Tissue Reaction to Intramuscular Injection of Resorbable Polymer Microparticles

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Tissue reaction to implantation of polymeric microparticles from resorbable polymer (polyhydroxybutyrate) is characterized by slight inflammatory reaction and pronounced progressive macrophage infiltration with the presence of mono- and multinuclear foreign body giant cells resorbing the polymeric matrix. No fibrous capsules were formed around the polymeric microparticles; neither necrosis nor other adverse morphological changes and tissue transformation in response to implantation of the PHB microparticles were recorded. The results indicate good prospects of using polyhydroxybutyrate for the construction of long-acting dosage forms as microparticles for intramuscular injection.

Key Words: *microencapsulation; resorbable polymers; polyhydroxybutyrate; tissue reaction*

The development of systems of regulated drug delivery is a prospective and rapidly developing trend in pharmacology. The main advantage of these systems is the possibility of maintaining of the needed drug level in the blood and/or tissues for a long time [2,6]. The most promising approach is construction of systems consisting of biodegradable microspheres (MS) and microcapsules, which can be used for deposition of a wide spectrum of drugs and injected into the circulation, subcutaneously, or intramuscularly, and adapted for oral use or inhalations [4]. The key aspect for the creation of long-acting dosage systems with regulated drug release is the choice of adequate material absolutely harmless for the body and characterized by a complex of certain physico-mechanical and biomedical properties, including degradation in biological media. Among bioresorbable materials used and developed at present time are polymers of monocarbonic acid derivatives (polylactides and polyglycolactides) and, from recent time, linear polyesters of microbiological origin: polyhydroxyalkanoates (PHA) [2,11]. Biocompatibility of β-hydroxyaminobutyric acid polyhydroxybutyrate (PHB) polymer, the most often used and best studied PHA type, was confirmed *in vitro* in cell cultures of different origin [7,8,11] and experimentally *in vivo* [5,9], but these studies were carried out with the use of large grafts in the form of suture material, films, or pins [12]. On the other hand, it is known that biocompatibility of biomaterials is determined not only by chemical structure, but largely by the graft size and shape [10].

Microspheres are considered to be preferable for the construction of long-acting drugs for injections. However, subcutaneous or intramuscular injections of MS can induce more intensive tissue response, because of large total surface area and small volume of MS [1,10]. The biocompatibility of polymeric MS and tissue reaction to their implantation was not described for PHA, despite numerous reports about their use for drug microencapsulation.

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Here we for the first time studied tissue reactions to implantation of polymeric PHB MS.

MATERIALS AND METHODS

Microspheres were made from PHB synthesized at Institute of Biophysics (molecular weight 150 kDa, crystallinity 72%, melting point 168°C) [11]. Microspheres were obtained by evaporating the solvent from emulsion (polymer solutions in dichloromethane with polyvinylalcohol and gelatin). The emulsion was dispersed with a mixer with a three-bladed propeller. After evaporation of the solvent, MS were collected by centrifugation and vacuum-dried. The MS microstructure was studied under a scanning electron microscope, the size distribution was studied using a Casy optical automated system for particles counting.

Sterile MS (20 mg in 0.3 ml saline) were injected intramuscularly into the thigh (adult female Wistar rats, 220-240 g). One day after MS implantation and then weekly 3 animals were sacrificed by inhalation narcosis overdose. General tissues reaction to MS implantation was studied by routine histological methods. Fragments of the muscle from the site of MS injection with adjacent tissues were collected; the material was fixed in 10% formalin and embedded into paraffin; 5-10-μ sections were sliced from the blocks and stained with hematoxylin and eosin. Semithin sections were made from tissue fragments fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) and postfixed in 1% OsO_4 in the same buffer. The samples were dehydrated in ascending alcohols and acetone and embedded in Epon 812-Araldite M (mixture of epoxy resins; Serva). Semithin sections (1.5 μ) were sliced on a Reichert Um-03 ultramicrotome and stained with 1% methylene blue. The images were analyzed and morphometry of the sections was carried out using Image Analysis System (×100 and ×400, Carl Zeiss). The intensity and duration of inflammation, cell infiltration in the zone of MS injection, and the status of implanted MS were evaluated. Activity of cells was evaluated by their mean number in a visual field (10 visual fields were analyzed).

RESULTS

MS had regular spherical shape with well-developed "wrinkled" porous surface (Fig. 1). Fractions of microparticles of different diameter were discernible among MS: 33.3±2.2% MS were <2 μ in diameter (these MS are most suitable for injections), the fraction of 2-10-μ particles constituted 38.0±1.9%, and 29.0±2.3% microparticles were >10 μ. The maximum diameter of MS was 35 μ, the particle constitute ≤3-5% MS. The mean diameter of MS used in the experiment was 10 μ.

The microscopic picture at the site of MS injection 24 h postinjection was characterized by slight tissue edema with leukocytic infiltration. The MS cluster implanted intramuscularly can be regarded as a model of open porous graft. The nature and intensity of tissue reaction to the graft is characterized by the presence of specific cell types at the site of implantation [1,10]. Initial inflammatory response of tissue characterized by the presence of polymorphonuclear leukocytes (up to 20-25 per visual field) was noted 24 h after injection of MS. This tissue reaction was transient: the number of polymorphonuclear leukocytes decreased significantly one week postimplantation. Slight intensity of leukocytic infiltration during this period characterized tissue response to MS implantation as medium intense inflammatory reaction.

One week after implantation, tissue response to the foreign body presented as infiltration with fibroblastic cells and formation of a fine fibrous capsule at the interface between the zone of MS injection and intact muscle tissues.

Tissue reaction to the foreign body manifested in the presence of macrophages at the graft/tissue

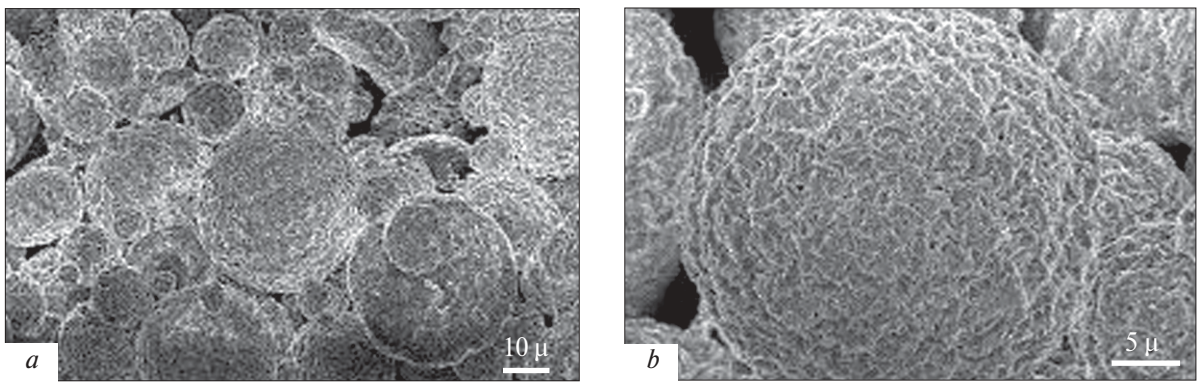

Fig. 1. Microspheres obtained from PHB.

interface [10]. It is known for several PHA types, including PHB, that phagocytic macrophages and foreign body giant cells (FBGC) forming as a result of macrophage fusion actively participate in polymer resorption *in vivo*. Our previous experiments with suturing myofascial wounds with monofilament PHB threads showed that macrophages phagocytosing damaged cells and tissues, as well as polymer particles and products of its destruction played an important role in the reparative response of tissues [8]. By the intensity and duration the tissue response to PHB implantation was comparable to the reaction to an inert surgical silk and was much less pronounced than the with reaction to resorbable catgut.

In addition to phagocytosis of resorbed biomaterials, macrophages initiate tissue granulation. It is characterized by fibroblast infiltration and forma-

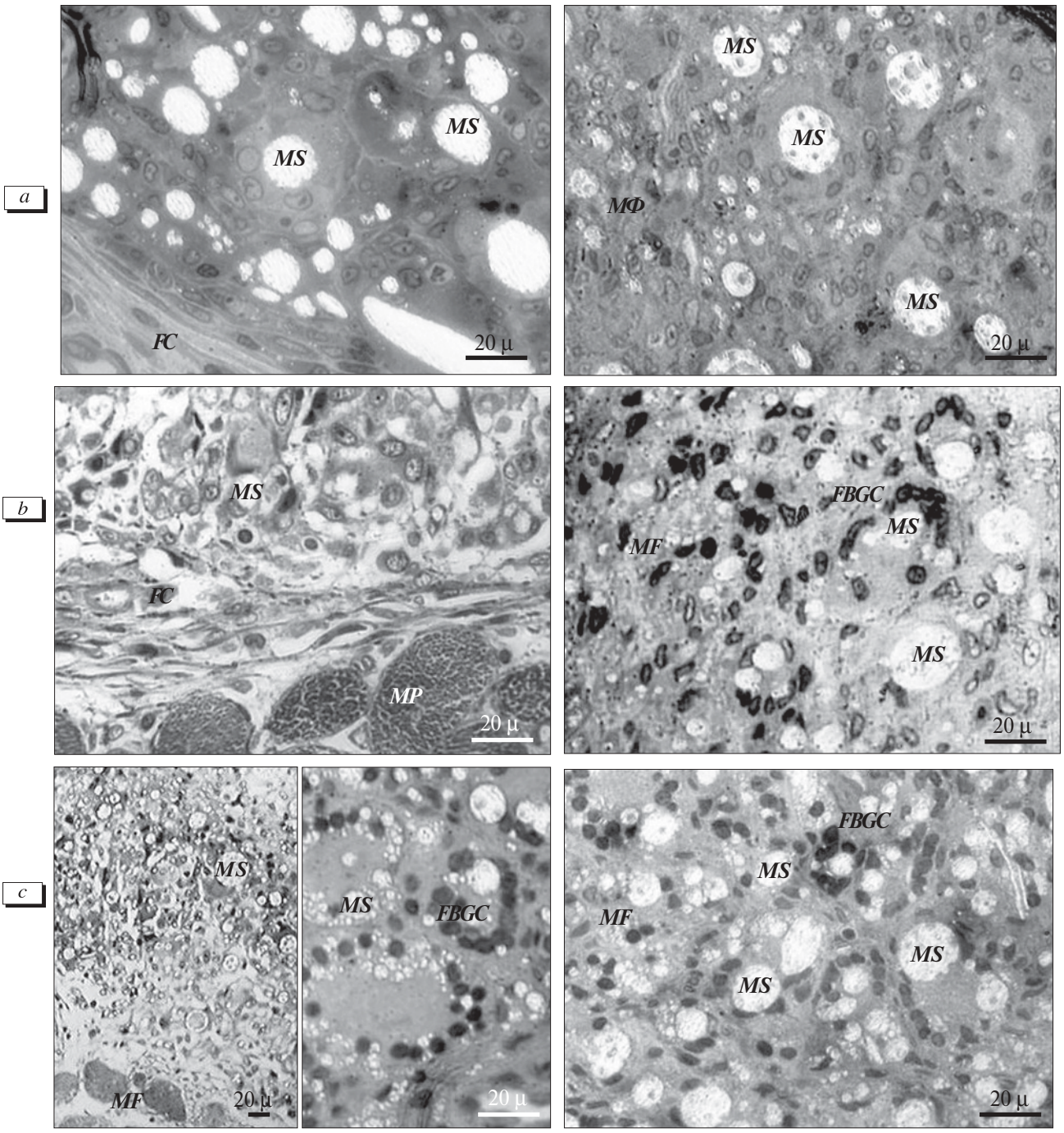

Fig. 2. Dynamics of the microscopic picture of tissues at the site of PHB MS implantation. *a*) 2 weeks; *b*) 5 weeks; *c*) 12 weeks. Semithin sections, methylene blue staining. *MP*: macrophages; *MF*: muscle fibers; *FC*: fibrous capsule.

tion of blood capillaries [3]. The grafts made from non-resorbable material are usually surrounded by a clearly discernible fibrous capsule. Grafts made from biocompatible and bioresorbable materials are not surrounded by thick fibrous capsule or the capsule undergoes involution [8]. After 2 weeks, the cluster of implanted MS was enveloped in a fine fibrous capsule from newly formed connective tissue (Fig. 2, *a*). During this period, the number of large (>10-15 μ) particles decreased significantly and was no more than 14% in the visual field. Active fibroblastic elements were identified in the capsule, the mean thickness of the fibrous capsule was <50-60 μ, it consisted of 4-6 layers of fibroblasts. This is in fact 3-fold lower in comparison with muscle tissue reaction to implantation of suturing material [8]. Newly formed tissue was intensively vascularized (Fig. 3), which indicated high biocompatibility of polymeric MS and favorable tissue reaction to implantation. The area of edema in the implantation zone decreased significantly, no necrotic sites were seen. Solitary leukocytic cells were seen among MS. The number of mature secretory phagocytic macrophages increased to 6.36± 0.42 per visual field on the inner side of the capsule adjacent to the graft and in the MS cluster zone (Fig. 2, *b*). An appreciable part of macrophage cytoplasm was occupied by lysosomes and phagosomes.

Macrophage infiltration in the zone of MS injection increased with time, the number of mononuclear macrophages and FBGC per visual field increased. After 5 weeks the number of mono- and polynuclear macrophages at the site of MS implantation increased (Fig. 2, *b*). The fraction of large particles decreased and was no more than 4-5% of the total number of MS in a visual field, this indicating destruction of the polymeric matrix. Accumulation of FBGC with 6-8 nuclei was noted around large MS (more than 10 μ in diameter). Thinned fibrous capsule, consisting of 2-3 layers of mature fibroblasts, was retained at the interface between intact muscle tissue and MS zone (Fig. 2, *b*). A trend to intensification of the macrophage reaction persisted 7-9 weeks after MS injection, the number of FBGC among microparticles increased the number of nuclei in them also increased. Macrophages grouped around large MS were clearly seen in the implantation zone. Introduction of cells into the MS surface matrix was seen in some cases, as well as destroyed (resorbed) zones on microparticle surface at the graft/tissue interface, which attests to biodegradation of the MS matrix. No fibrous capsules were seen at the MS graft/tissue interface and around individual MS. It is an important fact, as if

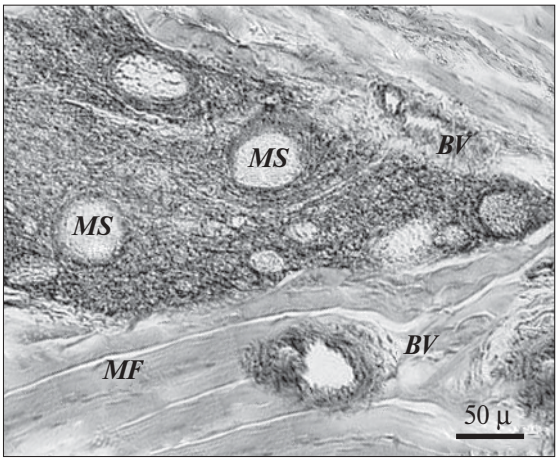

Fig. 3. Formation of blood vessels at the interface of MS injection 3 weeks after implantation. *MF*: muscle fibers; *BV*: blood vessels. Hematoxylin and eosin staining.

the fibrous capsules envelope polymeric MS, they can modify the rate of drug release from the polymeric matrix and cause its resorption.

A characteristic tissue reaction 11-12 weeks after MS injection was pronounced macrophage infiltration with numerous FBGC surrounding polymeric microparticles or grouped around a cluster of small MS (Fig. 2, *c*); large symplasts of FBGC with 10-12 and even more nuclei were seen. No fibrous capsules were detected at the interface between MS cluster and intact muscle tissues. Polymeric detritus (the product of destruction of larger particles) was present in some zones of MS cluster. The number of large MS (>10-15 μ in diameter) decreased to 2-3% and fragmented MS appeared. However, on the whole the majority of intact MS were present in tissues during a long period, indicating a sufficiently long process of MS resorption *in vivo* and the possibility of using PHB for the creation of long-acting dosage form for intramuscular injections.

Tissue response to implantation of polymeric MS from PHB was characterized by transient inflammatory reaction of slight intensity, pronounced progressive macrophageal infiltration with FBGC absorbing the polymeric matrix, and by granulation response with formation of well vascularized fine fibrous capsule at the interface between the zone of MS injection and muscle tissue, resorbed after several weeks. No formation of fibrous capsules enveloping the polymeric MS, necrotic and other undesirable morphological changes or tissue restructuring in response to implantation of polymeric particles was detected. These results indicate good prospects of using PHB for the creation of long-acting dosage forms in the form of MS for intramuscular injections.

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