AGE CHANGES IN THE CHOLERETIC EFFECT OF TRIMETHYLGLYCINE IN NORMAL ANIMALS AND ANIMALS WITH EXPERIMENTAL ATHEROSCLEROSIS

V. I. Zapadnyuk and T. N. Panteleimonova

UDC 616.127-005.4-084-092.9

KEY WORDS: trimethylglycine; bile secretion; experimental atherosclerosis

The principal pathway of cholesterol (ChS) metabolism in the body is the synthesis of bile acids (BA). It has been suggested that the physiological role of this process is removal of the excess of ChS from the body and maintenance of ChS homeostasis [10]. An important cause of accumulation of ChS in the blood and tissues, spontaneously and during aging or during long-term administration of ChS to experimental animals, may therefore be the inefficiency of this metabolic pathway [9, 11, 12]. The limited information available on the state of the external secretory function of the liver in experimental atherosclerosis is largely contradictory. Some workers consider that utilization of the ChS of lipoproteins (LP) for synthesis of BA and steroid hormones in experimental atherosclerosis is intensified, although the LP level remains much higher than normally in this condition [5], whereas others have not observed any increase in ChS excretion in the composition of the bile [4, 6].

The aim of this investigation was to study several parameters of the external secretory function of the liver and of lipid metabolism in animals of different ages under normal conditions and in experimental atherosclerosis, and an attempt was made to regulate biochemically the processes of bile formation and excretion by means of a methylated amino acid, namely trimethylglycine (TMG).

EXPERIMENTAL METHOD

Experiments were carried out on young (7 months) and old (26 months) rats and on adult (12 months) rabbits. The comparative choleretic effect of TMG was studied on rats, which were given TMG in a dose of 1.5 g/kg body weight (group 1), allochol in a dose of 0.1 g/kg (group 2), or dehydrocholic acid in a dose of 0.1 g/kg (group 3), for 14 days per os by means of a gastric tube; group 4 consisted of intact animals. Experimental atherosclerosis was produced in rabbits by administration of ChS (0.2 g/kg): for 90 days in the control, for 60 days in the experiment; for the next 30 days both ChS and TMG (1.5 g/kg) were given. At the end of the course of the preparations, under amobarbital anesthesia (1 ml of a 1% solution of amobarbital/100 g body weight) the animal's bile duct was cannulated [2]. Bile was collected for 4 h. The quantity of bile excreted during each hour of observation and the total volume were measured. The concentrations of BA and ChS in the bile [3] and concentrations of total, free, and esterified ChS [13] and the total fraction of β- and pre-β-LP in the blood serum [1] were determined.

EXPERIMENTAL RESULTS

In the intact old rats the quantity of bile excreted in 4 h did not differ significantly from that excreted in young animals. The BA concentration in the bile of old rats was higher than in that of young rats: 2.91 ± 0.09 and 1.77 ± 0.2 g/liter respectively ($p < 0.01$, Table 1).

Injection of choleretics into young rats led to an increase in the quantity of bile excreted compared with the control, whereas in old animals none of the substances injected changed this parameter. As a result, an age difference in the intensity of bile secretion was observed in the experimental rats.

Laboratory of Experimental Pharmacology, Institute of Gerontology, Academy of Medical Sciences of the USSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR D. F. Chebotarev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 7, pp. 30-32, July, 1987. Original article submitted October 2, 1986.

0007-4888/87/0007-0907$12.50

TABLE 1. Comparative Effect of Choleretic Agents on Intensity of Bile Secretion, BA Concentration in the Bile, and Total ChS in Blood Serum of Young and Old Rats

Group of animals	Experimental conditions	Intensity of bile secretion, mg/100 g/4 h	BA concentration in bile, g/liter	ChS concentration in blood serum, mM		
				total	free	esterified
Young	Control (11)	443,5±60,6	1,77±0,24	1,50±0,15	0,42±0,14	1,28±0,35
	TMG (7)	535,0±44,7	3,05±0,25[a]	1,14±0,04[a]	0,21±0,05	1,03±0,32
	Allochol (10)	551,0±44,7	2,08±0,12	1,45±0,15	0,31±0,03	1,17±0,20
	Dehydrocholic acid (6)	575,0±27,2[a]	2,52±0,06[a]	1,25±0,31[a]	0,39±0,12	0,86±0,31
Old	Control (10)	371,8±33,4	2,91±0,09[b]	2,90±0,30[b]	1,09±0,01[b]	1,75±0,2
	TMG (10)	368,0±40,0[b]	4,80±42[a, b]	1,50±0,25[a]	0,74±0,20	0,96±0,03[a]
	Allochol (8)	327,0±66,0[b]	4,60±0,85[b]	1,48±0,02[a]	0,94±0,08	0,74±0,18[a]
	Dehydrocholic acid (8)	303,5±55,2[b]	3,70±0,35[a, b]	1,80±0,20[a]	0,77±0,04	1,14±0,16[a]

Legend. a) Differences significant compared with control animals, b) differences significant compared with young animals. Number of animals in group shown in parentheses.

TABLE 2. Effect of TMG on Biochemical Composition of Bile and on Serum ChS and LP Levels in Rabbits with Experimental Atherosclerosis

Experimental conditions	Bile		Blood serum			
	ChS, mM	BA, mg/100 g	total ChS, mM	free ChS, mM	esterified ChS, mM	β- and pre-β-LP, g/liter
Control	0,09±0,001	21,0±3,1	15,5±1,5	5,8±1,0	9,9±2,5	27,09±3,2
TMG	0,13±0,008*	30,7±2,6*	5,9±0,6*	2,9±0,5	3,2±0,9*	19,17±1,8*

Legend. Asterisk indicates that differences are significant compared with the control.

This fact is evidently connected with age changes in the structure of the hepatocytes. During aging, for instance, a decrease in the surface area of the smooth and rough endoplasmic reticulum, structural reorganization of the sinusoids and a decrease in their number per unit area of the lobule, and a decrease in the area of contact between liver cells and sinusoids, and in the size of the lobes of the liver and its weight as a whole, are observed [8]. These and other age differences lead to disturbance of metabolic processes localized in the hepatocytes, and reduce the reserve capacity of the liver, as is particularly clearly observed under functional loading conditions, in this case by the use of drugs.

All the preparations studied increased the BA concentration in the bile of young and old rats. The BA concentration in the bile of young rats receiving TMG was higher, not only than in the control ($p < 0.05$), but also than in animals receiving allochol ($p < 0.05$). The BA level in old rats after injection of TMG also was higher not only than in the control, but also in animals receiving dehydrocholic acid. The age difference in the BA concentration discovered in intact rats was preserved in the groups of animals receiving TMG ($p < 0.001$), allochol ($p < 0.01$), and dehydrocholic acid ($p < 0.02$).

The total ChS concentration in the blood serum of both young and old rats fell after administration of all the choleretics tested, in young animals due to the free ChS fraction, and in old animals due to the esterified ChS.

In rabbits with experimental atherosclerosis receiving TMG the intensity of bile excretion gradually fell toward the 4th hour of observation, and did not differ from that in the control. The quantity of bile excreted during 4 h was the same in both groups, namely 16.5 ± 2.7 g/kg in the control and 16.4 ± 0.9 g/kg in the experiment ($p > 0.05$). However, the BA level in the bile of animals receiving TMG was higher than in the control: 30.7 ± 2.6 and 21.0 ± 3.1 mg/100 g respectively ($p < 0.05$, Table 2).

A statistically significant increase in the ChS concentration to 0.13 ± 0.008 mM was observed in the bile of rabbits receiving TMG, compared with 0.09 ± 0.001 mM in the control ($p < 0.05$).

Under the influence of TMG the intensity of the hypercholesterolemia and hyperlipoproteinemia in rabbits with experimental atherosclerosis was considerably reduced. For instance, the total ChS level fell from 15.5 ± 1.5 to 5.9 ± 0.6 mM ($p < 0.05$). The concentration of the most atherogenic fraction of LP (β- and pre-β-LP) fell correspondingly from 2709.0 ± 319.1 to 1917.0 ± 176.8 mg/liter ($p < 0.05$).

Thus TMG, in a dose of 1.5 g/kg, had a marked choleretic effect, which was not weaker that that of choleretic agents such as allochol and dehydrocholic acid. The action of TMG is directed toward activating processes of cholate formation, so that it can be classed as a true choleretic.

It can be tenatively suggested that one of the mechanisms of the antiatherosclerotic action of TMG, established by the writers previously [7] and confirmed by the present investigation, is increased transformation of ChS into BA and its excretion in the bile.

LITERATURE CITED

1. A. A. Pokrovskii (ed.), Biochemical Methods of Investigation in Medicine [in Russian], Moscow (1969).
2. L. S. Vasilevskaya, Vopr. Pitan., No. 3, 47 (1965).
3. L. P. Gromashevskaya, V. P. Miroshnichenko, and M. G. Kasatkina, Advances in the Laboratory Diagnosis of Internal Diseases [in Russian], Chernovtsy (1977), pp. 89-90.
4. I. M. Kozhura, V. M. Shevchuk, and P. S. Lyashchenko, Byull. Eksp. Biol. Med., No. 9, 1038 (1976).
5. I. B. Livshits, Regulatory-Adaptive Mechanisms under Normal and Pathological Conditions [in Russian], Leningrad (1982), pp. 66-69.
6. M. S. Martsevich, Byull. Eksp. Biol. Med., No. 10, 43 (1973).
7. T. N. Panteleimonova and V. I. Zapadnyuk, Farmakol. Toksikol., No. 4, 83 (1983).
8. A. S. Stupina, Textbook of Gerontology, ed. by D. F. Chebotarev et al. [in Russian], Moscow (1978), pp. 143-163.
9. L. K. Finagin, I. M. Kozhura, and V. M. Shevchuk, Nervous and Humoral Mechanisms of the Onset of the Principal Diseases of the Cardiovascular System [in Russian], Poltava (1979), p. 155.
10. G. Harvengt, F. Heller, and I. Desager, Pathol. Biol., 31, No. 4, 284 (1983).
11. I. R. Sabine, Cholesterol, New York (1977), p. 270.
12. G. Salvioli and R. Salati, G. Arterioscler., 7, No. 2, 235 (1982).
13. R. Searcy and L. Bergquist, Clin. Chim. Acta, 5, No. 2, 192 (1960).