METHODS

QUANTITATIVE ANALYSIS OF INTERACTION BETWEEN LOW-DENSITY LIPOPROTEINS AND THE PERFUSED RABBIT AORTA

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Interaction between low-density lipoproteins (LDLP) and blood vessel walls plays an important role in the pathogenesis of atherosclerosis. The specific receptor pathway for intracellular penetration of LDLP has been discovered and characterized in studies of fibroblasts and cells of the vessel wall in culture [5, 8, 14]. The technical conditions and procedures for studying the principal stages of LDLP metabolism have been worked out on cultures. However, the mechanism of interaction between LDLP and the whole vascular wall still remains incompletely studied. Only isolated publications deal with this problem [7, 11-13].

In this paper a quantitative method of evaluation of interaction between [^{125}I]-LDLP and perfused rabbit blood vessels *in situ* is suggested. The experimental conditions chosen enable the integrity of the endothelial lining of the perfused segment of the blood vessel to be maintained and interaction between circulating [^{125}I]-LDLP and the whole vessel wall to be studied within ranges of concentrations at which specific incorporation of labeled LDLP is exhibited.

EXPERIMENTAL METHOD

Experiments were carried out on 45 male Chinchilla rabbits weighing 2.5-3 kg deprived of food for 18 h. Under pentobarbital anesthesia and with artificial ventilation the descending portion of the thoracic aorta, the abdominal aorta from the celiac artery to the bifurcation, and the carotid arteries were perfused.

The perfusion solution consisted of medium 199 (from Gibco, USA) with 10% delipidized embryonic calf serum from the same firm. The density of the serum was adjusted to 1.215 g/ml with dry NaBr, delipidized by centrifugation twice at 48 h and 105,000g, freed from excess of salts by dialysis, sterilized by filtration, and kept at −20°C. LDLP (d = 1.019-1.063 g/ml) were isolated by preparative successive centrifugation from healthy blood donors' serum [10] and iodinated by the iodide-monochloride method, using ^{125}I [3]. The [^{125}I]-LDLP used in the work had a specific activity of 20-160 cpm/ng protein. The LDLP concentration was estimated as protein.

The perfusion system consisted of inner and outer recirculation systems (Fig. 1). The inner system, 4 ml in volume, was used to perfuse blood vessels at a rate of 10 ml/min and under a hydrostatic pressure of 133 kPa at 37°C. The inner system was oxygenated from the outer system in a chamber with Mark D604 semipermeable polypropylene membrane (from Radiometer, Denmark). The outer recirculation system, with a capacity of 150 ml, was filled with medium 199 and oxygenated with oxygen.

After perfusion the vessels were rinsed with medium 199 cooled to 0-4°C, excised, freed from periadventitial tissue, opened longitudinally, the area of the intimal surface was measured planimetrically, and the specimen was dried and weighed. The quantity of [^{125}I]-LDLP incorporated into the blood vessels was estimated radiometrically per square millimeter of intimal surface or per milligram weight of blood vessel.

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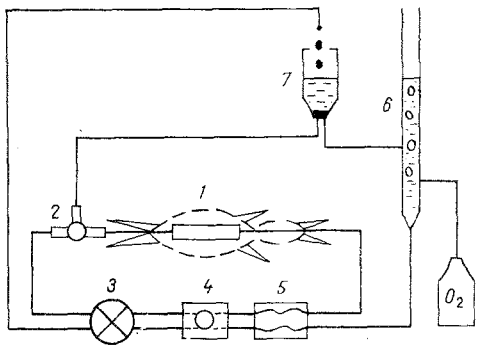

Fig. 1

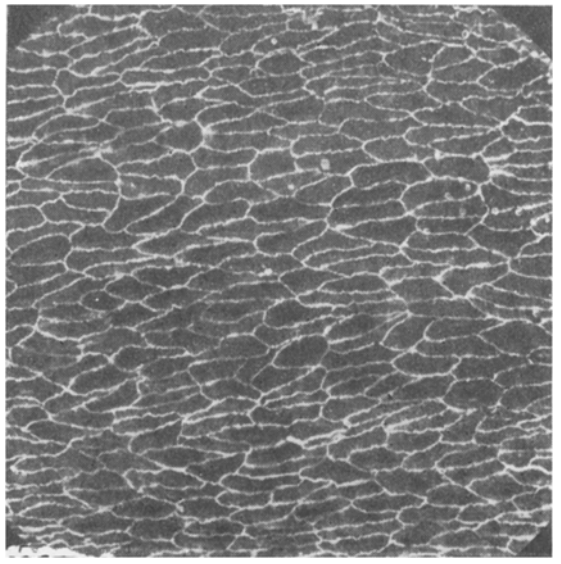

Fig. 2

Fig. 1. Scheme of perfusion system: 1) perfused rabbit blood vessel; 2) three-way cock; 3) peristaltic pump; 4) oxygenation chamber; 5) thermostat; 6) cuvette for oxygenation of external circulating solution; 7) reservoir for creating hydrostatic pressure.

Fig. 2. Luminal surface of rabbit's thoracic aorta after perfusion for 6 h *in situ*. Scanning electron microscopy, 200 ×.

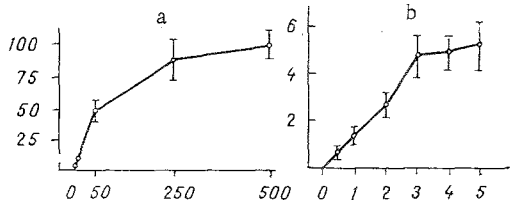

Fig. 3. Incorporation of [^{125}I]-LDLP into aorta as a function of their concentration in perfusion fluid (a) and of duration of perfusion (b). Abscissa: a) concentration of [^{125}I]-LDLP in perfusion fluid (in μg/ml), duration of perfusion 1 h; b) duration of perfusion (in h), concentration of [^{125}I]-LDLP 5 μg/ml. Ordinate, concentration of [^{125}I]-LDLP in tissue (in ng protein/mg).

The integrity of the endothelial lining of the vessels was verified under the scanning electron microscope as described previously [1, 2].

EXPERIMENTAL RESULTS

The endothelial lining of segments of the rabbits' aorta and carotid artery preserved its integrity at least during 6 h of perfusion (Fig. 2). Large zones of denudation of endothelium were not present, but the area of zones which lost single cells amounted to about 0.1% of the total area examined.

When incorporation of [^{125}I]-LDLP into the aorta was plotted against their concentration in the perfusion fluid and the duration of perfusion a curve with saturation was obtained (Fig. 3). This indicates a limited number of LDLP binding sites. Further evidence in support of this view is given by inhibition of incorporation of [^{125}I]-LDLP by excess of unlabeled LDLP. A 100-fold excess of LDLP inhibited incorporation of [^{125}I]-LDLP into the thoracic aorta on average by 37% and in the abdominal aorta by 34% ($P < 0.05$; Table 1). Re-

TABLE 1. Incorporation (in ng protein/mg tissue) of [^{125}I]-LDLP (concentration in perfusion fluid 5 μg/ml) in the Presence of an Excess (500 μg/ml) of Unlabeled LDLP (M ± m)

Experimental conditions	Thoracic aorta	Abdominal aorta	Vessel
Without excess of LDLP (n = 6)	1,09±0,19	1,47±0,60	1,31±0,37
With excess of LDLP (*n*=6)	0,69±0,07	0,98±0,05	1,09±0,19
P	<0,05	<0,05	>0,05

TABLE 2. Effect of Heparin (1 mg/ml) on Incorporation of [^{125}I]-LDLP (10 μg/ml) into Blood Vessels after Perfusion for 4 h (M ± m)

Vessel	Incorporation, pg protein/mm^2 surface		
	total	heparin-dependent	heparin-independent
Thoracic aorta (*n*=4)	613±136	226±44	386±56
Abdominal aorta (*n*=4)	358±56*	75±30*	283±87
Carotid artery (*n*=4)	314±66*	101±37*	213±56*

*P < 0.05 compared with values for thoracic aorta.

ceptor incorporation of [^{125}I]-LDLP into a confluent monolayer of aortic endothelial cells in culture was similar in character, and the internalization process reached saturation at a concentration of about 40 μg/ml and 2 h after the beginning of incubation; excess of LDLP depresses specific binding and internalization of [^{125}I]-LDLP by 20 and 32% respectively [14]. Injection of heparin into the blood vessels 4 h after the beginning of perfusion caused liberation of about 37, 21, and 32% of incorporated [^{125}I]-LDLP from the thoracic and abdominal aorta and the carotid artery respectively (Table 2). Experiments on cell cultures have shown that heparin can rupture the LDLP—receptor complex and liberate surface-bound LDLP [9]. Calculation showed that heparin liberates on average $545 \cdot 10^6$, $180 \cdot 10^6$, and $243 \cdot 10^6$ LDLP molecules from one square millimeter of surface of the thoracic and abdominal aorta and the carotid artery respectively (mol. wt. of LDLP is considered to be $3 \cdot 10^6$). According to previous observations made by scanning electron microscopy [1], the mean number of endothelial cells per square millimeter of luminal surface of the thoracic and abdominal aorta and carotid artery of the rabbit is 1340, 1450, and 1780 respectively. Consequently, for every endothelial cell in these vessels there are about $4.1 \cdot 10^5$, $1.3 \cdot 10^5$, and $1.4 \cdot 10^5$ heparin-dependent LDLP binding sites. We know [4] that there are between $2 \cdot 10^4$ and $4 \cdot 10^4$ LDLP receptors per fibroblast in culture. The number of LDLP receptors per endothelial cell in a confluent culture is $1 \cdot 10^4$ [6]. Calculations show that the number of binding sites for LDLP is greater *in situ* than *in vitro*. The level of binding of LDLP with the luminal surface of the arterial wall is evidently about an order of magnitude higher than the level of LDLP binding with a monolayer of endothelial cells in culture.

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