NANOTECHNOLOGIES

Passive and Active Target Delivery of Drugs to Ischemic Myocardium

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Silica nanoparticles as carriers for targeted drug delivery to the heart were studied. Studies of hemodynamic parameters of rats after intravenous infusion of silica nanoparticles showed no acute toxicity. Intravenous infusion of silica nanoparticles to animals with ischemia–reperfusion of the myocardium led to accumulation of the nanoparticles in the focus of injury, which attests to possibility of passive targeted drug delivery to the myocardium.

Key Words: *target delivery; drugs; nanoparticles; myocardium*

The development of methods for targeted drug delivery to damaged tissue by means of nanosized carriers is one of the most promising trends of modern pharmacology [1,5].

This approach has many advantages: regulated treatment of focal pathological processes (tumor growth, inflammation, and ischemia), reduction of drug toxicity and other side effects, increase of drug solubility and stability, improvement of biocompatibility, regulated release of the drug.

We assume that local accumulation of nanoparticles charged with the drug in reversibly damaged compartments of the heart can be attained by selective binding of annexin V on the nanoparticle surface (Fig. 1).

Annexin V is an endogenously produced protein binding to phosphatidylserine in the presence of calcium ions. Phosphatidylserine is usually located on the inner surface of the plasmalemma, but in sublethal injury to the cell it is translocated to the outer surface of the cell [6]. Specific interactions between annexin V fixed to the nanoparticle and phosphatidylserine theoretically should lead to nanoparticle fixation to the cardiomyocyte membrane and subsequent accumulation of these nanoparticles in the ischemic focus of the heart. This is how active delivery is realized.

The other mode of target delivery is its passive variant. In this case the drug is selectively accumulated due to sharply increased capillary permeability, which is paralleled by easy release of nanoparticles charged with the drug outside the vascular bed.

We studied the possibility of targeted drug delivery to the ischemic myocardium.

MATERIALS AND METHODS

Nanodispersed silica particles (NSP) with specific surface evaluated by the BET method by low-temperature adsorption of nitrogen, from 170 to 380 m^2/g and mean size of particles from 6 to 13 nm [13] were proposed as the initial transporters for anti-ischemic drugs. It was previously shown that NPS are characterized by

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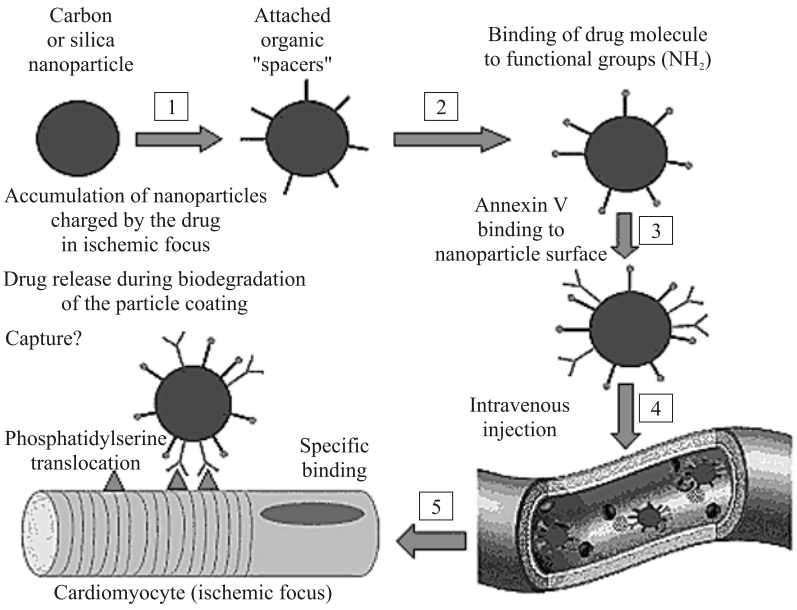

Fig. 1. Algorithm of targeted delivery to the myocardium.

good biocompatibility and biodegradation [7]. A method was developed for NSP surface modification by amino groups. The method included 3-amino-propyl tri-ethoxysilane chemosorption from gaseous phase in a flow reactor. The spacer was synthesized using 3-(re-amino)octanoic acid.

The biocompatibility of NSP was experimentally evaluated by the acute hemodynamic effects of the nanoparticle suspension in Wistar rats (200-250 g) narcotized with chloral hydrate (420 mg/kg). NSP ($nSiO_2$) and NSP bound to sodium fluorescein ($nSiO_2$+SFl) suspensions in saline were used. The final concentration of NSP in suspension was 2 mg/ml. Binding of SFl to nanoparticles surface was carried out by the chemical assembly method [2]. The concentration of SFl in $nSiO_2$+SFl suspension was about 0.013 mg/ml. The biocompatibility of the carrier was studied as described previously [4]. Principal possibility of passive targeted delivery of NSP to damaged myocardium was studied in special experimental series. Sham-operated animals and animals with 30-min myocardial ischemia followed by reperfusion were intravenously infused with NSP, after which silica content was measured in heart and liver specimens by atomic absorption spectroscopy.

RESULTS

In order to evaluate biocompatibility, hemodynamic parameters were recorded: systolic BP, diastolic BP, mean BP, pulse BP (PBP), and heart rate (HR). The mean BP before the preparation injection was 120±35 mm Hg, PBP 31.7±5.4 mm Hg, and HR 402±20 bpm. After the first and subsequent injections of the prepara-

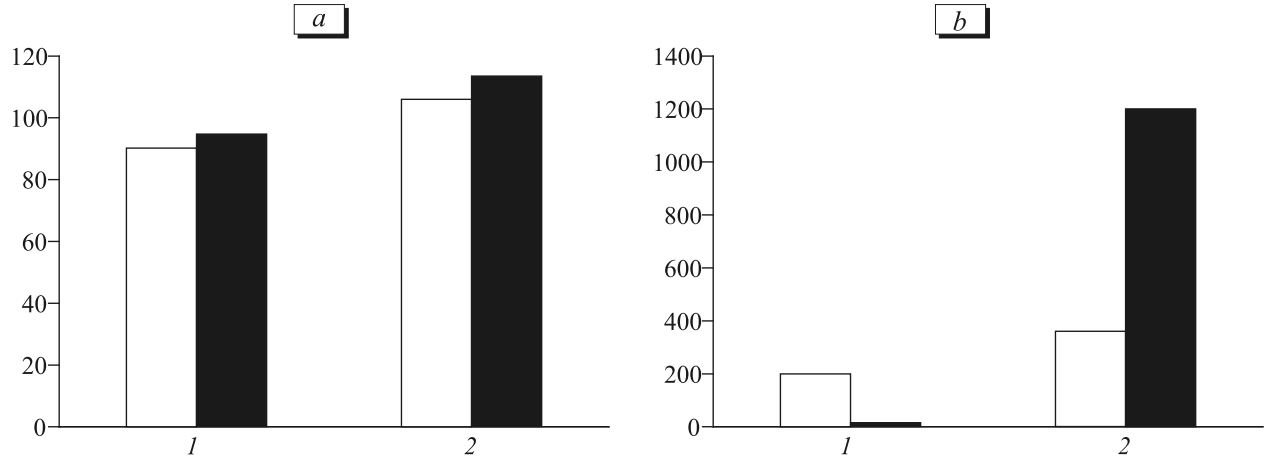

Fig. 2. Histogram of fluorescence time course (arb. units) in the control (*1*) and after injection (*2*) of NSP with immobilized fluorescein (*a*) and cardiogreen (*b*). Light bars: heart; dark bars: liver.

tion, the mean BP and HR, as well as the rate of respiratory movements virtually did not change. However, PBP gradually increased in the course experiment and reached 46±7 mm Hg by the end of observation, which significantly surpassed the initial level ($p<0.05$). The significance of correlation between pulsed pressure and period of experiment was confirmed by high values of r coefficient of correlations, its mean value being 0.82. Hence, intravenous infusion of NSP specimens caused negligible changes in the hemodynamic parameters, indicating sufficient biocompatibility of this nanomaterial.

We developed a method and studied the distribution of NSP bound to SFl and indocyanine green, layered onto NSP by molecular laying through ionic and covalent bonds. Differences in fluorescence levels for the liver and heart were demonstrated for SFl and indocyanine green (Fig. 2). It should be noted that UV exposure of organ specimens for detection of NSP labeled by SFl involved a significant contribution of autofluorescence, which could impede analysis of the target signal. Despite this, we observed predominant accumulation of SFl-labeled NSP in the lung, spleen, and liver, *i.e.* in organs of the reticuloendothelial system. The use of indocyanine green allowed us to rule out the effect of high autofluorescence, because this fluorophore was stimulated in the near infrared band. The highest levels of NSP labeled by indocyanine green were found in the liver and kidney. The results recommend the infrared fluorophores for fluorescent label of nanoparticles and studies of their biodistribution.

Intravenous injection of NSP with a diameter of 10 nm to animals with myocardial ischemia–reperfusion led to selective accumulation of these NSP in the focus of injury (Fig. 3). This fact suggested that fixation of cardioprotective drugs (*e.g.*, bradykinin, erythropoietin, ATP-sensitive K-channel openers, vascular endothelium growth factor, *etc.*) to nanoparticle surface could lead to selective increase of drug concentrations in damaged myocardium. This hypothesis was in good agreement with previous data [8] obtained with adenosine-charged liposomes 134 nm in diameter on the myocardial ischemia/reperfusion model in rats. We showed that the effect of passive target delivery to ischemic myocardium was realized with smaller nanoparticles with a basically different structure.

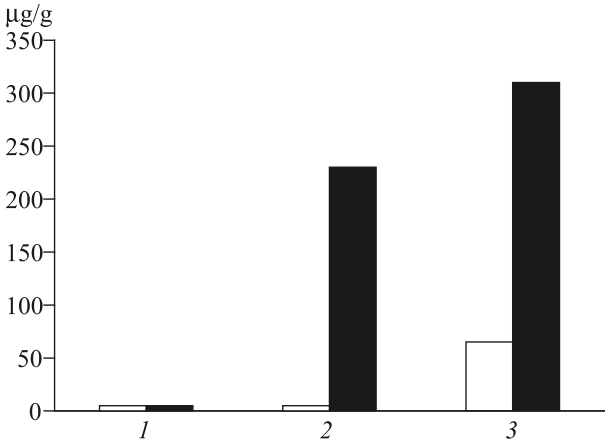

Fig. 3. Silica content in the myocardium (light bars) and liver (dark bars) (atomic absorption spectroscopy). *1*) basal level (group 1); *2*) NSP (group 2); *3*) ischemia–reperfusion+NSP (group 3).

Hence, the concepts of passive and active delivery can be adapted to development of methods for drug transport to ischemic myocardium. Nanodispersed silica is a promising carrier for target delivery of drugs due to its low price, easy functionalization, biocompatibility and biodegradation; the NSP biodistribution can be effectively evaluated by infrared fluorescent label.

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