble gel-forming components of the fibrous matter (β-1,3-D-glucan with a low degree of polymerization forming 15–20 % of dry matter) can interact with bile acids and affect the formation of micelles. Such substances could interfere in this way with the absorption of cholesterol (Vahouny *et al.* 1980). Mushroom sterols (0.2 % of dry matter) can reduce cholesterol absorption by competitive inhibition (Ikeda *et al.* 1988). Other substances present in oyster mushroom, such as lignin and pectin (2 and 6 % of dry matter, respectively; Story 1985), undigested protein residues (Sugano *et al.* 1988), chitin (5 % of dry matter) that could be transformed in the gastrointestinal tract to chitosan (Sugano *et al.* 1980, Zemek *et al.* 1987), can increase the excretion of bile acids by their ability to bind them. Increased excretion can in turn reduce the pool of bile acids in the liver and enhance cholesterol catabolism to bile acids in this organ (Havel 1988). It is highly probable that fruiting bodies of oyster mushroom contain monacolin K (Gunde-Cimerman *et al.* 1993), an inhibitor of the key enzyme of cholesterol biosynthesis – HMG-CoA reductase. Reduced cholesterol absorption (and biosynthesis) and acceleration of cholesterol catabolism have major impact on restricted accumulation of cholesterol in the plasma and liver. It

is highly probable that the detailed mechanism of hypocholesterolaemic effect of oyster mushroom will, in general, be similar to the effect of a combination of cholestyramin and HMG-CoA reductase inhibitors-type pharmaceuticals.

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Reprint Requests

RNDr. Pavel Bobek, CSc., Research Institute of Nutrition, Limbová 14, 833 37 Bratislava, Slovak Republic.