Prophylactic and Corrective Action of the Polyethylene Oxide Polyox WSR-301 in Rats with Experimental Lipoidosis

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Two once-weekly intravenous injections of the polyethylene oxide Polyox WSR-301 (yielding a blood concentration of the order of 5×10^{-6} g/ml) led to a 38% decrease in the area occupied by sudanophilic lesions in the aortic arch of rats fed an atherogenic diet for two weeks. Perfusion under constant pressure of the formalin-fixed vascular system in the posterior part of the body with physiological saline and then with polyethylene oxide (10^{-5} g/ml) was without effect in normal rats and in those with mild lipoidosis, but reduced hydrodynamic vascular resistance by 9-14.5% in rats with pronounced lipoidosis. Intravenous injection of polyethylene oxide into anesthetized rats with pronounced lipoidosis in doses that were subthreshold for normal rats (blood concentrations of the polymer were of the order of 10^{-7} g/ml) caused a 20% decrease in the total peripheral resistance to blood flow, with a 17-20% rise of the blood flow rate in the carotid artery.

Key Words: *atherosclerosis; polyethylene oxide; hemodynamics*

Local hemodynamic abnormalities are a characteristic feature of atherosclerosis. The composition and permeability of the vessel wall become altered even at low values of the shear stress suffered by the vessel wall, provided the blood flow along the vessel undergoes microperturbations. If the shear stress is substantial, the blood flow may damage the vessel wall and thus promote fat deposition. In an area where the formation of atherosclerotic plaque has already started, secondary flows will cause it to spread [6]. Substances capable of "laminating" blood flow may therefore prove effective in the prevention and treatment of atherosclerosis. A class of compounds - high-molecular polymers - that are able to modify the microcomposition of flowing liquids, including blood, has now been identified. These compounds are effective not only in tubes *in vitro* (Tomes' effect, 1948) but also *in vivo*, in living organisms, where they reduce the total peripheral resistance to blood flow by reducing its microperturbations [4,9]. The purpose of the study described here was to explore the possibility of preventing and correcting the atherosclerotic process biomechanically by acting on the blood flow structure.

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MATERIALS AND METHODS

The study was conducted on male Wistar rats weighing 160-180 g and included three series of tests. In the first series, rats received two intravenous injections, at a 7-day interval, of the polymer polyethylene oxide (PEO) Polyox WSR-301 (Union Carbide) in a dose of 2.5×10^{-4} g/ml (0.2 ml) or of physiological saline in the same volume (control group). Concomitantly, the rats of both

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Table 1. Hydrodynamic Resistance (Arbitrary Units) to Perfusion with Saline or Polyethylene Oxide Solution (PEO) Offered by Vessels of the Posterior Part of the Body in Rats Fed an Atherogenic or Normal Diet

Perfusion solution	Perfusion pressure, mm Hg		
	80	100	120
Normal diet			
Control rats:			
Saline	1.48±0.02	1.42±0.05	1.49±0.03
Saline	1.49±0.03	1.43±0.04	1.41±0.05
Test rats:			
Saline	1.51±0.04	1.51±0.06	1.52±0.08
PEO	1.52±0.13	1.49±0.07	1.39±0.05
Atherogenic diet causing mild lipoidosis			
Saline	1.50±0.07	1.47±0.04	1.42±0.02
PEO	1.53±0.09	1.43±0.03	1.46±0.06
Atherogenic diet causing pronounced lipoidosis			
Saline	1.51±0.08	1.46±0.07	1.54±0.06
PEO	1.05±0.14*	1.23±0.12*	1.32±0.03*

Note. *$p<0.05$ relative to perfusion with saline.

groups were administered vitamin D_2 and cholesterol by the oral route (via a gastric tube) in daily doses of 320,000 U and 40 mg per 100 g body weight, respectively, 6 days per week for 2 weeks [5]. Thereafter, portions of the aortic arch were removed, fixed, and stained with Sudan red, and the number of intersections of the stained areas with ribs of a standard grid with a step of 1 mm was counted [1].

In the second series, the posterior part of the body was perfused under constant pressure [7] with a 7% formalin solution and then, at each of the pressures used (80, 100, and 120 mm Hg), with saline first and with a PEO solution (10^{-5} g/ml) later in the test group and again with saline in the control group, measuring the amount of outflowing perfusate. Thereafter, the rates of perfusate volume flow were calculated using calibration curves plotted for the given perfusion system and for each of the solutions used, and the hydrodynamic resistance of the vasculature was computed. There were three groups of rats in this series: rats fed the atherogenic diet described above for 6 days, those fed this diet for 12 days (with a one-day interruption), and those fed a normal (standard) diet (controls). In this series, the severity of sudanophilic lesions in the aortic arch was evaluated by examining its morphology.

In the third series, three groups of rats were also used, which had received the same treatment as the corresponding groups in the second series, but were then anesthetized with Nembutal (50 mg/kg intraperitoneally) and infused with the PEO solution in the jugular vein in a concentration of 10^{-5} g/ml at a rate of 0.15 ml/min over 1 min (yielding a PEO concentration of the order of 10^{-7} g/ml in the blood) or with saline by the same route (controls). In this series, we recorded systemic arterial pressure (from the left carotid artery using a Statham P-23 sensor), heart rate, and stroke volume (by means of tetrapolar rheography using an RPG-102 rheoplethysmograph) and calculated the total peripheral vascular resistance. In addition, blood flow rates in the right carotid artery were measured with ultrasonic Doppler anemometry employing a blood flow indicator. The data were expressed in units of volume blood flow using the calibration curves obtained in tests where isolated segments of the same vessels as above were perfused at a constant flow rate by means of a perfusion pump (LKB).

The results were subjected to statistical treatment by Student's t test and are presented below as means and standard errors of the means.

RESULTS

The rats injected with PEO developed less pronounced lipoidosis than did the control animals injected with saline. Thus, the mean area of sudanophilic lesions amounted only to 12.7±3.1% of the aortic arch surface in the 35 test rats as compared to 20.6±2.4% in the 25 controls ($p<0.05$). This suggests that even rare and widely spaced PEO injections may be effective in preventing the development of atherosclerotic lesions.

The results obtained in this test series are in good agreement with the previously reported decreases in the number of atherosclerotic plaques in rabbits and birds fed an atherogenic diet and injected intravenously with Separan [8,10], a polymer chemically distinct from PEO. Recently,

Table 2. Systemic Arterial Pressure and Blood Flow in the Carotid Artery before (Baseline), at the Time of, and after Injection of Saline (I) or Polyethylene Oxide Solution (II–IV) into Rats Fed a Normal Diet (I and II) or an Atherogenic Diet Causing Mild (III) or Pronounced (IV) Lipoidosis

	Time, min				
	baseline	injection		after injection	
	0	0.5	1	2	6
Systemic arterial pressure, mm Hg					
I	113±4	115±4	113±4	112±4	114±4
II	101±1	99±6	94±6	94±4	106±5
III	110±2	109±2	103±3	99±2	108±6
IV	96±2	100±4	92±4	90±3*	91±4
Blood flow rate, ml/min					
I	2.7±0.8	2.6±0.8	2.6±0.8	2.8±0.8	2.4±0.8
II	3.7±1.1	4.9±1.3	5.4±1.4	5.0±1.4	4.8±1.2
III	2.7±0.4	3.2±0.4	4.3±0.5	3.0±1.1	2.4±0.7
IV	4.6±0.6	5.0±0.6	5.6±1.6*	5.6±1.3*	5.1±1.1

Note. *$p<0.05$ in comparison with baseline values.

similar results have been obtained for rabbits using PEO [3]. The similarities between the action of polymers differing in chemical nature but not in the physical properties of their molecules (which are relatively linear, flexible, and long) can be taken as further evidence that their prophylactic effects are hydrodynamic in nature.

Could it be that polymers such as PEO can also be beneficial as therapeutic agents? In an attempt to answer this question, we carried out the second and third series of perfusion tests (as described above) on rats with different degrees of experimental lipoidosis.

In the second series, where the posterior part of the body was preliminarily perfused with formalin solution, we were testing fixed vessels that had lost the ability to change their radius. Table 1 summarizes the results recorded for the 23 rats fed the standard diet (including 12 controls perfused with saline twice and 11 test rats perfused with saline and then with the PEO solution) and for the 21 rats fed the atherogenic diet during 6 (10 rats) or 12 (11 rats) days. Sudanophilic lesions in the aortic arch were found to occupy 13.2±2.1% of its surface area in the group fed the atherogenic diet for 6 days and as much as 37.5±2.6% in the group fed this diet for 12 days ($p<0.01$). Perfusion with cell-free saline minimizes blood flow microperturbations which in a real vascular system are mainly produced by erythrocytes. For this reason, the substitution of the PEO solution for saline proved ineffective in the normal (control) rats and in those with mild lipoidosis. In the group with pronounced lipoidosis, produced by the 12-day exposure to the atherogenic diet, such substitution led to significant decreases (by 9-14.5%) in hydrodynamic vascular resistance. This suggests that eliciting changes in the local hydrodynamics may help not only to prevent disease but also to eliminate existing lesions.

The isolation from human donor blood, as well as from bovine and rat blood, of biopolymers capable of diminishing the turbulent friction of liquid flowing in a tube [2] validated the idea that the circulatory system possesses an endogenous mechanism for reducing resistance to blood flow. Being hydrodynamic in nature, this mechanism is coupled to *in vivo* changes in the blood flow structure. Possibly, biopolymers of this kind are deficient in the body under certain pathological conditions so that doses of an exogenously added biopolymer that are ineffective in health may prove beneficial in disease.

In the third series, systemic hemodynamic parameters were measured in anesthetized rats - 23 normal animals fed the standard diet (10 of them were injected with saline and 13 with the PEO solution) and 15 fed the atherogenic diet; 8 of the latter rats had mild lipoidosis (only 14.4±2.5% of the aortic arch area was affected) and 7 had pronounced lipoidosis (38.7±2.2%). In this series, too, the PEO doses subthreshold for normal rats were without effect in the rats with mild lipoidosis. In the group with pronounced lipoidosis, however, such doses lowered the systemic arterial pressure by 13% (Table 2). In the latter group the stroke volume tended to increase, the heart rate remained virtually unchanged, while the estimated values of peripheral vascular resistance decreased by 20% on average ($p<0.05$). Blood flow rates in the carotid artery significantly increased (by 17-20%) only in the group with pronounced lipoidosis (Table 2).

Thus, high-molecular linear polymers that act on the blood flow itself seem to be not only ef-

fective in preventing experimental lipoidosis but also capable of optimizing hydro- and hemodynamic parameters in animals with established lipoidosis, a feature which opens up prospects for therapeutic application of such biopolymers.

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Adaptation to Altitude Hypoxia Limits Lipid Peroxidation during Inflammation and Stress

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Preadaptation of rats to altitude hypoxia results in reduced activation of lipid peroxidation during subsequent stress, inflammation, or both, as compared to hypoxia-unadapted animals, with the result that secondary changes in organs and tissues of adapted rats are much less pronounced and conditions are created for alleviating the acute inflammation and the stress reaction.

Key Words: *adaptation to hypoxia; lipid peroxidation; inflammation; stress*

Activation of lipid peroxidation (LPO) is now known to be one of the main injurious factors in stress and may determine the development of secondary changes in organs and tissues [2,8]. It should be noted that the deleterious effects from LPO activation in inflammatory diseases are intensified by leukocyte-generated free oxygen radicals released into the inflammatory focus [13-15]. This is an important point, since the inflammatory process is usually accompanied by a marked stress reaction [5]. Recent research has shown that stress-associated damage can be significantly reduced by suppressing LPO through adaptation of the organism to hypoxia [8,9].

Accordingly, we assumed that enhancing the antioxidative potential of animals by adapting them to hypoxia should mitigate the secondary changes arising in their tissues during stress, inflammation, or both.

MATERIALS AND METHODS

Male white rats weighing 160-180 g were used; 40 of them served as controls and 80 were adapted to altitude hypoxia in a pressure chamber by exposing them to an "altitude" of 5500 m for 6 h per

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