BIOPHYSICS AND BIOCHEMISTRY

Investigation of Mass-Transfer in Occluded Rat Artery *In Vivo*

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The release of radiolabeled immunoglobulins from distal end of blocked rat carotid artery was studied *in vivo*. The elimination half-time was 8-9 min for a 4-5-cm long segment of blocked artery and 16-24 min for a 7-8-cm long segment. Surprisingly high rate of convection in completely blocked arteries under conditions of pulsatile pressure has been demonstrated.

Key Words: *thrombus; occlusion; blood vessel*

Complete arterial occlusion (obturating thrombus or clamping during surgery) often occurs in clinical practice. An area with presumably lowered mass-exchange is formed between the site of occlusion and the nearest proximal bifurcation [1]. As we are aware, there is no evidence on the intensity of convection in this zone of "stagnation". However, there are indirect indications of intense mass-exchange in this zone. Specifically, without convection blood should coagulate due to the presence of a thrombogenic surface (thrombus or damaged vascular wall), which has not been observed in clinical practice. Angiographic investigations of arterial thrombi showed that an X-ray contrasting agent is washed from the blocked segment within several seconds [10]. However, it is unclear whether this is due to microarteries that cannot be visualized by angiography or/and by intense convection in a completely occluded blood vessel.

In order to estimate the intensity of convection in the area between the thrombus surface and the nearest proximal orifice we measured the release rate of radiolabeled immunoglobulins G (IgG) from occluded artery into the bloodstream. IgG were chosen because they do not cross vascular wall and circulate in the blood for a long time period [7].

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MATERIALS AND METHODS

High-purity rabbit IgG were labeled with ^{125}I using Iodogen (Pierce) according to the manufacturer's recommendations. At least 97% of radioactivity was incorporated into IgG. The specific activity of the IgG preparation was 800 000 cpm/mg. The preparation was stored as aliquots at -18°C.

Experiments were performed on rabbits (body weight 3.5-4.5 kg) under sodium thiopental anesthesia (20 mg/kg). A segment of the right carotid artery (8-9- or 5-6-cm long) was isolated from surrounding tissues. Small arterial branches were cut with UHF-coagulator. The artery was clamped at a distance of 4-5 cm (group 1) or 7-8 cm (group 2) from the nearest proximal bifurcation and a ligature was applied 0.5 cm downward the clamp. Radiolabeled IgG (15 μl) was injected in the segment between the clamp and ligature, the ligature was tightened, and the clamp was removed (Fig. 1).

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The moment when the clamp was removed was regarded as the start of experiment. Blood samples (0.5 μl) were collected from the right femoral artery during the first 12 min at 2-min intervals and during 2 h at 10-20-min intervals. Blood radioactivity was measured in a Minigamma 125 counfer (LKB). Blood pressure in the right femoral artery was monitored with an HP1280C sensor and a Hewlett-Packard HP7758D pressure monitor. The pressure remained constant throughout the entire observation period.

The rate of spontaneous IgG clearance from circulation was determined in 3 rabbits by injecting 5 mg of radiolabeled IgG into the marginal ear vein and measuring the radioactivity of blood (0.5 ml) collected from the right femoral artery during 3 h at 30-min intervals. Blood radioactivity was measured in a Minigamma 125 counter (LKB).

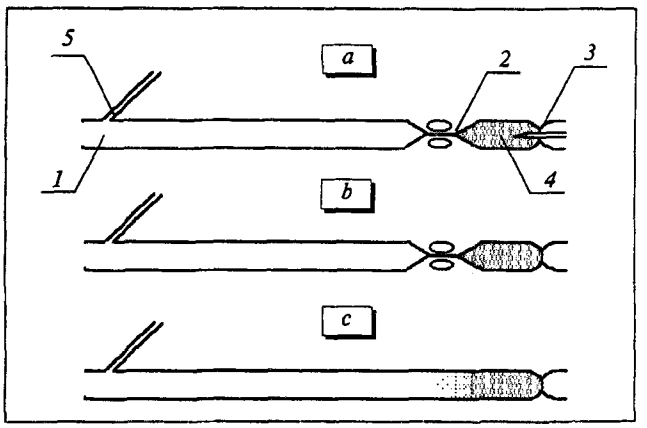

Fig. 1. Experimental scheme. *1)* carotid artery; *2)* first ligature; *3)* second ligature; *4)* labeled IgG; *5)* proximal arterial bifurcation. *a)* injection of labeled IgG; *b)* withdrawal of a microsyringe needle; *c)* start of experiment.

RESULTS

Kinetic characteristics of release and accumulation of radiolabeled IgG in systemic circulation have been obtained. The values were approximated by exponential curves with characteristic time constants for the label accumulation. Typical curves illustrating the increase in the concentration of radiolabeled IgG in systemic circulation and half-elimination times are shown in Table 1 and in Fig. 2. For the 4-5-cm long blocked segment this time was 8-9 min and for the 7-8-cm long segment it was 16-24 min.

Figure 3 illustrates spontaneous clearance of ^{125}I-IgG from the circulation. After the first 30 min, 70% of radiolabeled IgG remains stable for at least 90 min. Thus, the contribution of spontaneous clearance to the accumulation of ^{125}I-IgG in the bloodstream during the experiment can be neglected.

The method employed in the present study enabled us to determine the rate of the clearance of radiolabeled protein from blocked vessel into systemic circulation *in vivo*. The results obtained indicate that the time required for the release of macromolecules from a blocked blood vessel into systemic circulation depends on the length of blocked segment: half-elimination time was 8-9 min for 4-5-cm long segment and 16-24 min for 7-8-cm long segment. Practically all labeled IgG was washed within the first 2 min from a 2-cm long blocked segment of rabbit femoral artery (data not shown). Since our approach (isolation of the artery from surrounding tissues) precludes washout of the label via *vasa vasorum*, we have suggested that elimination half-time characterizes the intensity of convection in a blocked vessel. The convention determines not only the washout rate, but also the rate of delivery of a compound from the circulation to the site of blocking. Convection in a blocked vessel may be caused by dilatation wave emerging in elastic blood vessel under conditions of pulsatile pressure. Blood viscosity, vascular wall elasticity, and blood pressure gradient are probably the major parameters determining the intensify of such a convection.

The results can be compared to the data on application of trombolytic agents in clinical practice.

TABLE 1. Parameters of Experimental Animals

Animal No	Weight, kg	Elimination half-time, min	Length of arterial segment, cm	Blood pressure, mm Hg
1	4.9	7.71±0.8	4.5	150/100
2	3.8	10.7±0.8	4.5	200/120
3	3.8	9.1±1.3	4.5	200/100
4	4.1	7.1±1.6	5	180/120
5	3.9	14.2±1.1	8	160/100
6	3.7	30.3±1.9	7.5	170/100
7	3.8	17.8±1.5	7.5	180/100
8	3.6	21.4±1.9	6.5	150/100

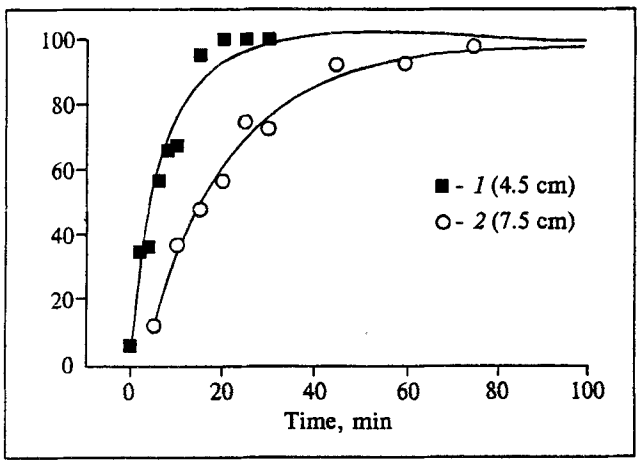

Fig. 2. Typical curves showing an increase in the concentration of radiolabeled IgG in systemic circulation. Experiment No. 3 (*1*) and No. 7 (2), half-elimination time 9.1±1.3 and 17.8±1.5 min, respectively. Here and in Fig. 3: ordinate: % of the initial IgG concentration.

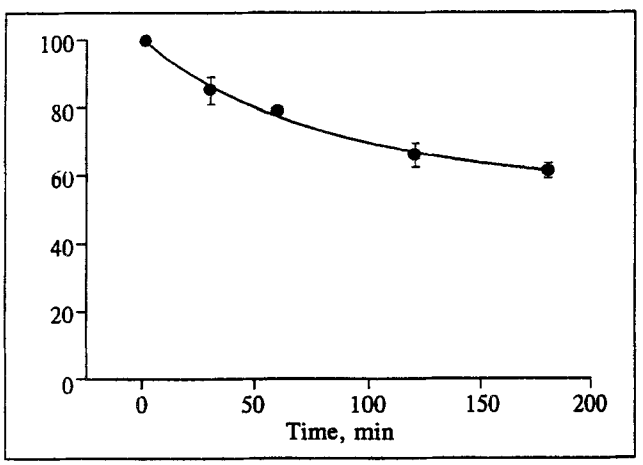

Fig. 3. Spontaneous clearance of radiolabeled IgG (n=3).

In the absence of convection, half-dose of thrombolytic preparation will pass a distance of 1 cm for 50 hours [6]. This time is sufficient for inactivation of the agent by the inhibitor of tissue-type plasminogen activator (PAI 1) [8] (at physiological concentrations of PAI 1 half-clearance for the enzyme is 100 sec) [8]. Thrombolytic agent is eliminated from the blood predominantly via the liver; for instance, half-clearance time for urokinase plasminogen activator and plasminogen tissue activator is 5-10 min [3,5]. Nevertheless, thrombolytic preparations have been successfully used for lysis of obstructive thrombi [4].

Moreover, effective dose of urokinase for thrombolysis in coronary and aortocoronary bypass is $2\text{-}3\times10^6$ IU upon intravenous administration [2], $1.2\text{-}2.7\times10^6$ IU using transthrombus technique [9], and 1.2-5.5 mln IU upon intra-arterial administration [11], indicating a similar time during which a thrombolytic agent reaches the thrombus irrespective of the distance between the site of administration and thrombus. Thus, clinical observations indicate an intense mass-exchange in blocked segment of artery.

We would like to draw the attention of researchers to a surprisingly high rate of convection in artery *in vivo* under conditions of pulsatile pressure as well as to the physiological significance of this phenomenon and its physical causes.

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