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CONCENTRATION OF ERYTHROCYTE-BASED MAGNETIC CARRIERS IN THE BLOODSTREAM

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UDC 615.2/3.014.6:612.111.014.426/.033

KEY WORDS: targeted drug transport, magnetic erythrocytes, angio-prostheses, abdominal aorta.

Oriented transport of drugs to a target organ is a promising modern therapeutic and prophylactic technique which enables the action of a drug to be localized, the administered dose to be reduced, and, consequently, its toxic effect to be minimized. Drugs are best transported in biocompatible containers. Erythrocytes [3, 6, 8, 9] are among the most suitable of containers. Erythrocytes "loaded" with a drug can circulated in the bloodstream for up to 10-20 days [1, 4-6, 8]. If, besides a drug, a magnetic material is also introduced into the erythrocyte, such a magentic erythrocyte (ME) can be concentrated locally in a magnetic field [2, 9].

The writers postulated that ME, injected into the bloodstream, may be concentrated in an assigned region of the vascular bed with the aid of the field of a permanent magnet. To test this hypothesis, erythrocytes "loaded" with colloidal magnetite were used, and concentrated in experiments *in vitro* and *in vivo*.

EXPERIMENTAL METHOD

Human erythrocytes were used. ME were obtained by hypotonic lysis in the presence of magnetic material, after which the integrity of the membranes was restored [2]. The magnetic material introduced into the erythrocytes consisted of colloidal magnetite (particle size 10-40 nm), stabilized with dextran T-40 (from Pharmacia, Sweden) [7], which was obtained by precipitation from ferrous and ferric chloride in the presence of dextran [7]. ME were fixed with 1% glutaraldehyde (from Merck, West Germany) for 1 h at 20°C, then washed with phosphate saline buffer (PSB), containing 138 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, and 1.5 mM KH_2PO_4 (pH 7.4), by centrifugation at 1000 g for 5 min. The fixed ME were suspended up to a 10% suspension in PSB containing 5 mg/ml of bovine serum albumin (from Sigma, USA) and incubated for 3 h at 20°C, after which they were washed with PSB. A segment of prosthesis to be used for angio-plastic of the femoral artery (Ftorlon† – Lavsan‡, diameter 8 mm, length 40 mm, from the "Sever" Leningrad Production Combine, USSR) was carried out be means of an SP-3 pump (East Germany). The prosthesis was perfused with 30 ml of a 1% suspension of ME (rate of perfusion 500 ml/min). A magnetic field was created by a permanent magnet made from $SmCO_5$, measuring 15 × 38 × 8 mm, with an energy product of over 160 kj/m^3 (the magnet was provided by the All-Union Research Institute of Electromechanics). During perfusion the magnet was fixed on part of the prosthesis. The degree of concentration of ME in the prosthesis was determined from the reduction in the number of cells circulating in the perfusion system. The number of cells was determined by means of a cell counter (Coultronics, France). For the experiments *in vivo*

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 0007-4888/85/0012-1684 $09.50

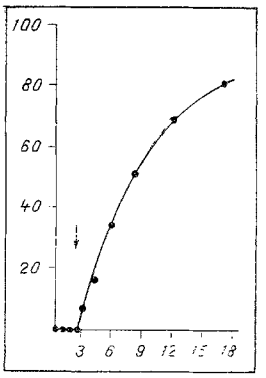

Fig. 1. Kinetics of ME accumulation on prostesis in region of magnet. Abscissa, duration of perfusion (in h): ordinate, number of cells held up on segment of prosthesis (in % of original number). Arrow indicates time of application of magnet to prosthesis. Typical dependence (six experiments).

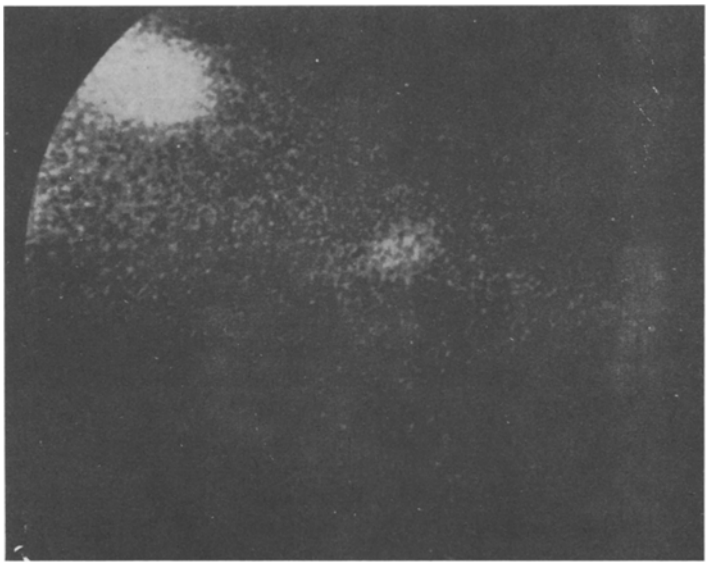

Fig. 2. Distribution of labeled ME in region of abdominal aorta 25 min after injection.

ME were labeled with sodium pertechnetate ($Na^{99m}TcO_4$ was estimated in ME with a "Rackgamma 1270" counter, LKB, Sweden), by determining radioactivity in the ME suspension and in the supernatant. For the experiment *in vivo*, a midline laparotomy was performed on a dog weighing 14 kg under intravenous hexobarbital anesthesia. The infrarenal portion of the abdominal aorta was then isolated for a distance of 12 cm from the bifurcation of the aorta. The permanent magnet used in the experiments *in vitro* was fixed to this part. Labeled ME were injected through a catheter into a vein of the left forelimb. The distribution of ME was recorded by means of a gamma-camera (Picker Nuclear Dynacamera, USA).

EXPERIMENTAL RESULTS

Erythrocytes loaded with colloidal magnetite possess ferromagnetic properties and can move in the magnetic field of a permanent magnet at a rate of 10-30 μ/sec. To discover

whether ME could be concentred in fluid flowing at near-phyciological velocities, they were perfused through a segment of the vascular prosthesis to which the magnet was fixed.

The kinetics of ME accumulation on the prosthesis in the region of the magnet is illustrated in Fig. 1. With a linear perfusion velocity of 16 cm/sec the ME could be concentrated in the magnetic field. During 15 h of perfusion up to 80% of the eythrocytes were held up in the region of the prosthesis.

To study the possibility of concentration of ME in an actual blood flow, an experiment was carried out *in vivo* on a dog with ME labeled with ^{99}Tc. Incorporation of radioactive label into ME amounted to 25-50% of the original quantity introduced. Labeled ME were injected into the venous blood flow and their distribution was recorded in the segment of the abdominal aorta to which the magnet was fixed.

The distribution of labeled ME in the region of the abdominal aorta 25 min after injection is shown in Fig. 2. The bifurcation of the abdominal aorta, and the femoral and caudal arteries can be seen in Fig. 2 because the concentration of labeled ME in the main vessels is higher than in the surrounding tissues. Accumulation of ME can be seen in the segment of aorta to which the magnet was fixed.

Thus ME, injected intravenously, can be concentrated by a magnetic field in a trunk vessel with high blood flow velocity.

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