CHANGES IN MORPHOLOGY AND PERMEABILITY OF PERFUSED RABBIT ARTERIES DURING ACUTE ELEVATION OF THE INTRAVASCULAR PRESSURE

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It has been postulated on the basis of the results of epidemiologic and clinical observations that a high arterial blood pressure (BP) is a positive risk factor of atherosclerosis, which accelerates the development of morphological or clinical manifestations of the disease [3, 12]. According to the Framingham study, the number of cases of ischemic heart disease (IHD) recorded in the population of men with essential hypertension was 5 times higher than in the normotensive group [4]. According to autopsy data, this difference was greater still: In patients with hypertension the frequency and degree of arterial atherosclerosis are 10 times higher than in the control, normotensive group for the same age [4]. However, it has not yet been explained how and under what conditions a high BP accelerates the development of athersclerosis.

Chronic or acute hypertension combined with hypercholesterolemia does not induce the development of atherosclerosis in the arteries of animals [6]. However, hypertension with a high plasma cholesterol level stimulates the development of typical lipofibrous plaques in zones of mechanical injury or denudation of arteries in animals [6]. These data led to the conclusion that a high BP does not affect zones with intact, undamaged endothelium, but rather accelerates the development of atherosclerosis in zones where loss of or injury to the endothelium has already occurred.

The aim of this investigation was to study whether a short-term increase of intravascular pressure causes morphological injuries to the endothelium or accelerates the accumulation of ^{125}I-labeled low-density lipoproteins (^{125}I-LDL) in the wall of perfused arteries of healthy rabbits, and how a raised hydrostatic pressure affects ^{125}I-LDL transport in denuded areas of perfused arteries.

EXPERIMENTAL METHOD

Experiments were carried out on 42 male chinchilla rabbits weighing 2-3 kg. The thoracic aorta was perfused in anesthetized, artificially ventilated animals. Segments of the vessel 3-4 cm long were cannulated. Perfusion was carried out under standard conditions [1, 10]. The perfusion medium consisted of medium 199 with 10% delipidized calf serum (Gibco, USA). Perfusion of the aorta by this method, under a physiological pressure of 100 mm, enabled the endothelium to be kept morphologically intact for 4-5 h [2, 5]. Elevation of the pressure in the vessels while the peristaltic pump was in operation was produced by compressing the drainage tube partially. The intravascular pressure was measured by means of a strain-gauge transducer of a Polyphysiograph (Schwarzer, West Germany). The vessels were prepared for scanning electron microscopy by the method described previously [2]. The morphology of the endothelium was studied outside the zone of the ostia. Specimens were studied in the Philips-500 scanning electron microscope. The number and area of zones of de-endothelization, the number of argyrophilic cells and of craters and stomata, and the area of regions of endothelium with stigmata were counted on the microscope screen [2]. The density of endotheliocytes per square millimeter was determined by counting the cells in 50 random areas each of 0.08 mm^2. The area and length of the cells were measured on the MOP-3 semiautomatic image analyzer. In each segment 200 random cells were analyzed.

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TABLE 1. Mean Number of Endotheliocytes per 1 mm^2 Luminal Surface of Rabbit Thoracic Aorta under Increased Intravascular Pressure (M ± m)

Fixation	Pressure, mm Hg		
	100	150	250
Under normal pressure (100 mm Hg)	1475±40 (56)	1410±24 (56)	1390±26* (56)
Under raised pressure (150 and 200 mm Hg)	1475±40 (56)	1385±57 (16)	1375±21* (16)

Legend. Total area on which density of endotheliocytes was determined is shown (in mm^2) in parentheses. Here and in Tables 2 and 3: $P < 0.05$ compared with data under a pressure of 100 mm Hg.

Interaction of ^{125}I-LDL with the vascular wall was determined quantitatively by the method described previously [1]. ^{125}I-LPL with a density of between 1.019 and 1.067 mg/ml, obtained from human serum by preparative ultracentrifugation in a dose of 5 μg/ml, were used. The LDL were labeled by the iodine monochloride method. To investigate the effect of an excess of unlabeled LDL on ^{125}I-LDL uptake, two parallel perfusion systems were used, in one of which unlabeled LDL were added in a dose of 250 μg protein/ml instead of ^{125}I-LDL. De-endothelization was carried out with a Foggarty's balloon catheter [10], and the completeness of de-endothelization was verified on the scanning electron microscope. Incorporation of ^{125}I-LDL into the vessel wall was determined by measuring radioactivity of a segment of the aorta on a Gamma-counter (Searle 1175, USA), after which the result was expressed per picogram protein of ^{125}I-LDL/mm^2 of luminal surface of the vessel. The experimental results were subjected to statistical analysis. The significance of differences was estimated by Student's test.

EXPERIMENTAL RESULTS

Unlike in previous investigations [1, 10] perfusion of the rabbit aorta in situ was carried out at different hydrostatic pressures, i.e., during varying of the diameter of the vascular lumen. It was therefore necessary to determine whether the morphology of the endothelial lining changes in response to stretching the lumen of the vessel. We calculated the average number of cells per 1 mm^2 of luminal surface during perfusion under a pressure of 100-250 mm Hg (Table 1). For quantitative morphometry the vessels in group A were fixed under a pressure of 100 mm Hg, those in group B under a pressure equal to that during perfusion. The mean cell density in the monolayer began to decrease very slightly but significantly ($P < 0.05$) only under a pressure of 250 mm Hg, by both methods of fixation. Under low magnification of the scanning electron microscope regional redistribution of the number of cells could not be detected during stretching of the luminal surface by a pressure of 100-250 mm Hg. Sample analysis of the polymorphism of the endothelial cells relative to area and diameter revealed no significant change in the redistribution of the cells by size in arteries perfused under a pressure of 100-250 mm Hg. The mean area of the endothelial cell likewise remained unchanged with a change of pressure in the perfused vessel.

During a short-term rise of hydrostatic pressure, a significant increase was found in the number of lesions under pressures of 150 and 250 mm Hg, mainly due to an increase in the number of argyrophilic cells and of stigmata. Incidentally, a rise of pressure led to a significant increase in size of the deendothelized zones only under a pressure of 250 mm Hg (Table 2).

Incorporation of ^{125}I-LDL into the aorta was increased by 1.7 times ($P < 0.05$) when the pressure was raised from 100 to 150 mm Hg, to reach a maximum (an increase of 2.2 times) under a pressure of 200-250 mm Hg (Table 3). An increase in intravascular pressure stimulated specific, receptor uptake of ^{125}I-LDL very slightly, but activated nonspecific, i.e., not controlled by an excess of unlabeled LDL, incorporation of ^{125}I-LDL into the aorta about threefold. Perhaps this stimulation of uncontrolled transport of ^{125}I-LDL was partly due to an increase in the number of dying argyrophilic cells and a defect of intercellular junctions (stigmata, craters). Nvertheless, under a pressure of 250 mm Hg the endothelium in the vessels maintained an unchanged level (about 30%) of receptor-determined uptake of labeled LDL.

TABLE 2. Morphology of Endothelial Lining of Rabbit Aorta under Increased Intravascular Pressure (M ± m)

Pressure, mm Hg	Area examined, mm²	Area of de-endothelized zone, %	Number of de-endothelized zones per 1 mm²	Number of argyrophilic cells per 1 mm²	Number of craters per 1 mm²	Number of stomata per 1 mm²	Area of zones with stigmata, %
100	77	0,1±0,02	0,34±0,08	1,1±0,3	0,5±0,2	1,0±0,2	5,6±1,5
150	77	0,27±0,15	0,69±0,19	3,7±0,9*	0,4±0,2	1,6±0,4	10,9±3,3
200	22	0,11±0,07	0,81±0,23	4,7±2,0	0,6±0,2	0,8±0,6	18,7±5,1*
250	77	0,33±0,09*	1,36±0,27*	6,2±1,1*	0,3±0,1	2,1±0,8	23,6±5,3*

TABLE 3. Incorporation of ^{125}I-LDL into Wall of Rabbit Aorta (in pg protein ^{125}I-LDL/mm²) under Increased Intravascular Pressure (M ± m)

Aorta	Perfusion fluid	Number of experiment	Pressure, mm Hg			
			100	150	200	250
Native	^{125}I-LDL + excess of LDL	9	115,3±14,0	195,4±16,1*	248,9±18,2*	247,7±10,5*
Native		8	69,4±8,9	—	—	180,3±24,7*
De-endothelized	^{125}I-LDL	7	1181,3±275,5	—	—	2365,3±418,9*

Uptake of ^{125}I-LDL in the denuded zones of the aorta was much more marked: under a pressure of 250 mm Hg accumulation was increased 20-fold compared with the normal vessel, perfused under a pressure of 100 mm Hg. These results are in agreement with the accelerated transport of other proteins and macromolecules in denudation zones observed previously [9-11]. These data suggest that the "atherogenic risk" or a raised intravascular pressure is linked primarily with the more rapid filtration of atherogenic LDL in the region of the luminal surface, where morphological defects of the endothelial lining are found. The injurious effects and accelerated transport of lipoproteins associated with a raised intravascular pressure are perhaps even more marked in atherosclerotic vessels, where the probability of macroscopic and microscopic lesions of the endothelium is increased [1, 7, 8].

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