Suppression of Experimental Alimentary Atherosclerosis with Drag Reducing Polymers

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It has long been accepted that disturbances of lipid metabolism play a fundamental role in experimental atherogenesis (EA) and their normalization has been regarded as the major goal of antiatherogenic therapy. At the same time, the published data indicate that there is a close relationship between EA and the hydrodymanic action of blood flow on the vessel wall, particularly the effect of increased blood flow turbulence at the sites of arterial branching and curvature. Since control over the blood biomechanics is associated with considerable difficulties, the preparations which reduce the hydrodynamic resistance of the blood (drag-reducing agents, DRA) have not been employed in modern antiatherogenic therapy, although suppression of EA with high-molecular-weight linear polymers has been reported [6-8]. The principle underlying the effect produced by long linear molecules of these agents is consistent with the "Toms phenomenon" which is well known to hydrodynamicists and is associated with laminarization of the flow and decrease in pseudoturbulence of flow of any fluid (including the blood), leading to a reduction of the hydrodynamic resistance of the blood and thus diminishing the effect of the blood flow on the vascular wall.

The objective of this study was to demonstrate the antiatherogenic effect of the polyethylene oxide WSR-301 (Union Carbide, USA), a DRA with a molecular weight of 4 mln daltons, and to perform a morphological characterization of plaques formed at aortic ostia orifices against the background of DRA administration.

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MATERIALS AND METHODS

Experiments were performed on adult chinchilla rabbits weighing 2.5-3 kg. During a 3-month period the animals received: 1) a cholesterol-rich diet (250 mg/kg) together with standard rabbit chow (group I, 12 animals); 2) a weekly intravenous injection of the DRA with a resulting blood concentration of 2×10^{-6} g/ml and standard rabbit chow (group II, 11 rabbits); and 3) the cholesterol-rich diet and the DRA injection (group III). Judging from the literature and our studies, this dose of DRA is hydrodymnamically most effective: it does not alter the major metabolic parameters of the blood (pH, pO_2, pCO_2, glucose concentration, osmolarity and asymptomatic viscosity), provokes no increase in the permeability of the blood-brain barrier, and has no effect on the cerebral blood flow or pO_2 of the brain tissues, but it does reduce the systemic arterial pressure (by 10-15%) while increasing the cardiac output, minute circulatory volume, and aortic blood flow without altering the heart rate [1-5,9].

Biweekly determinations of the plasma cholesterol level (mg/dl, by the Ilca method), osmolarity (in a Knauer microsmometer, Germany), and

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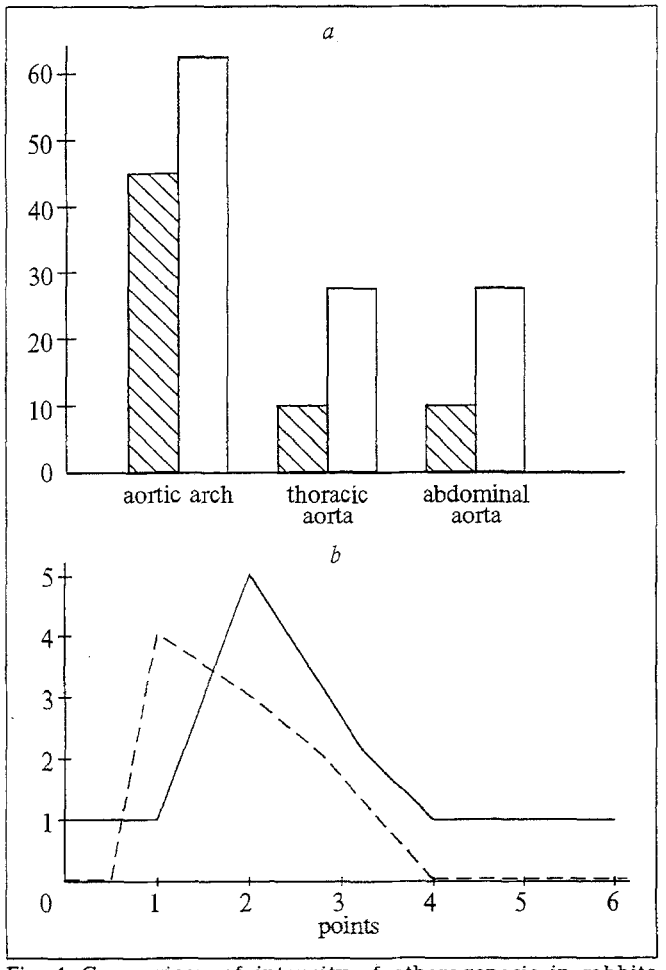

Fig. 1. Comparison of intensity of atherogenesis in rabbits (control group I), receiving only a high cholesterol diet (white bars) and experimental rabbits receiving the same diet + i.v. injection of DRA polymer (hatched bars). *a*) affected area in different regions of aorta (%); *b*) magnitude of aoortic lesions (6-point scale). Solid line group I; dashed line: group III.

hematocrit (in a hematocrit centrifuge) were performed in all animals. Corrections of DRA and cholesterol doses were done biweekly according to the body weight changes.

After decapitation, the aorta was excised, photographed, fixed in 10% formaldehyde, stained with Sudan by the standard method. The areas (%) of EA lesions were determined in aortic arch, thoracic and abdominal segments of the aorta, the total magnitude of EA lesions was evaluated using a 6-point scale, and macroscopic peculiarities of EA plaques formed at the aortic ostia were documented.

RESULTS

The cholesterol-rich diet induced a considerable increase in the arterial plasma cholesterol concentration, irrespective of DRA administration. In group I rabbits, plasma osmolarity increased to 310-312 mOsm/l (Table 1). Thus, this model of EA has a side effect consisting of blood hyperosmolarity, which is known to lead to secondary changes in the shape of erythrocytes, other blood cells, and vascular endothelial cells.

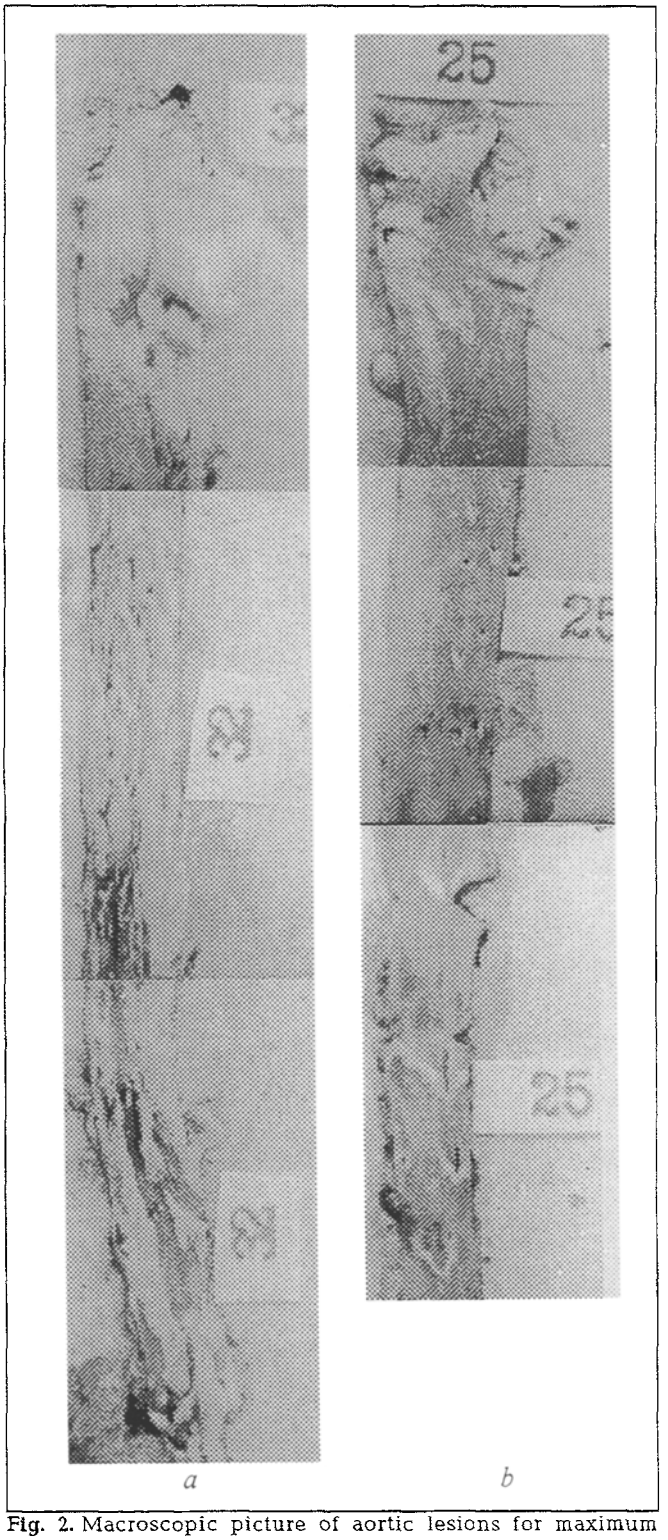

Fig. 2. Macroscopic picture of aortic lesions for maximum atherogenesis in group I (control), receiving only the high-cholesterol diet (*a*) in comparison with maximum atherogenesis discovered in experimental group receiving the same diet + i.v. injection of DRA polymer (*b*). Unstained specimens, ×1.5.

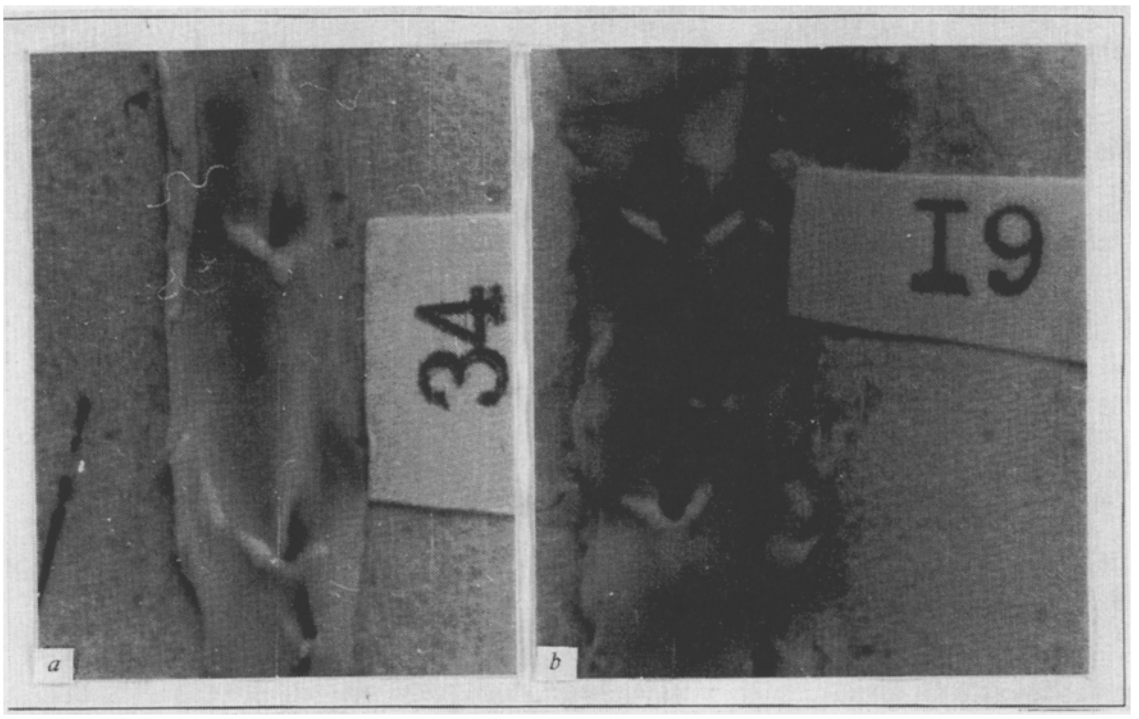

Fig. 3. Morphological peculiarities of plaques formed in EA around ostia of abdominal aorta branches. *a*) formation of a plaque shaped like a check mark, the "beak" distal to ostium (group I, cholesterol–rich diet); *b*) formation of plaque shaped like two oval deposits of cholesterol without involving into the process the aorta distal to its branch (group III, same diet + i.v. injection of DRA polymer). Unstained specimen, ×6.

In group II rabbits, which were administered DRA, plasma osmolarity and cholesterol level remained unchanged compared with the initial values. In both series the hematocrit was unchanged.

Macroscopic examination of the aortas in all series confirmed the observations [6-8] indicating the possibility of suppressing EA with DRA even in the presence of very high cholesterol levels (Table 1). The most potent suppression of EA with DRA occurred in the thoracic and abdominal aortas (Fig. 1, *a*, *b*), where the pseudoturbulent blood flow may have been affected by DRA to a greater extent, increasing blood flow laminarity and preventing the appearance of zones of stasis. In the aortic arch the effect of DRA was statistically significant, though less potent (Fig. 1, *a*). Moreover, in group III rabbits, plaques were developed even in cases where they were not found in the descending aorta. We think that this may be due to the highest turbulence of blood flow in the aortic arch, where the DRA concentrations employed in this study were insufficient to attain a complete normalization of the flow.

In contrast to group I, where 4.5- and 6-point EA was developed, in not one rabbit of group III did the EA intensity reach these values (Fig. 1, *b*). The maximum number of rabbits in group III had 1 point EA, while in group I the maximum num-

TABLE 1. Plasma Cholesterol Level and Osmolarity of Rabbits with Experimental Atherosclerosis ($M \pm m$)

Group	Background	Time of determination, weeks					
		2	4	6	8	10	12
		Cholesterol level, mg/dl					
I. Control	101.6±14.1	645.7±89.6*	835.8±69.5*	1066.4±98.7*	857.6±130.4*	857.6±130.4*	812.6±118.8*
854.6±131.0*							
II. Control	107.6±14.3	134.7±15.2	85.7±11.9	80.8±11.3	148.1±24.2	114.2±10.1	
III. Experimental	125.9±15.9	696.7±86.9*	964.4±10.7*	423.9±75.2*	676.2±11.0*	905.2±91.1*	
		Osmolarity, mOsm/liter					
I. Control	291.3±2.9	—	—	—	309.7±2.6*	311.8±1.8*	307.8±10.3*
II. Control2	291.3±2.9	—	—	—	292.8±2.1	300.8±3.2	305.3±2.5
III. Experimental	291.3±2.9	—	—	—	296.4±1.3	302.8±2.1	299.0±2.9

Note. Asterisk denotes significant differences ($p<0.01$).

ber of rabbits exhibited 2 point EA. No rabbits in group II developed EA.

The appearance of lipid spots and whitish EA plaques elevating over the intima varied in group I and group III. In the least damaged aortas the plaques were small, always confined to the aortic arch and occasionally localized at the ostia of the thoracic and abdominal aorta. The most intense EA was developed by group I rabbits: a larger area of their aortas was covered with plaques, they were more elevated over the intima, fused, occluded some ostia, and disfigured the aortic surface (Fig. 2, a). In group II, the maximum degree of EA was never associated with such damage to the aorta (Fig. 2, *b*).

The longitudinally alternating grossly normal and damaged aortic areas were terminated by plaques having the shape of a check mark with the "beak" located distal to the ostium and oriented along the blood flow, which was typical of group I animals (Fig. 3, *a*). In group III rabbits, atherosclerotic plaques not only occupied a smaller area of the aortic surface but also differed in shape and were always confined to the ostium. These plaques consisted of two separate oval foci that did not form a "beak" (Fig. 3, *b*). It can be assumed that the DRA elicits the strongest effect on the most nonstationary blood flow at the ostia, which is well documented in the literature. This specific feature of EA plaques was observed in both minimally and rather pronounced EA. Only in 2 rabbits with the most pronounced EA-related aortic damage, which was comparable to the moderate degree EA recorded in group I, atherosclerotic plaques were formed distal to the ostium and their "beaks" were less apparent than the lateral foci. Occasionally, the "beak" lay asymmetrically to the central axis of the ostium.

This study has confirmed the effect of DRA on the intensity and morphological peculiarities of EA. We thus postulate that the development of drugs capable of altering the biomechanic influence of the blood flow on the vessel wall could substantially broaden the possibilities of current antiatherogenic therapy.

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