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EFFECTS OF EXOGENOUS CHOLESTEROL AND AMIDATED CELLULOSE ON FAT AND STEROLS CONCENTRATION IN FAECES

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The increased conversion of cholesterol to bile acids in the liver compensates for the loss of bile acids in faeces. This leads to lower cholesterol levels in the blood. An alternative to bile acid sequestrants is sorbents of sterols prepared by amidation of polysaccharides.

There was observed the hypocholesterolemic effect of octadecylpectinamide in rats that were kept on diets containing cholesterol in the amount of 10 g/kg. Amidated pectin reduces cholesterol in the blood serum and significantly increases levels of natural sterols and bile acids in faeces. Therefore we investigated the effect of feeding cholesterol and amidated oxidized monocarboxycellulose (MCC), and carboxymethylcellulose (CMC) on the concentration of fat and sterols in the feces of rats. We used 30 female Wistar rats aged 3 months and weighing ~ 250 g. After leveling period (4 weeks), rats were divided into 6 groups of 5 animals each. The first group was control, diets of groups (2–6) were supplemented with cholesterol at 10 g/kg at expense of palm fat, diets of 3 and 4 groups supplemented 30 and 60 g/kg of amidated MCC, respectively, instead of conventional cellulose (in total 60 g/kg), and 5 and 6 groups — 30 and 60 g/kg of amidated CMC, respectively. The experiment duration was 4 weeks. Faeces were collected during the last 5 days of the experiment.

It is established that the cholesterol and coprostanol concentration in faeces are significantly increased in all rats which were fed diets supplemented with cholesterol, and coprostanol increases significantly with the consumption of amidated cellulose. Amidated MCC and CMC increase significantly the fecal fat output. They decrease significantly concentration of lithocholic, α-muricholic acids and the total bile acids in faeces.

The results show considerable difference between amidated cellulose and amidated pectin, which significantly increases the fecal excretion of cholesterol and to a lesser extent - of bile acids.

Possibilities of the removal of dietary fat from the organism using the amidated cellulose (that provide a hypolipidemic and hypocholesterolemic effects) will be verified again in the near future.

Keywords: CHOLESTEROL, FAT, BILE ACIDS, AMIDATED CELLULOSE, FAECES, RATS

ДІЯ ЕКЗОГЕННОГО ХОЛЕСТЕРОЛУ І АМІДОВАНОЇ ЦЕЛЮЛОЗИ НА КОНЦЕНТРАЦІЮ ЖИРУ І СТЕРОЛІВ У ФЕКАЛІЯХ

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Збільшення обсягів перетворення холестеролу до жовчних кислот у печінці компенсує втрату жовчних кислот у фекаліях. Це призводить до зниження холестеролу в крові. Альтернативою секвестрантам жовчних кислот є сорбенти стеролів, отриманих шляхом амідування полісахаридів.

Був відмічений гіпохолестеролемічний ефект при застосуванні октадецилпектиаміду на щурах, що утримувалися на раціонах, у складі яких входить холестерол у кількості 10 г/кг. Амідований пектин знижує вміст холестеролу в сироватці крові та вірогідно збільшує рівні природних стеролів й жовчних кислот у фекаліях. Тому далі досліджували дію згодовування холестеролу та амідованої окисненої монокарбоксицелюлози (МСС) й карбоксиметилцелюлози (СМС) на концентрацію жиру й стеролів у фекаліях щурів. Використовували 30 самок щурів лінії Вістар віком 3 місяці і вагою ~ 250 г. Після зрівняльного періоду (4 тижні) щурі були розділені на 6 груп по 5 тварин у кожній. Перша група залишалась контрольною, 2–6 групи взамін 10г/кг пальмового жиру одержували адекватну кількість холестеролу, 3 і 4 групи взамін звичайної целюлози (у загальному обсязі 60 г/кг) ще одержували відповідно по 30 і 60 г/кг амідованої МСС, а 5 і 6 — 30 і 60 г/кг амідованої СМС. Тривалість дослідного періоду — 4 тижні. Для аналізів збирали фекалії впродовж останніх 5 днів.

Встановлено, що у фекаліях усіх щурів, які поїдали холестерол вірогідно збільшуються концентрації холестеролу й копростанолу та вірогідно зростає копростанол при споживанні амідованої целюлози. Амідовані МСС і СМС вірогідно збільшують фекальний вихід жиру. Вони вірогідно зменшують у фекаліях концентрацію літохолевої, α-муріхолевої та загальної суми жовчних кислот.

Наведені результати суттєво відрізняють амідовані целюлози від амідованого пектину, який вірогідно збільшує фекальне виведення з організму холестеролу і в меншій мірі — жовчних кислот.

Можливості видалення жирів з організму з допомогою амідованої целюлози, що передбачають гіполіпідемічний і гіпохолестеролемічний ефекти, ще раз будуть перевірені найближчим часом.

Ключові слова: ХОЛЕСТЕРОЛ, ЖИР, ЖОВЧНІ КИСЛОТИ, АМІДОВАНА ЦЕЛЮЛОЗА, ФЕКАЛІЇ, ЩУРИ

ДЕЙСТВИЕ ЭКЗОГЕННОГО ХОЛЕСТЕРОЛА И АМИДИРОВАННОЙ ЦЕЛЛЮЛОЗЫ НА КОНЦЕНТРАЦИЮ ЖИРА И СТЕРОЛОВ В ФЕКАЛИЯХ

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Увеличение объемов преобразования холестерола в желчные кислоты в печени компенсирует потерю желчных кислот в фекалиях. Это приводит к снижению холестерола в крови. Альтернативой секвестрантам желчных кислот являются сорбенты стеролов, полученных путем амидирования полисахаридов.

Был отмечен гипохолестеролемический эффект при применении октадецилпектиамида на крысах, содержавшихся на рационах, в состав которых входил холестерол в количестве 10 г/кг. Амидированный пектин снижает содержание холестерола в сыворотке крови и достоверно увеличивает уровни естественных стеролов и желчных кислот в фекалиях. Поэтому далее исследовали действие скармливания холестерола и амидированной окисленной монокарбоксицеллюлозы (МСС) и карбоксиметилцеллюлозы (СМС) на концентрацию жира и стеролов в фекалиях крыс. Использовали 30 самок крыс линии Вистар в возрасте 3 месяца и весом ~ 250 г. После уравнительного периода (4 недели) крысы были разделены на 6 групп по 5 животных в

каждой. Первая группа оставалась контрольной, 2–6 группы взамен 10г/кг пальмового жира получали адекватное количество холестерола, 3 и 4 группы взамен обычной целлюлозы (в общем объеме 60 г/кг) еще получали соответственно по 30 и 60 г/кг амидированной МСС, а 5 и 6 — 30 и 60 г/кг амидированной СМС. Продолжительность исследовательного периода — 4 недели. Для анализов собирали фекалии в течение последних 5 дней.

Установлено, что в фекалиях всех крыс, поедавших холестерол, значительно увеличиваются концентрации холестерола и копростанола и достоверно возрастает копростанол при употреблении амидироованной целлюлозы. Амидированные МСС и СМС достоверно увеличивают фекальный выход жира. Они достоверно уменьшают в фекалиях концентрацию литохолевой , α-мурихолевой и общей суммы желчных кислот.

Приведенные результаты существенно отличают амидированные целлюлозы от амидированного пектина, который достоверно увеличивает фекальное выведение из организма холестерола и в меньшей степени - желчных кислот.

Возможности удаления жиров из организма с помощью амидированной целлюлозы, предусматривающие гиполипидемический и гипохолестеролемический эффекты, еще раз будут проверены в ближайшее время.

Ключевые слова: ХОЛЕСТЕРОЛ, ЖИР, ЖЕЛЧНЫЕ КИСЛОТЫ, АМИДИРОВАННАЯ ЦЕЛЛЮЛОЗА, ФЕКАЛИИ, КРЫСЫ

Hypercholesterolemia and obesity belong to the main risk factors of coronary heart disease, which is the leading cause of deaths worldwide. Several methods for the treatment of hypercholesterolemia exist, mainly fat restriction, the use of inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase (statins), and sequestrans that bind bile acids in the small intestine and interrupt their enterohepatic circulation [1]. Increased conversion of cholesterol to bile acids in the liver compensates for the loss of bile acids in faeces, which results in reduction of cholesterolemia. An alternative to bile acid sequestrants are sorbents of sterols prepared by amidation of polysaccharides. In our recent study, the hypocholesterolemic effect of octadecylpectinamide was examined in rats fed diets containing cholesterol at 10 g/kg [4]. Amidated pectin supplied at 20, 40 and 60 g/kg significantly decreased serum cholesterol from 3.32 (control) to 1.23 μmol/ml in a dose-dependent manner. In a previous experiment, amidated pectin significantly increased the faecal excretion of natural sterols, and to a lesser extent also excretion of bile acids [2]. The aim of the present experiment was to compare the faecal output of sterols (both neutral and acidic), and fat in rats fed amidated celluloses prepared from oxidized monocarboxycellulose and carboxymethylcellulose. Female rats were chosen as the experimental animals, as female rats are more susceptible to changes in serum cholesterol by dietary means than male rats [3, 8].

Materials and methods

N-octadecylamide of oxidized monocarboxycellulose (MCC) and carboxymethyl-cellulose (CMC) were prepared by esterification with methanol, and amino-dealkoxylation of methyl esters with n-octadecylamine. The product of the reaction was washed twice with 80 % ethanol, petroleum ether, 0.2 M HCl in 80 % ethanol, and again with 80 % ethanol. MCC, CMC and their derivatives were analyzed for C, H and N on the instrument EL III Universal CHNOS Elemental Analyzer (Elementar Analysensysteme GmbH, Germany). The degrees of substitution (DS) were calculated on the basis of N and C contents.

Thirty female Wistar rats at 3 months of age weighing ca 250 g were housed in a temperature- and humidity controlled room. Rats were fed a commercial ST-1 diet (Velaz Ltd, Lysolaje, Czech Republic), supplemented with microcrystalline cellulose and palm fat at 60 g/kg. The rat diet ST-1 ingredients were soybean meal, meat and bone meal, fish meal, wheat, maize, oats, wheat bran, limestone,

dicalcium phosphate, salt, and supplements of vitamins, trace elements and amino acids. The diet contained crude protein, fibre, fat and ash at 208, 41, 19, and 54 g/kg, respectively. After 4 weeks, the rats were randomly divided into 4 groups of 5 animals each. The diets 2, 3 and 4 were supplemented with cholesterol at 10 g/kg at expense of palm fat, and diets 3 and 4 with amidated MCC and CMC, respectively, at 60 g/kg at expense of microcrystalline cellulose (Table 1). The experiment duration was 4 weeks. Faeces were collected during the last 5 days of the experiment.

The faecal neutral sterols and bile acids were determined in freeze-dried samples by gas chromatography on a 25 m fused silica capillary column (CP-SIL 5CB, Varian, USA). Norcholic acid (the internal standard) was added, and samples were then subjected to n-butylation and trimethylsilylation according to Batta et al. [5]. Standards of sterols were purchased from Sigma-Aldrich (Prague) and Steraloids (Newport, USA). The faecal lipids were extracted with 2:1 chloroform-methanol and determined gravimetrically [7].

Table 1

Composition of control and experimental diets (g/kg)

Ingredient	Diet					
	1	2	3	4	5	6
Cholesterol	0	10	10	10	10	10
Palm fat[a]	60	50	50	50	50	50
Amidated oxidized MCC	0	0	30	60	0	0
Amidated CMC	0	0	0	0	30	60
Cellulose	60	60	30	0	30	0
Diet ST-1	880	880	880	880	880	880

Note: [a] — Palm fat contained myristic, palmitic, stearic, oleic, and linoleic acid at 2, 60, 27, 10, and 1 %, respectively

Results and discussion

According to the elemental analysis, the amidated derivative of MCC contained N, C and H at 1.63, 53.63, and 8.56 % (m/m), respectively. Corresponding values in the derivative of CMC cellulose were 2.38, 60.74, and 10.08 % (m/m). Resulting degree of amidation of N-octadecylamides of MCC and CMC was 20.6 and 58.6 mol. %, respectively.

The cholesterol and coprostanol concentration in faeces significantly increased in all rats that were fed diets supplemented with cholesterol (Table 2). Supplementation of diets with amidated celluloses significantly increased concentration of coprostanol and total neutral sterols. There was no treatment effect on other neutral sterols (campesterol, stigmasterol, β-sitosterol and β-sitostanol, i.e. plant sterols present in components of the ST-1 diet for rats).

Table 2

Effect of cholesterol and amidated celluloses on concentration of neutral sterols[a] in faeces of rats

Cholesterol (g/kg)	0	10	10	10
Amidated MCC (g/kg)	0	0	60	0
Amidated CMC (g/kg)	0	0	0	60
Cholesterol	5.73 ±0.31[c]	53.04 ±3.58[b]	59.39 ±1.75[b]	55.44 ±8.71[b]
Coprostanol	2.55 ±0.20[e]	10.48 ±2.02[d]	20.21 ±3.85[c]	28.23 ±6.20[b]
Other neutral sterols[b]	4.20 ±0.15	5.49 ±0.29	5.29 ±0.39	5.19 ±0.36
Total neutral sterols	12.48 ±0.51[d]	69.01 ±4.21[c]	84.85 ±4.44[b]	88.86 ±10.68[b]

Note: [a] — μmol/g DM, [b] — Plant sterols and traces of epicoprostanol, Data are means and SD for 5 rats per diet. Values in the same row with different superscripts are significantly different ($P<0.05$)

Muricholic acids represented 41.8 % of the total bile acids in faeces of rats fed the basal diet. The concentration of cholic and chenodeoxycholic acid, i.e. primary bile acids, represented 2.2–11.1 % of the total bile acids in faeces. The bile acids concentration in faeces significantly increased in rats fed diets supplemented with cholesterol. Supplementation of diets with amidated celluloses did not significantly affect the bile acids concentration in faeces (Table 3).

Table 3

Effect of cholesterol and amidated celluloses on concentration of bile acids[a] in faeces of rats

Cholesterol (g/kg)	0	10	10	10
Amidated MCC (g/kg)	0	0	60	0
Amidated CMC (g/kg)	0	0	0	60
Isolithocholic	0.29 ±0.01^{c}	0.50 ±0.06^{b}	0.52 ±0.05^{b}	0.48 ±0.02^{b}
Lithocholic	0.59 ±0.10^{d}	3.01 ±1.12^{b}	1.66 ±0.27^{c}	1.78 ±0.35^{c}
Deoxycholic	1.02 ±0.11^{c}	1.44 ±0.56^{bc}	1.61 ±0.40^{bc}	2.00 ±0.34^{b}
α-muricholic	0.44 ±0.03^{c}	2.34 ±0.86^{b}	0.73 ±0.13^{c}	0.70 ±0.09^{c}
Cholic	0.31 ±0.02^{c}	0.09 ±0.09^{d}	0.45 ±0.02^{b}	0.64 ±0.05^{b}
Chenodeoxycholic	0	0.16 ±0.23^{c}	0.06 ±0.14^{c}	0.41 ±0.06^{b}
β-muricholic	0.92 ±0.04^{c}	1.53 ±0.20^{b}	1.51 ±0.05^{b}	1.58 ±0.28^{b}
12-ketolithocholic	0.52 ±0.02^{c}	0.59 ±0.08^{c}	0.74 ±0.09^{b}	0.63 ±0.09^{bc}
ω-muricholic	0.60 ±0.14^{c}	1.61 ±0.44^{b}	0.99 ±0.11^{bc}	1.22 ±0.56^{bc}
Total bile acids	4.69 ±0.13^{c}	11.27 ±1.83^{b}	8.27 ±0.75^{b}	9.44 ±1.60^{b}

Note: [a] — μmol/g DM

Data are means and SD for 5 rats per diet. Values in the same row with different superscripts are significantly different ($P < 0.05$)

Cholesterol supplementation significantly increased faecal output of cholesterol, neutral sterols, bile acids, total sterols, and non-significantly increased faecal output of fat (Table 4). Amidated cellulose had no significant effect on faecal output of cholesterol, neutral sterols and total sterols, but significantly decreased output of bile acids. Amidated MCC and CMC significantly increased faecal output of fat.

Table 4

Effect of cholesterol and amidated celluloses on faecal concentrations of dry matter and fat, and faecal output of fat and sterols

Cholesterol (g/kg)	0	10	10	10
Amidated MCC (g/kg)	0	0	60	0
Amidated CMC (g/kg)	0	0	0	60
Faecal DM (%)	66.87 ± 4.74	67.37 ± 0.06^{b}	63.97 ± 2.92	60.62 ± 2.90
Faecal fat (%)	4.30 ± 0.36^{c}	4.77 ± 1.12^{b}	12.43 ± 1.32^{a}	7.83 ± 0.78^{b}
Faecal output (per day)				
fat (mg)	304.3 ± 46.4^{c}	378.2 ± 0.86^{b}	885.0 ± 233.1^{a}	633.2 ± 33.0^{b}
cholesterol (μmol)	27.0 ± 3.4^{b}	282.7 ± 0.09^{d}	266.6 ± 43.4^{a}	243.9 ± 44.6^{a}
neutr. sterols (μmol)	58.9 ± 7.0^{b}	367.3 ± 0.23^{c}	380.1 ± 55.0^{a}	389.0 ± 40.8^{a}
bile acids (μmol)	22.2 ± 2.8^{c}	59.0 ± 0.20^{b}	36.9 ± 5.0^{b}	41.5 ± 8.1^{b}
total sterols (μmol)	81.1 ± 9.7^{b}	426.3 ± 0.08^{c}	417.1 ± 58.4^{a}	430.6 ± 47.6^{a}

Note: Data are means and SD for 5 rats per diet. Values in the same row with different superscripts are significantly different ($P<0.05$)

In the present study, amidated celluloses increased faecal concentration of coprostanol, but did not influence faecal output of cholesterol. The increase in faecal excretion of fat was the most pronounced effect of amidated celluloses. Diets used in the present experimental contained 76.7 g of fat per kg. Daily intake of feed in groups 1, 2, 3 and 4 was 17.1, 18.8, 17.0 and 17.0 g per a rat, respectively. It can be calculated that output of fat in faeces represented 23.2, 26.2, 67.9 and 48.6 % of the dietary fat intake in rats of the group 1, 2, 3 and 4, respectively. The accumulated data suggest that saturated fat probably has a greater impact on blood cholesterol than the dietary cholesterol [6]. Palmitic acid, which is present in palm fat is a hypercholesterolemic fatty acid [9]. The excretion of bile acids was lower in rats fed diets supplemented with amidated celluloses, presumably because of the lower synthesis of bile acids in the liver, in response to lower availability of fat. Amidated celluloses thus differ from amidated pectin, which significantly increased faecal excretion of cholesterol and to a lesser extent also excretion of bile acids [2].

The analyses of faecal sterols illustrate extensive microbial modification of sterols. Coprostanol is the product of bacterial hydrogenation of cholesterol. Secondary bile acids, i.e. other than those synthesized in the liver, predominate over cholic and chenodeoxycholic acid.

Conclusion

Amidated celluloses are efficient sorbents of dietary fat, but not neutral and acidic sterols. The removal of dietary fat from the body suggests a hypolipidemic and hypocholesterolemic effect of amidated celluloses, which should be verified in the near future.

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