Distribution and Resorption of Polymeric Microparticles in Visceral Organs of Laboratory Animals after Intravenous Injection

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Microparticles obtained by using ^{14}C-labeled resorbable hydroxyaminobutyric acid polymer were injected into the caudal vein of laboratory animals without negative aftereffects for their growth and development and without changes in the macro- and microstructure of organs and tissues. The distribution of microparticles in the viscera and the dynamics of accumulation of carbon-containing polymer degradation products in the viscera were studied. The main targets for the particles are liver tissues, as well as renal and splenic tissues. The polymeric matrix of the microparticles is most actively destroyed in the spleen and liver. The presence of high-molecular-weight polymeric matrix in organs indicates the integrity of microparticles and the possibility of long-term (up to 12 weeks) functioning of polymeric particles *in vivo*.

Key Words: *resorbable polyhydroxybutyrate; microparticles; intravenous injection; ^{14}C; visceral tissues*

The development of systems for controlled drug delivery is a prospective rapidly developing trend in modern pharmacology [11]. Drug systems in the form of biodegraded microspheres and microcapsules can be injected subcutaneously, intramuscularly, and intravenously [5,12]. The substances for the development of long-acting dosage forms should be not only biocompatible, but also blood-compatible (should cause no thromboses, thromboembolism, or antigenic response, no destruction of blood cells and plasma proteins, and should not modify their mechanical physical characteristics) [11].

Bioresorbable polyesters of microbiological origin, polyhydroxyalcanoates (PHA), are regarded as prospective material for the use in various spheres, including pharmacology [1]. The prospects of PHA use in medicine and creation of long-acting drug systems were discussed [7,8,10]. Studies of PHA for drug deposition, recently unfolded at Institute of Biophysics, resulted in the development of experimental forms, films, tablets, and microparticles [4,6]. Tissue reactions to intramuscular injection of PHA particles were studied [12]. The possibility of using matrices for deposition of antitumor drugs was demonstrated for rubomicin hydrochloride [5].

Important aspects in the development of drug systems in the form of bio- and blood-compatible microparticles are the need in the choice of the optimal administration route for the dosage form, studies of the migration routes and location of the particles *in vivo*, and of regularities bioresorption of the matrix material. This determined the goal of our study – distribution of polymeric microparticles in the viscera of laboratory animals after intravenous injection and location of the polymeric matrix biodegradation products.

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MATERIALS AND METHODS

The particles were obtained using ^{14}C-labeled polyhydroxybutyrate (β-hydroxyaminobutyric acid polymer) synthesized at Institute of Biophysics (Bioplastotan trademark) [3]. Radioactive (^{14}C) microparticles obtained by evaporation of the solvent from a 3-component polymeric emulsion as described previously [4] were used in the experiment. The animals (adult female Wistar rats, 200-240 g) from Breeding Center of Institute of Cytology and Genetics of the Russian Academy of Sciences were kept in a vivarium on standard rations in accordance with the instruction regulating handling and experimental studies on animals [2]. Sterile suspension of microspheres (5 mg in 0.5 ml saline; microspheres ≤4 μ in diameter) were injected into the caudal vein. Intact animals served as controls. The animals were sacrificed by inhalation narcosis overdosage 3 and 24 h after injection and then daily (5 per term). The viscera were isolated, examined, weighed, and after drying and fragmentation their radioactivity was measured. The samples (100 mg) were placed in plastic vials (PerkinElmer/Paccard) with 15 ml dioxane scintillation fluid (1 liter of dioxane contained 10 g 2,5-diphenyloxasole, 0.25 g 1,3-di-2,5-phenyloxasole benzene, and 100 g naphthalene). The radioactivity of the samples was measured on a TRI-CARB 2100TR scintillation counter (Packard BioScience Company). The content of radioactive carbon was analyzed in the heart, lungs, liver, kidneys, bone marrow, and blood without consideration for the radioactivity of soft and hard tissues and metabolites released. The resorption of polymeric matrix and accumulation of ^{14}C PHA degradation products were studied using methanolysis of dry tissue samples. Fatty acid methyl esters were measured on a GCD plus chromatomass-spectrometer (Hewlett Packard). High-molecular (intact) polymer was detected in organs by chloroform extraction from tissue samples and hexane precipitation. The resultant polymer was methylated and chromatographed as described previously (sensitivity: 10^{-11} g). The general reaction of tissues to microparticles was studied by routine histological methods. The material was fixed in 10% formalin and embedded in paraffin; 5-10-μ sections were sliced from the blocks and stained with hematoxylin and eosin.

RESULTS

All animals were healthy after intravenous injection of the microparticles and later throughout the experiment (they actively ate the fodder). No differences were detected between animal body weights and weights of the viscera in the control and experimental groups. Macroscopic examinations of the viscera and histological studies of sections revealed no negative shifts at all terms.

The dynamics of specific radioactivity of the viscera throughout the experiment is presented in Figure 1. Three hours after injection of microparticles into circulation, the maximum specific radioactivity of ^{14}C (13,680±117 cpm×g) was recorded for heart tissues. Radioactivities of renal tissues (6520±81 cpm×g) and lung tissue (4280±65 cpm×g) ranked next. Radioactivity of hepatic and splenic tissues was similar (3400±58 cpm×g). The lowest radioactivity was recorded in the blood (1820±43 cpm×g) and bone marrow (580±75 cpm×g). The picture drastically changed after 24 h: the radioactivity of heart tissues decreased 2-fold, while that of hepatic tissues increased virtually 5.5 times. Radioactivity of splenic tissues increased 1.8 times, which was virtually the same as in the heart at this term. After 1 week, the content of the label increased in hepatic tissue and dropped in the heart and lungs. After 1 month, specific radioactivity of the heart tissues decreased 4-fold in comparison with its initial level and was 3240±57 cpm×g. Radioactivity of hepatic tissues also decreased during this period (15,120±90

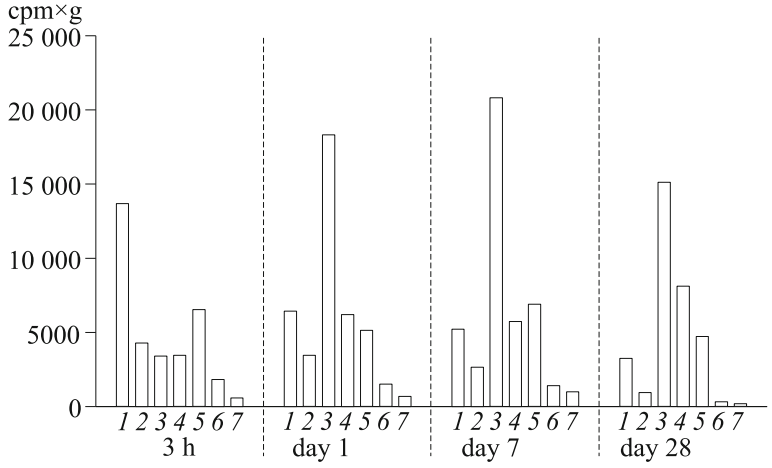

Fig. 1. Dynamics of specific radioactivity of visceral tissues of animals intravenously injected with ^{14}C-labeled polymeric microparticles. Here and in Figs. 2: *1*) heart; *2*) lungs; *3*) liver; *4*) spleen; *5*) kidneys; *6*) blood; *7*) bone marrow.

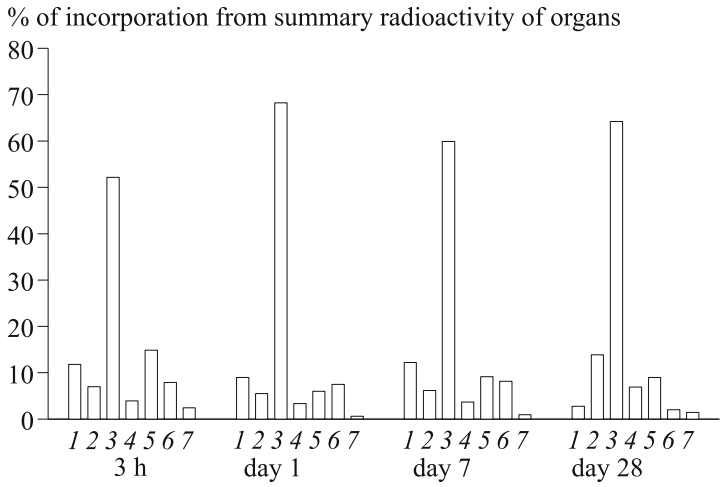

Fig. 2. Dynamics of label accumulation in animal organs after intravenous injection of ^{14}C-labeled microparticles.

cpm×g). This was paralleled by an increase in label level in splenic tissues, presumably because of accumulation of the polymer resorption products in this organ, because in addition to hydrolytic enzymes, the macrophages absorbing cell elements actively function in it. As we know, macrophages are active destroyers of polyhydroxybutyrate [12].

Thus, visceral tissues differently absorbed microparticles injected into circulation. This was even more clearly demonstrated by *in vivo* analysis of the label accumulation with consideration for organ weight (Fig. 2). Incorporation of ^{14}C in animal viscera was paralleled by its marked accumulation in the liver. Three hours after injection of microparticles, the level of ^{14}C-labeled polymer in the organs was 80%, the highest level of radioactivity (52.14%) was recorded in the liver. The remaining 47.86% radioactivity, recorded for all the rest organs, were distributed as follows: 11.8 and 6.97% in the heart and lungs, respectively; 3.94 and 14.89% in the spleen and kidneys. The dynamics of label accumulation throughout *in vivo* circulation of microparticles was opposite, despite the differences in specific radioactivities of tissues (Fig. 1). One day after injection of microspheres, radioactivity of the liver increased 1.5 times, while that of the rest organs decreased. The most pronounced reduction was recorded for the kidneys (2.5 times).

Starting from day 28 and later summary radioactivity dropped (Fig. 3). This means that the polymer was destroyed and the ^{14}C products of polymer degradation were eliminated from the body. Three hours after injection of microparticles, summary radioactivity of organs was 38,720±2575 cpm (80% of the injected dose radioactivity). Later, as the microparticles circulatied in the organism with the blood flow, the summary label incorporation into organs somewhat increased. One day and 1 week after injection, this parameter increased to 43,700±2778 cpm, which was close to the injected dose. After 4 weeks, radioactivity

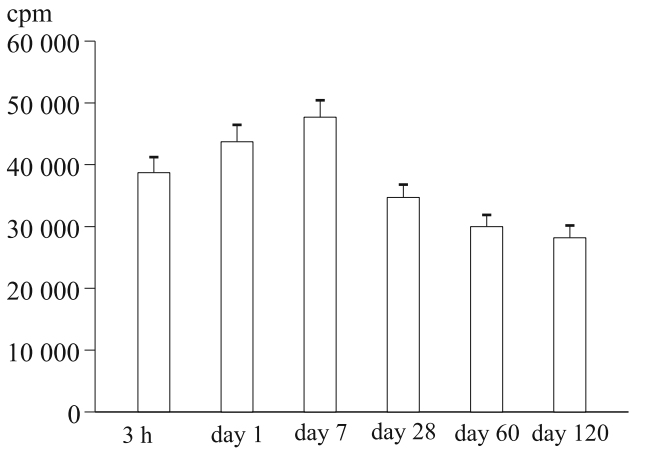

Fig. 3. Dynamics of summary accumulation of ^{14}C in animal organs.

of organs significantly decreased, which we attributed to starting biodestruction of the polymeric matrix and partial elimination of the label from the body with carbon-containing products. Summary radioactivity of organs 120 days after injection of particles decreased to 28,210±2054 cpm.

It is noteworthy that the recorded ^{14}C levels in the organs do not fully reflect the picture of the microparticle accumulation in organs, because PHA, as biodegradable polyesters, are subjected to bioresorption *in vivo* under the effect of enzymes and blood and tissue cells, while the resultant products of polymer degradation are excreted with metabolites [9]. The label levels recorded in the tissues reflect the integral values of summary radioactive carbon of the high-molecular-weight polymeric matrix proper (intact microparticles and ^{14}C products of polymer destruction).

Polymer biodegradation in various organs and the lifespan of microparticles were evaluated by chromatographic analysis of organ tissues. The content of the analyzed polymeric substance changed in all organs during the experiment. After 8 weeks this parameter

TABLE 1. Results of Chromatographic Analysis of Hydroxybutyrate Monomer Methyl Esters in the Rat Organ Tissues

Organ	Hydroxybutyrate content, 10^{-4} mg/organ		
	week 1	week 8	week 12
Heart	1527	132	75
Lungs	1694	111	56
Liver	5861	4889	197
Spleen	606	251	14
Kidneys	189	124	43

TABLE 2. Residual Content of High-Molecular-Weight Polymer in Rat Organ Tissues

Organ	Hydroxybutyrate content, 10^{-4} mg/organ		
	week 1	week 8	week 12
Heart	420	30	5
Lungs	600	5	6
Liver	360	110	80
Spleen	6	10	5
Kidneys	16	20	7

decreased virtually by one order of magnitude for the heart and lungs in comparison with week 1. We think that this was caused by washing out of the microparticles introduced by the blood flow into the smaller circulation circle during the initial period of experiment, but not by destruction of the polymeric matrix in these organs. The content of polymeric substance 8 weeks after injection of the microparticles decreased 1.5 times in the kidneys and 1.2 times in the liver. A more marked reduction of the parameter was observed in the spleen (2.5 times). After the next 4 weeks, at the end of the experiment, the values decreased 2-fold in the heart and lungs, 3-fold in the kidneys, 18- and 25-fold in the spleen and liver, respectively, in comparison with the previous term (8 weeks) (Table 1).

These data indicate different intensity of PHA degradation in animal organ tissues and evidence the presence of high-molecular-weight polymer in the organs by the end of the experiment, which indicates that some microparticles remained intact. However, as the current spectrometric methods for detection of high-molecular-weight PHA are based on the registration of fatty acid methyl esters (polymer-forming monomers), obtained by pre-hydrolysis of the polymer to monomers and their subsequent methylation, these data may sum up the hydroxyaminobutyric acid monomers, formed from high-molecular polymer by methanolysis, and the products (monomers) of its natural biological degradation under the effect of enzymes and organ cells.

In order to detect high-molecular-weight (intact) polymer in organ tissues, its extraction from the samples and detection were carried out. The concentrations of high-molecular-weight intact polymer in the organs were thus determined for different periods of observation (Table 2). The maximum content of the polymer 1 week after injection of the microparticles was recorded in the lungs, heart, and liver compared to its concentrations in the spleen and kidneys. However, the decrease in this parameter 8 weeks postinjection in the lungs and heart corresponded to the data presented in Table 1 and more probably indicated washing out of the particles than their degradation in these organs. This conclusion is confirmed by measurements of tissue radioactivity.

Quite different data by the term of 12 weeks after injection of microparticles are worthy of note. Chromatography of tissues showed significantly higher levels of the polymeric substance (Table 1) in comparison with the volume of high-molecular-weight polymer isolated from the organs (Table 2). This fact suggests that by the end of the experiment the greater percentage of radioactive polymer was present *in vivo* in the form of hydroxyaminobutyric acid monomers and low-molecular-weight products of its degradation.

Analysis of the data also indicated that fragments of polymeric microparticles were present in animal organs at various terms of observation (Table 2). The content of the polymer in organs was not very high, particularly in the liver and spleen; together with high radioactivity of tissues, this indicated high intensity of the polymeric matrix destruction in these organs.

In general, the studies demonstrated the possibility of using the developed polymeric microparticles from polyhydroxybutyrate for intravenous injection. Visceral tissues were characterized by different cumulative activity towards adsorption of polymeric microparticles, and the intensity of the polymeric matrix degradation in them was different. The presence of high-molecular-weight polymeric matrix in organs indicated intactness of microparticles and suggested that polyhydroxybutyrate can be used for long-term (up to 12 weeks) delivery of drugs to visceral organs by intravenous injection.

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