SPECIFICITY OF THE EFFECT OF POLYUNSATURATED PHOSPHATIDYLCHOLINE DEPENDING ON MODE OF ADMINISTRATION AND SPECIES OF ANIMAL

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Despite almost 30 years of experience in the use of polyunsaturated phospholipids and, in particular, the most widely spread of them, phosphatidylcholine (PCh), in the treatment of experimental and clinical atherosclerosis [5, 7, 11], many problems relating to the mechanism of action, mode of administration, and criteria for the evaluation of its efficacy still remain unsolved, and this still limits its practical application to some degree. Although data on the use of polyunsaturated PCh (PUPCh) by intravenous injection are basically consistent in character and point to positive changes in the plasma lipoprotein (LP) system and in the state of the aorta [1, 3, 5, 17], the results of investigations in which PUPCh was given perorally are contradictory. In particular, there are reports both of a decrease [7, 11] and of no change [4], or even an increase [6] in the plasma cholesterol (Chs) concentration.

The aim of this investigation was to study dependence of the action of PUPCh on the mode of its administration to two species of animals (rats and rabbits) and on the metabolic status of the animal.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats aged 3 months. The control group (I) received a standard diet for 18 months, the rats of group II received additionally a special ration with 4% cholesterol, such as is used as a model of atherosclerosis in rats [11]. The diet of the rats of group III also included 0.5% of soy PCh (Nattermann), and rats of group IV received 0.5% PCh only.

In the other series of experiments rabbits with alimentary atherosclerosis after being kept for 2 months on an atherogenic diet and 1 month on the standard diet, were used as the test object Animals of one group received 170 mg PUPCh/kg body weight perorally, and those of the other group received PCh intravenously in a dose of 50 mg/kg on alternate days in the form of an emulsion stabilized with detergent of plant origin [17]. The course of treatment lasted 20 days.

At the end of the experiments the concentrations of plasma lipids were determined by enzymic methods and by the use of the Centrifichem-400 automatic analyzer. Concentrations of Chs, PL, and individual classes of PL (PCh and sphingomyelin) in the erythrocytes were determined after extraction of the lipids and their fractionation by two-way thin-layer chromatography [8].

The liver and the entire aorta of the rats were fixed with 4% formaldehyde for morphological investigation. Sections of liver and aortic tissue were stained with Sudan III, hematoxylin-eosin, and Van Gieson's stain [16]. Material from the thoracic and abdominal aorta, embedded in paraffin wax, was studied in the form of 3-mm segments The severity of lesions in the rabbit aorta was assessed by means of an "IBAS" automatic image analyzer.

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TABLE 1. Changes in Blood Lipids and Degree of Damage to Aorta of Rabbits with Alimentary Atherosclerosis after Injections of PUPCh (M ± m)

Parameters	Receiving PUPCh (experiment)		Receiving 0.9% NaCl (control)	
	before treatment	after treatment	before treatment	after treatment
Plasma cholesterol, mg/dl	473±21	317+18	481+31	439+20
Cholesterol of LHDL	19+5	27+4	18+4	17+4
Per cent involvement of aorta	—	48+12	—	87+14

TABLE 2. Parameters of Blood Lipids (in mg/dl) in Rabbits with Alimentary Atherosclerosis during Treatment by Peroral Administration of Polyunsaturated PCh (M ± m)

Parameters	Receiving PUPCh		Control	
	before treatment	after treatment	before treatment	after treatment
Cholesterol	518±24	471±39	480±29	451±32
Triglycerides	110±22	95±14	121±29	103±14
Receiving PUPCh	18±6	16±4	17±4	17±5

TABLE 3. Changes in Lipid Composition of Blood Plasma and Erythrocytes in Rats Receiving Cholesterol in Response to Peroral Administration of PUPCh (M ± m)

Parameters	Groups of animals			
	control (n = 6)	receiving cholesterol (n = 10)	receiving cholesterol + PUPCh (n = 6)	receiving PUPPCh (n = 7)
Plasma, mg/dl				
Cholesterol	103±10	134±11	508±42	425±39
Triglycerides	161±42	88±23	124±28	116±19
Erythrocytes (molar ratio)				
Chs/PL	0,91±0,04	0,93±0,04	1,14±0,05	1,0±0,04
PCH/sphingomyelin	3,21±0,12	2,07±0,09	3,28±0,13	3,92±0,14

EXPERIMENTAL RESULTS

Table 1 gives the results of a course of treatment of rabbits with alimentary atherosclerosis by the PUPCh preparation given by intravenous injection. Treatment with PUPCh led to a decrease in the total Chs concentration and an increase in the content of cholesterol of high-density LP (HDL) compared with initial values and also with the control, and also to a significant fall (almost by half – from 87 to 48%) in the percentage involvement of the aorta.

Similar results were obtained previously after intravenous injection of PUPCh into rabbits in the form of the preparation "Lipostabil" [11], or of positively charged micelles [1]: a fall in the cholesterol level of apoB-containing LP and erythrocytes and in the percentage involvement of the aorta was noted. There is also evidence of the protective action of injection of PUPCh against the development of alimentary hyperlipoproteinemia when used parallel with cholesterol feeding [3, 17].

Meanwhile, in most investigations the more convenient peroral method of administration of PUPCh in the form of "Lipostabil" capsules has been used. Despite the positive effect of this preparation [7, 11, 12], it nevertheless is not on the list of the most widely used antiatherogenic agents [2], possibly because in some cases it has no hypocholesterolemic action [4, 6].

To ascertain the causes of this contradictory attitude toward the use of PUPCh in the treatment of atherosclerosis, we also studied a group of rabbits receiving PUPCh perorally in a dose of 170 mg/kg daily, which is 5-10 times greater than the clinical dose [12].

It will clear from Table 2 that peroral treatment of experimental atherosclerosis in rabbits with PUPCh had no effect on the blood lipids, by contrast with its hypolipidemic effect observed in rabbits, chimpanzees, and rats [7, 11, 16]. In these investigations, however, the doses of PUPCh as a rule were not below 500 mg/kg, i.e., 3 times greater than those which we used and, on average, 20 times higher than the clinical doses [12]. This dose-dependent difference in the effect of PUPCh is due to differences in the manner of its penetration into the blood stream, when its hydrolysis products may sub-

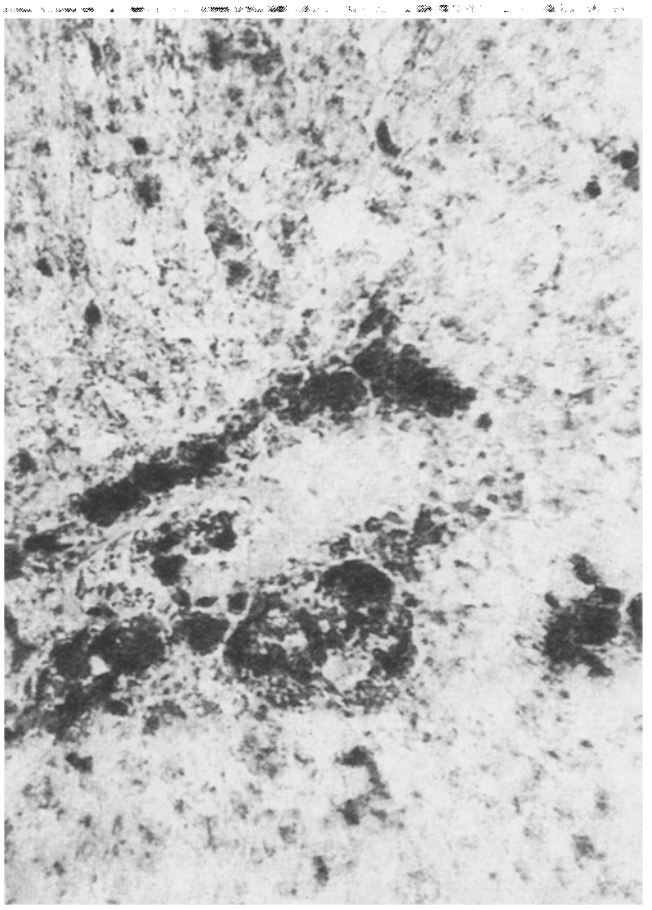

Fig 1. Rat liver after 18 months on cholesterol diet combined with soy phosphatidylcholine (group III).

sequently recombine with other lipid fragments, and the probability of this resynthesis of PUPCh depends on its initial quantity. It must also be remembered that differences are found in the pharmacokinetics of PUPCh in different animals, and this is manifested in particular in rats, for whereas with doses of 0.5-1 g/kg (in addition to a cholesterol diet) the Chs level fell [11, 16], peroral administration of particularly high doses of PUPCh (over 7 g/kg [4]) led,on the contrary, to a hypercholesterolemic effect.

To study the pattern of action of PUPCh in rats when administered perorally in addition to cholesterol feeding, i.e., to study not the therapeutic, but rather the protective action of PUPCh, the next series of experiments was undertaken.

As Table 3 shows, keeping rats for 18 months on a cholesterol diet led to only a small rise of the plasma cholesterol level (group II). Meanwhlle, the effect of addition of PUPCh both against the background of this diet (group III) and without it (group IV), was unexpected, for in these animals the plasma Chs level was almost 4 times higher than in animals receiving cholesterol alone In these same groups of animals, especially III, the molar ratio of Chs/PL and also of PCh/sphingomyelin was increased in the erythrocytes, evidence of incorporation of both PCh and cholesterol into the cell membranes. The parallel rise of these two coefficients under these circumstances shows that the degree of incorporation of cholesterol was stronger. The concentration of triglycerides showed no significant change in all groups of animals.

Morphological analysis found no changes in either the thoracic or the abdominal aorta of rats receiving Chs and/or PUPCh. Only in one rat of group III (receiving Chs + PUPCh) was lipid infiltration in the wall of the aorta observed (in the region of the intima and media).

Meanwhile, in all groups except group I, fatty degeneration of the liver was observed. Whereas after cholesterol feeding (group II) only slight deposition of fat was observed in the liver, in rats receiving PUPCh (group IV) these changes were more marked, and the greatest degree of lipid infiltration was observed after administration of ChS combined with PUPCh (group III; Fig. 1).

Thus both biochemical and morphological criteria show that in experiments conducted in this manner long-term feeding of rats on a cholesterol-containing diet, unlike in the majority of such experiments, did not cause accumulation of Chs in either the blood or the aorta. Meanwhile addition of PUPCh to the diet, with or without cholesterol feeding, led to hypercholesterolemia, with accumulation of Chs in erythrocyte membranes and to fatty degeneration of the liver.

This action of PUPCh resembles the effect of a diet rich in triglycerides: an increase in concentrations of lipids [10] and cholesterol (parallel with fatty infiltration of the liver) in the blood serum of pigs [9], rats [14], and rabbits [10]. According to Thompson [17], the action of perorally administered phospholipids can be reduced essentially to the effect of the fatty acids present in their composition. Increased biosynthesis of Chs and its secretion into the blood stream are in all probability a nonspecific physiological response to the need to form an additional amount of LP to ensure circulation of the lipid material introduced.

The mechanism of the difference between the results of our own experiment and those of most similar experiments [1, 3, 5, 17], in which with rare exceptions [4, 6] a hypocholesterolemic effect of perorally administered PUPCh was observed, is not yet clear. However, in all these studies there is one noteworthy fact: the presence of atherosclerosis or hyperlipoproteinemia, toward the prevention or minimization of which the action of PUPCh was aimed. In our own experiments, in the absence of atherosclerosis, perorally administered PUPCh played a different role – that of a source of fatty acids [17]. One reason for this could be the unusually long duration of the experiment (1.5 years), combined with the high activity, characteristic of rats [13], of the reversed cholesterol transport system. This could lead to some degree of adaptation, protecting the aorta, on account of the ever-increasing load on the liver.

The results of the present study show that peroral administration of PUPCh, first, is not to be recommended when the reversed Chs transport system is actively functioning, and second, can be effective, probably, mainly in combination with other approaches.

Conversely, intravenous injection of PUPCh directly into the blood stream is in fact an effective method of treatment of atherosclerosis as a result of direct incorporation and stimulation of the high-density LP system [12], with consequent activation of reversed Chs transport.

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LITERATURE CITED

1. E. A. Borodin, M. É. Lanno, É. M. Khalilov, et al., Byull. Éksp. Biol. Med., No. 2, 164 (1985).
2. V. S. Repin, Molecular-Cellular Mechanisms of Development of Atherosclerosis [in Russian], No. 6, Moscow (1987).
3. C. W. Adams, Y. H. Abdulla, O. B. Bayliss, et al., J. Path. Bact., No. 1, 77 (1967).
4. S. Bennet-Clark, V. E. Clark, and D. M. Small, Am. J. Physiol., **241**, 422 (1981).
5. M. Friedman, S. O. Byers, and R. H. Rosenman, Proc. Soc. Exp. Biol. Med., **95**, 586 (1957).
6. H. Greten, H. Raetzer, A. Stiel, et al., Atherosclerosis, **36**, 81 (1980).
7. A. N. Howard and Y. Patelsky, Phosphatidylcholine, ed. by H. Peeters, New York (1976), pp. 187-200.
8. M. Kates, Techniques of Lipidology, Amsterdam (1973).
9. D. M. Kim, J. Schmec, and K. T. Lee, Atherosclerosis, **52**, 101 (1984).
10. D. Kritschevsky, S. A. Tepper, D. M. Klurfeld, et al., Atherosclerosis, **50**, 253 (1984).
11. F. Leuschner, H. H. Wagener, and B. Neuman, Drug Res., **26**, 1743 (1976).
12. S. S. Ma[illegible]nd E. S. Nalivaiko, Phosphatidylcholine: Effect on Cell Membrane and Transport of Cholesterol, ed. by A. I. A[illegible]akov and K. J. Gundermann, Bingen am Rhein (1989), pp. 193-200.
13. M. Nagashima, J. Lipid Res., **27**, 706 (1986).
14. K. A. Narayan, J. J. McMullen, and D. P. Butler, Atherosclerosis, **23**, 1 (1976).
15. G. Salvioli, R. Lugli, and E. Gaetti, Phosphatidylcholine Effect on Cell Membrane and Transport of Cholesterol, ed. by A. I. Archakov and E. J. Gundermann, Bingen am Rhein (1989), pp. 27-32.
16. L. Samochoviec, D. Kadlubowska, and L. Rozewicka, Atherosclerosis, **23**, 307 (1976).
17. G. R. Thompson, A. Jadhav, M. Nava, et al., Eur. J. Clin. Invest., **6**, 241 (1976).