ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 43 (1997) 19-28

European Journal of Pharmaceutics and Biopharmaceutics

Biocompatibility of ABA triblock copolymer microparticles consisting of poly(L-lactic-co-glycolic-acid) A-blocks attached to central poly(oxyethylene) B-blocks in rats after intramuscular injection

Birgit Ronneberger[a], Thomas Kissel[a,*], James M. Anderson[b,c]

[a]*Department of Pharmaceutics and Biopharmacy, Philipps-University, Marburg, Ketzerbach 63, D-35032 Marburg, FRG*
[b]*Department of Macromolecular Science Case Western Reserve University, Cleveland, Ohio, USA*
[c]*Institute of Pathology, Case Western Reserve University, Cleveland, Ohio, USA*

Received 15 May 1995; accepted 16 June 1996

Abstract

Biocompatibility is one of the key factors for the development of new polymer delivery systems. Three ABA-triblock copolymers consisting of lactic-co-glycolic-acid A-blocks and poly(oxyethylene) B-blocks were studied after intramuscular injection in rats to investigate the influence of different polymer compositions and molecular weights on the tissue reactions by histological analysis of the injection site. The intramuscular injection of poly(lactide-co-glycolide) microparticles (PLG) was used as control system. In the present study, the tissue reaction was evaluated over a 56-day implantation period in rats characterizing the following factors: inflammation, necrosis, damage to the surrounding tissue, foreign body reaction and collagen deposition and fibrous capsule formation surrounding the microparticles at the injection site. Throughout the implantation period, all polymers showed a normal foreign body reaction and healing response. The foreign body reaction of all three ABA-triblock copolymers is mainly a granulation tissue type of healing response with the presence of macrophages, fibroblasts and foreign body giant cells. New small blood vessels were detected. Neither necrosis nor significant muscle damage could be identified in the histology slides examined. These results suggest that microparticles prepared of ABA-triblock copolymers can be considered as a biocompatible and a biodegradable delivery system.

Keywords: Biocompatibility; Microparticles; ABA triblock copolymer; Poly(lactide-co-glycolide) copolymer; Poly(oxyethylene); Inflammation; Foreign body reaction; Histology in rats

1. Introduction

Parenteral depot systems for drug delivery, especially the prolonged delivery of peptides and proteins like erythropoitein (EPO) and interleukin-2 (IL-2), are the object of intensive research efforts. Biodegradable matrices for drug delivery systems have to fulfill several requirements. Biocompatibility, which is defined as the ability of a material to perform an appropriate host response in a specific application [1], is one of the key factors for the development of new polymeric delivery systems. An ideal material should be well tolerated, elicit minimal inflammatory tissue reactions and when its use is not longer required, the material should be degraded and absorbed.

The degradation rate of the polymer and the release behavior as a function of time are important design criteria. Therefore, biodegradable polymers often contain hydrolytically labile bonds such as ester groups. The most frequently used biodegradable polymers for drug delivery are polymers of lactic acid, glycolic acid and their copolymers, abbreviated as PLG. The main mechanism of biodegradation is thought to be random

* Corresponding author. Tel.: +49 6421 285880; fax: +49 6421 287016.

0939-6411/97/$17.00
PII S0939-6411(96)00006-9

hydrolytic chain cleavage in the bulk of the polyester [2]. The involvement of enzymes in the degradation of PLG is still a matter of controversy and seems to play only a minor role [3]. The cleavage of polyesters was shown to proceed more rapidly in the core of the device [4], probably due to autocatalytic acceleration of the ester hydrolysis by degradation products. This phenomenon causes additional complications for acid-labile drug substances.

The release of proteins from PLG is not well documented in contrast to peptides. Mechanical, physical and chemical properties of the device influence the release of peptides and proteins as well as the biodegradation but the release factors are still incompletely characterized [5]. Apart from the higher molecular mass of proteins, compared to peptides, which complicates the attainment of continuous release profiles, proteins are more sensitive to chemical and physical degradation, leading to denaturation and loss of pharmacological activity. Several proteins are known to form aggregates when exposed to small amounts of water during the initial phase of release [6].

To overcome the above mentioned problems, the development of new degradable biomaterials was undertaken to obtain materials showing faster degradation times (ca. 2 weeks), better compatibility with proteins (e.g. less aggregates) and degradation products, better mechanical properties and to improve the release pattern.

An ideal aqueous environment for proteins provides hydrogels and by manipulation of the cross link density both the degree of hydration and protein release can be adjusted [7]. Hydrophilic poly(oxyethylene) domains introduced into hydrophobic polyesters like L (+)-lactic-co-glycolic-acid (LPLG) are thought to increase the polymer degradation- and the release-rate by generating a hydrogel-like environment [8]. ABA-triblock-copolymers composed of L-lactic-co-glycolic-acid (PLG) A-blocks and poly(oxyethylene) (PEO) B-blocks have shown interesting mechanical and swelling properties under in-vitro degradation conditions [9,10]. Nevertheless, the correlation of in-vitro to in-vivo degradation behavior has not been reported to date.

Important for the determination of the in vivo biocompatibility of biodegradable polymers studied so far is the cellular reaction at the polymer/tissue interface. Placing a biodegradable material in an biological environment initiates a response to this injury and mechanisms are activated to effect a healing reaction. Inflammation, wound healing and foreign body reaction are considered parts of the tissue responses to injury. Among others, intensity and/or time duration of the inflammatory response determine the biocompatibility and the success as a drug delivery system. In general, the biocompatibility of a material with tissue has been reported in terms of acute and chronic inflammatory responses, especially the formation of FBGCs and of fibrous capsules seen over various time periods following implantation [11]. The size [13], shape [17] and chemical and physical properties of the polymer [18] may be responsible for variations in the intensity and duration of the inflammatory and wound healing process.

Polymers of lactic acid and glycolic acid and copolymers of these have been used for many years as surgical suture material and they are applied for controlled release of drug delivery systems [12]. In various studies their histological biocompatibility was characterized, and these materials have been demonstrated to possess excellent biocompatibility [13–16].

The most commonly used method of evaluating the biocompatibility of tissue adjacent to implanted materials as a function of implant time has been the histologic evaluation. The inflammatory response is a series of complex reactions involving various types of cells. The predominant cell type present in the inflammatory response varies with the age of the injury. In general, neutrophiles predominate during the first several days following injury and then are replaced by monocytes as the predominate cell type [11].

In the present study the influence of different compositions of PLG A-blocks and PEO B-blocks in ABA block copolymers on the degradation rate and biocompatibility was examined. The polymer or its degradation products might be toxic and/or could cause mechanical irritation. The toxicity of PEO decreases as molecular weight increases [19]. High doses in the range of grams of injected PEG have consequences such as elevated osmolarity, acidosis, elevated total plasma calcium. Modest quantities have not been associated with detectable toxicity [20].The intramuscular injection of poly(lactide-co-glycolide) microparticles (PLG) was used as control. The tissue reaction to intramuscular injection of microparticles in rats was performed by the evaluation of histological slides of the implantation sites using light microscopy.

2. Materials and methods

2.1. Materials

ABA triblock copolymers were synthesized by bulk polymerization using aluminium triisopropoxide as catalyst in a bulk copolymerization process according to the method described previously [9]. The structure of the ABA polymers was confirmed by nuclear magnetic resonance spectroscopy. All ^{1}NMR spectra were obtained from $CDCl_3$ solutions containing TMS as reference at 25°C on a Jeol JNMR-FX 100 nuclear magnetic resonance spectrometer. Resomer® RG 503 50/50 D,L-lactide/glycolide copolymer was obtained from Boehringer Ingelheim (Ingelheim, Germany).

Table 1
Composition and properties of ABA-triblock-copolymers consisting of L-lactic-co-glycolic-acid (LLA/GA) A-blocks and poly (oxyethylene) (PEO) B-blocks and of poly(lactide-co-glycolide) (PLG)

Code	Molar composition (mol%) LLA/PEO/GA[a]	M_w g/mol[b]	M_n g/mol[b]	I[b]	M_w PEO g/mol[b]
ABA1:	70/7/23	26 000	14 400	1.80	1000
ABA2:	62/21/17	18 000	6300	2.86	4000
ABA3:	58/26/16	31 000	13 500	2.30	10 000
PLG	50/–/50	38 700	15 700	2.47	–

[a] Polymer composition, determined by ^{1}H-NMR.
[b] M_w/M_n measured by GPC.

The molecular weight data were obtained by gel permeation chromatography on Merck size exclusion columns (Lichrogel PS mix and Lichrogel PS 40). Molecular weight was calculated by the universal calibration method [21] using polystyrene as reference material (Merck, M_W 3250, 5100, 19 600, 34 500 and 87 000). The composition and properties of the polymers used in this paper are summarized in Table 1.

2.2. Methods

2.2.1. Preparation of microparticles

The microparticles were prepared by a modified solvent evaporation method [22]. Briefly, 20 ml of a 20% (w/v) polymer solution in dichloromethane were emulsified into 400 ml of an aqueous phase containing 0.05% w/v methylcellulose (400 cps) under stirring (8000 rpm) for 10 min. The evaporation of the solvent was carried for up to 14 h at room temperature. Subsequently, the microparticles were centrifuged, washed twice with distilled water, collected and lyophilized over 24 h. The size distribution analysis was performed using a Master Sizer (Malvern Instruments, UK). The average diameter D [4,3] of the microparticles was approximately 30 μm.

2.2.2. Injection of microparticles

All animal studies were approved by the Hessian Committee of Animal Protection. Young male and female Sprague-Dawley rats (Charles River, Sulzfeld, Germany) of 300 g were used for this study. The animals were kept in air-conditioned animal facilities and had free access to tapwater and standard laboratory feed. Groups of $n = 2$ per time point were injected 20 mg of the same microparticles into the gastrocnemius muscle of the hind legs. The microparticles were suspended in a sterile injection vehicle, consisting of 0.5% (w/w) carboxymethylcellulose, 0.1% (w/w) pluronic F 68 and isotonic sodium chloride solution. For better identification, the injection sites were shaven. Sterilized disposable syringes with 20-gauge needles containing 20 mg microparticles in 0.2 ml 0.5% carboxymethylcellulose were used for injection. Prior to injection, the material was vortexed for 30 s to suspend the microparticles in the injection vehicle.

2.2.3. Sample preparation

At predetermined time points (1, 6, 10, 14, 21, 28, 35, 42 and 56 days after injection) two rats per particle batch were sacrificed with an overdose of carbon dioxide. Tissue samples from the injection site were removed using a scalpel, fixed in buffered formalin and embedded in paraffin or methylmethacrylate. Finally the tissue-blocks were thinly sectioned (5 μm tissue sections) using a microtome (Supercut, Reichart-Jung, Germany). Glass slides were prepared using standard histological procedures [23]. The slides were stained with hematoxylin and eosin [24] for the examination of cellularity and cell counting. Massons trichrome stain [25] was used to identify fibrous capsule formation and collagen deposition.

2.2.4. Histopathological analysis

The tissue reaction was evaluated by light microscopy (Polyvar, Reichart-Jung, Germany) using the following criteria: Inflammation, damage of the surrounding tissue, foreign body reaction and collagen deposition in the fibrous capsule surrounding the implant of microparticles.

The extent of the inflammatory response was assessed by two independent observers using a scoring system [20], which is shown in Table 2: 0 (no inflammation), 1 (mild), 2 (moderate), 3 (extensive), 4 (very extensive) at magnification × 400. A scale was determined for each slide. Four areas of the slide were chosen and the arithmetic mean was calculated. The collagen deposition and appearance of FBGC was evaluated using a subjective scale. The results were shown in Table 3.

3. Results

ABA microparticles of different composition were studied in a 56 days implantation period to analyze the inflammatory responses in rats. The histopathological evaluation was performed by scoring the tissue reaction at the implantation site. All polymers showed a classic foreign body reaction and healing response [27]. The tissue reaction is limited to the implant site. The predominant cell type present in the injection site varies

Table 2
Relative rating scale for inflammatory response to the injection of ABA microparticles

Score	Monocytes/macrophages (no/field)[a]	PMNs (no/field)[a]
0 No inflammation	0	0
1 Mild	0–200	0–10
2 Moderate	200–400	10–30
3 Extensive	400–600	10–30
4 Very extensive	>600	>30

[a] Each slide was observed by light microscopy (magnification × 400). The most dense inflammatory cell areas were chosen in the vicinity of each implantation site. The number of cells was counted for each area, with at least four areas evaluated per slide and a score was given to each slide.

with the time after injury. Neither necrosis nor significant muscle damage could be identified in the histology slides examined.

The number of inflammatory cells, especially macrophages and FBGC around the microparticles increased due to the injection. Neither acute (characterized by polymorphonuclear leukocytes) nor chronic inflammation was identified after 6 days of implantation. The foreign body reaction of all three ABA-triblock copolymers was mainly a granulation tissue type of healing response with the presence of macrophages, fibroblasts and foreign body giant cells. New small blood vessels were detected. The end-stage healing response to the implanted microparticles was the formation of a fibrous capsule.

The relative scores of the extent of the inflammatory response, the appearance of FBGC and collagen deposition are listed in Table 3. Selected views of intramuscular implant sites in rats are shown in Figs. 1–3.

In the ABA implantation sites more inflammatory cells were observed compared to PLG implantation sites, but the foreign body reaction was comparable and was not considerably different from the reaction to PLG microparticles. After the abatement of the acute reaction to the implantation process, the injection sites of ABA showed stable inflammatory scores first of all. After 21 days, an increase in the number of cells as time increased could be observed. This might be due to a change of the surface area of the microparticles. At least the scores were stable again.

The histological examination of the ABA implantation sites is summarized below:

Day 1: The initial tissue reaction to injected microparticles was a localized, demarcated inflammatory reaction. The implantation sites of all polymers con-

Table 3
Histological evaluation of the inflammatory response to ABA microparticles

Polymer	Implantation period (days)	Inflammatory response	FBGC	Collagen deposition
ABA 1	1	Acute	None	None
ABA 2	1	Acute	None	None
ABA 3	1	Acute	None	None
ABA 1	6	Moderate	Minimal	Slight
ABA 2	6	Mild-moderate	Moderate	Moderate-slight
ABA 3	6	Mild	Minimal	Minimal-moderate
ABA 1	10	Mild	Minimal	Slight-moderate
ABA 2	10	Moderate	Moderate	Slight-moderate
ABA 3	10	Mild	Moderate	Minimal-moderate
ABA 1	14	Mild	Minimal	Moderate
ABA 2	14	Moderate	Moderate-extensive	Moderate-extensive
ABA 3	14	Mild	Moderate-extensive	Moderate
ABA 1	21	Mild	Minimal	Moderate
ABA 2	21	Mild-moderate	Minimal	Extensive-moderate
ABA 3	21	Mild	Moderate-extensive	Moderate-extensive
ABA 1	28	Mild	Minimal-moderate	Moderate
ABA 2	28	Moderate	Minimal	Moderate-extensive
ABA 3	28	Mild	Moderate-extensive	Moderate-extensive
ABA 1	35	Mild-moderate	Moderate	Extensive
ABA 2	35	Mild-moderate	Minimal	Moderate
ABA 3	35	Nd	Nd	Nd
ABA 1	42	Nd	Nd	Nd
ABA 2	42	Nd	Nd	Nd
ABA 3	42	Moderate	Moderate	Moderate-extensive
ABA 1	56	Mild-moderate	Moderate-extensive	Moderate-extensive
ABA 2	56	Nd	Nd	Nd
ABA 3	56	Moderate	Moderate-extensive	Moderate-extensive

Nd not done.

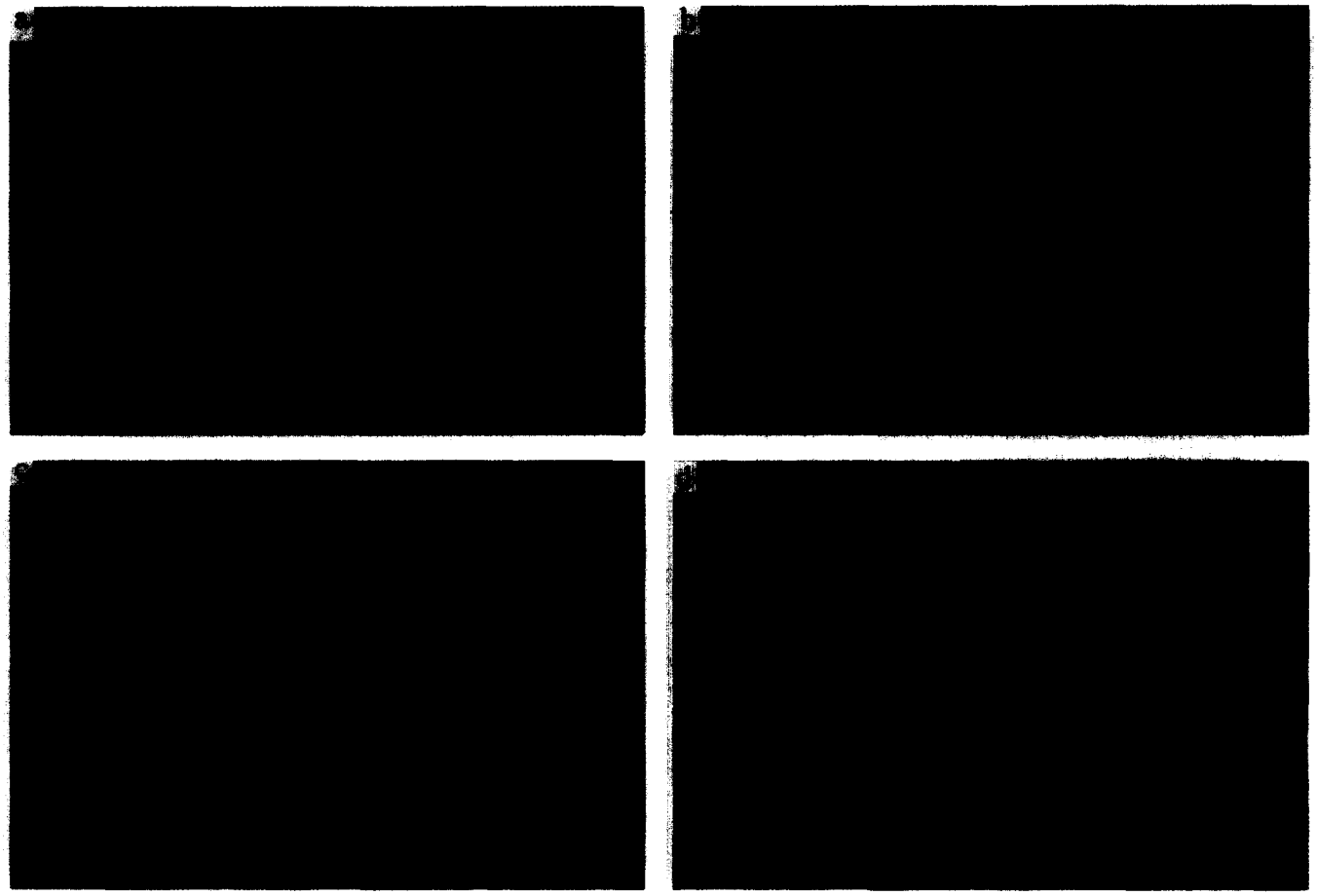

Fig. 1. Histology of the ABA1 microparticle implantation sites in rats, original magnification 100 ×. (a) 10 days implant site, haematoxylin and eosin stain, (b) 28 days implant site, haematoxylin and eosin stain, (c) 56 days implant site, haematoxylin and eosin stain, (d) 35 days implant site, Masson's trichrome stain.

tained microparticles in a spherical configuration. The main characteristics of acute inflammation at the implant/tissue interface was the accumulation of leukocytes. Predominantly polymorphonuclear leukocytes migrated into the interstices between the microparticles. The center of the injection sites showed microparticles but minimal to no cellular infiltration. The histological picture of all injection sites was consistent with an acute inflammatory response to a biomaterial at 1 day implantation time (Fig. 3a).

Day 6: The microparticles caused a tissue reaction within a granulation tissue characterized by macrophages, foreign body giant cells and fibroblasts. An early development of a fibrous capsule could be identified for ABA1 but not for ABA2. Collagen deposition was present for all three types of microparticles. There was no evidence of acute or chronic inflammation (Fig. 2a).

Day 10: Microparticles were surrounded by macrophages and FBGCs. Fibroblasts were oriented parallel to the implantation sites (Fig. 1a). The collagen deposition was low to moderate. The injection site of ABA2 microparticles was enclosed in a collagenous fibrous capsule (Fig. 2b).

Day 21: The injection sites showed collections of spherical microparticles. The tissue response consisted of a foreign body reaction with the presence of macrophages, FBGC and fibroblasts (Fig. 2c). The skeletal muscle appeared to be normal. In the interstices of the implant sites fibroblast infiltration with moderate (ABA1, ABA3) or extensive (ABA2) collagen deposition was observed. PMNs were not present. The size of ABA microparticles and their morphology was changing, indicative of an onset of polymer mass loss.

Day 28: The tissue response was a classic foreign body reaction with macrophages as the predominant cell type. The cell density was relatively low for ABA1. No degradation could be identified (Fig. 1b). For ABA2 a high number of macrophages and fibroblasts was seen. This was due to the fact that the degradation of microparticles was extensive, only some intact microparticles were present. In the case of ABA3, degradation started in the center of the injection site. The collagen deposition was moderate for ABA1 (Fig. 1d) and moderate to extensive for ABA2 and ABA3.

Day 35: The classical foreign body reaction with macrophages, foreign body giant cells and fibroblasts was present for all implant sites. Extensive collagen

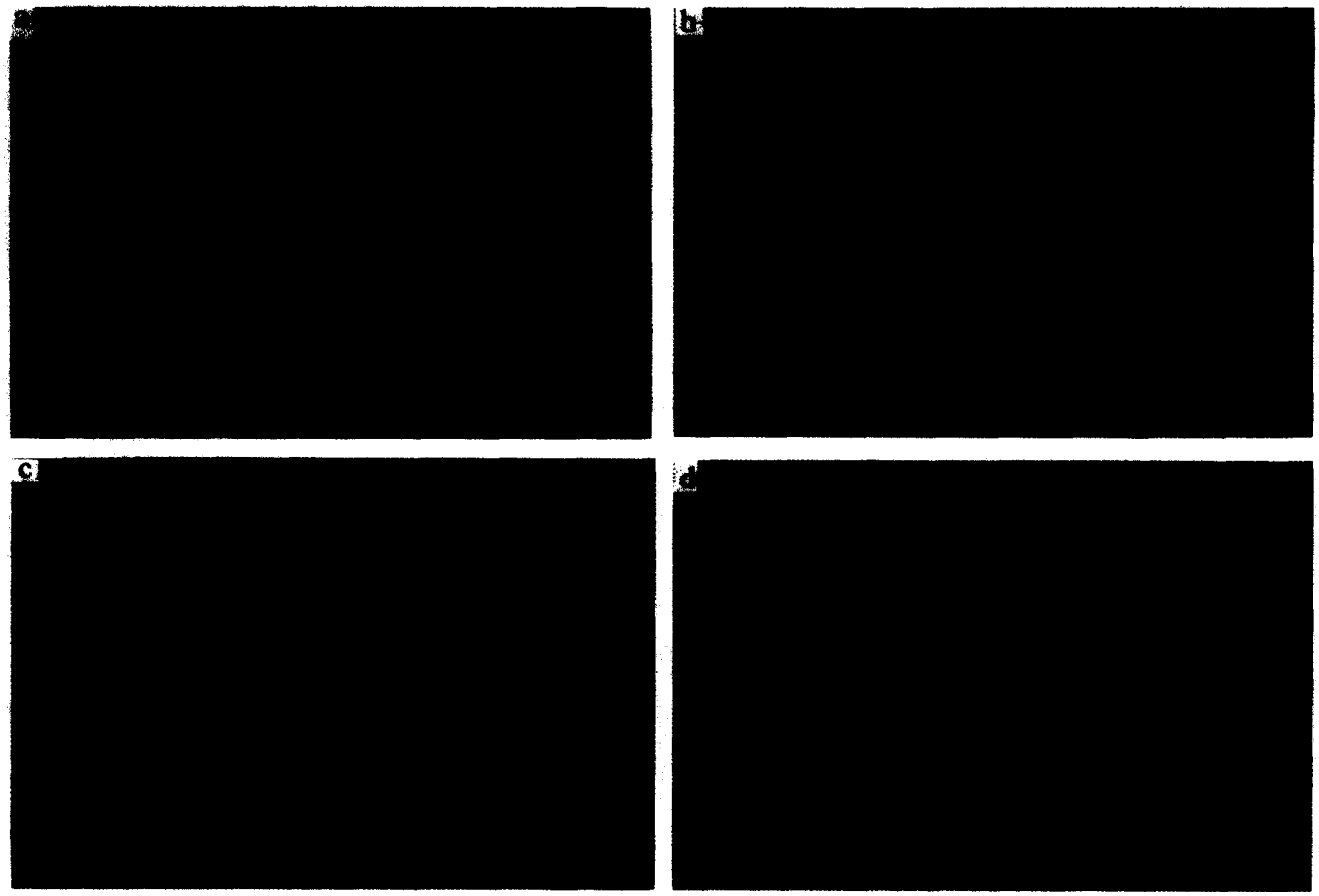

Fig. 2. Histology of the ABA2 microparticle implantation sites in rats, original magnification 100 ×. (a) 6 days implant site, haematoxylin and eosin stain, original magnification 63 ×, (b) 10 days implant site, Masson's trichrome stain, original magnification 40 ×, (c) 21 days implant site, haematoxylin and eosin stain, original magnification 100 ×, (d) 35 days implant site, haematoxylin and eosin stain, original magnification 100 ×.

deposition was observed for ABA1. Microparticles prepared from ABA1 and ABA3 were still intact in contrast to microparticles prepared of ABA2. Mainly fragments of ABA2 microparticles were seen (Fig. 2d) and the collagen deposition was moderate.

Day 42: The tissue material of the implant sites included macrophages and fibroblasts. In the interstices of the implant sites fibroblast infiltration with collagen deposition (Fig. 3c) was observed and newly formed blood vessels were seen.

For ABA1, granulation tissue was found, many macrophages could be, but only some FBGCs were identified. In the case of ABA3, the number of macrophages increased, due to the degradation of the microparticles, which were swollen. The collagen deposition was moderate to extensive.

Day 56: In the case of ABA1, inflammatory cells were still identified and no degradation of microparticles could be seen (Fig. 1c). The collagen deposition was moderate to extensive. The implant of ABA3 induced a high number of macrophages (Fig. 3b). New blood vessels could be identified and no fibrous capsule could be seen. Microparticles were swollen, but still present.

4. Discussion

Many factors, such as surface properties, size and degradation products from the polymer can affect the extent and magnitude of inflammatory reactions [23]. A histological investigation was performed using ABA microparticles of different composition and the inflammatory responses in rats were analyzed as shown in Fig. 4. The histological investigation of implanted microparticles demonstrated the development of a fibrous capsule of varying thickness and degree of inflammation in dependance of the polymer composition. The tissue reaction to microparticles was a normal defense reaction, which depends also on the extent of the injury or defect created during the implantation procedure [23].

In addition, it was reported that a lower degradation rate should result in a less severe foreign body reaction [18]. Microparticles prepared of different ABA polymers exhibited differences in their degradation rate due to the hydrophilicity of the copolymer and the mechanical stability of the microparticles. The hydrophilic behavior was mainly controlled by the copolymer com-

Fig. 3. Histology of the ABA3 microparticle implantation sites in rats, original magnification 100 × . (a) 1 day implant site, haematoxylin and eosin stain, (b) 56 days implant site, haematoxylin and eosin stain, (c) 42 days implant site, Masson's trichrome stain.

position, the mechanical stability was influenced by the molecular weight and the length of the chains of the molecules [28]. The hydrophilicity of the polymers decreased with an increase in amounts of lactide. That is the reason why the 70/7/23 ABA triblock copolymer degraded more slowly compared to the other polymers. ABA2 and ABA3 have a similar composition, but different initial molecular weights. As shown by light microscopy, microparticles prepared from ABA2 appeared to degrade more rapidly. Microscopic observations showed that ABA1 and ABA3 microparticles remained intact throughout the implantation period. Microparticles prepared from ABA2 exhibited the fastest degradation rate compared to ABA1 and ABA3, since 35 days post implantation only fragments of ABA2 microparticles could be detected. Visscher et al. [29] could not correlate cell counts of macrophages or giant cells at the implantation site with the degradation rate of PLG-microparticles. These findings suggest that the degradation rate of PLG-microparticles is mainly influenced by hydroylitic cleavage of the polyester and only to a lesser extend by the biological environment at the injection site. In this paper, ABA2 with a lower molecular weight seems to degrade faster, due to more rapid molecular weight decay with subsequent mass loss of the polymer from the injection site.

Other factors, such as the chemical and physical properties of the polymer and the degradation products may cause alterations during the inflammatory response. Therefore, the interactions of the surface of the microparticles and the surrounding tissue are important factors in the biological interaction. Studies of tissue reactions to microparticles, prepared from poly(lactide)glycolide have shown, that the material is biocompatible [11,26]. Therefore, PLG was used as control material. The tissue reaction of ABA and PLG was comparable.

Particle size seems to play a role in the tissue reaction to implanted materials [9]. Usually, particles ranging in size from 10 μm or larger are not phagocytosed by granulocytes in the acute inflammatory reaction [28]. The microparticles used in the study had an average diameter of ≈ 30 μm. Williams reported [30] that, generally, a pure polymer of high molecular weight in the form of a monolithic solid will behave as an inert foreign body and will elicit the minimal fibrous response. Deviations from this behavior are noted when the devices are implanted in particulate form. A collec-

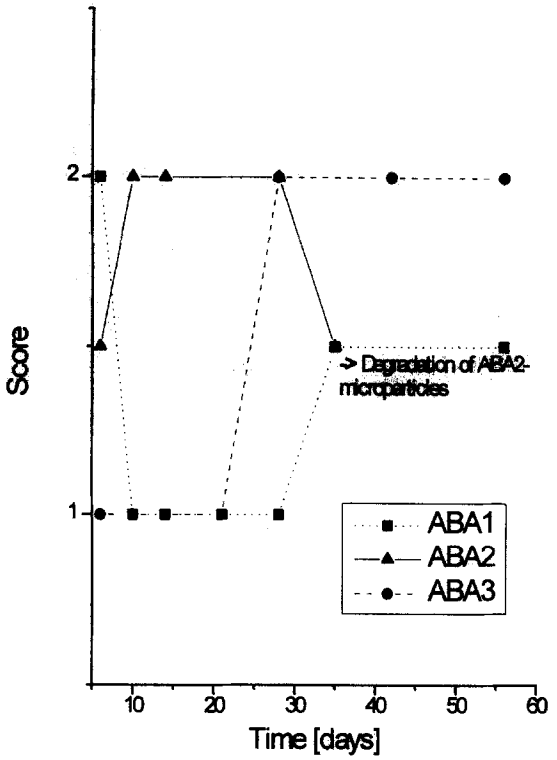

Fig. 4. Inflammation score of ABA1, ABA2 and ABA3 microparticles in rats with implantation time.

tion of particles may influence the tissue reaction by physical irritation [31].

Under in-vitro conditions an increase of PEO content led to an accelerated degradation rate of ABA block copolymer films [10]. Degradation rates could be adjusted by varying the PEO content and the lactic/glycolic ratio. In contrast to the ABA films, microparticles would be an appropriate application form of parenteral depot systems. Therefore we extended our investigations to ABA microparticles described in this study. A comparison of degradation data obtained under in vitro conditions with those from histological examination of injection sites is notoriously difficult, since the latter method is only qualitative in nature. Factors, such as shape and surface properties of the implant as well as the formation of fibrous capsules may complicate the picture.

In the ABA implantation sites more inflammatory cells were observed compared to PLG implantation sites, but the foreign body reaction, which was described above, was similar and was not considerably different from the reaction to PLG microparticles. It appeared that the introduction of PEO-blocks was not a critical factor for the tissue response in this study. No local toxicity from ABA microparticles was seen. The foreign body reaction was classic with fibroblast activity and FBGC formation. There has not been an indication for severe necrosis or significant muscle damage in response the implanted material.

The predominant cell type was the macrophage. The macrophage infiltration towards the implantation site was important, because this could initiate a cascade of responses. Macrophages and other inflammatory cells, which were present, may secrete potentially polymer damaging agents. In addition, the secretory products of macrophages such as polypeptide hormones, complement components and enzymes are of particular importance for the sequence of events during the inflammatory response [34]. Therefore, macrophages could be regarded as some kind of control cells in the inflammatory reactions to implants. FBGCs, which were formed, when a material could not be phagocytosed by macrophages, were also found. It has been suggested that size and/or surface area of the artificial implants is critical to the formation of FBGCs in vivo [26]. Material with rough surfaces are known to evoke preferential FBGC formation [35]. There have been several studies demonstrating the functions of the different cell types [36]. Papadimitriou has suggested that FBGCs have less attachment receptors than macrophages resulting in less phagocytic activity. However, little is known about the function of FBGCs. The presence of fibroblasts was often connected with the phase of wound healing. The cell was specialized for the establishment and maintenance of the extracellular matrix [32]. Fibroblasts migrate into the wound. Postlewaite and Kang [37] reported that fibroblasts secrete rapidly an extensive fibronectin matrix and shortly thereafter large amounts of collagen type III are deposited in association with this matrix. Fibronectin might be essential in the organization of this matrix.

Often macrophages were unable to phagocytose and to remove the material. Alterations in the foreign body reaction might be due to differences in the degradation rate. ABA contained structures which were hydrolysable. Schakenraad et al. [32] studied PLG with different composition. The inflammatory reactions were more severe for polymers having a higher degradation rate. The lower the degradation rate, the less severe was the foreign body reaction. An increase in inflammatory cells occurred when degradation started.

At later time points, when degradation of the microparticles started, a higher number of macrophages was seen indicating that the chemical composition and the initial molecular weight determined the onset of a more intensive foreign body reaction mediated by macrophages. Parts of the implantation sites were replaced by collagen tissue. The formation of a fibrous capsule might cause problems, because it can function as a barrier hindering the diffusion of substances [33]. In the present paper, a thin fibrous capsule surrounding the implant of microparticles was at least noted at day 10 after implantation for all three polymers. Special differences in capsule thickness could not be observed. Yamaguchi [23] described the formation of a fibrous

capsule surrounding subcutaneous implants of naltrexone beads in rats at day 7 post implantation. In the case of 65/35 PLG microparticles the appearance of a fibrous capsule was reported at day 15 post implantation [26].

The implantation of a biomaterial and the resulting interaction between cells and microparticles involved complex reactions, such as cell activation, immigration and release of substances. The role and the mechanism of activation of various cell types are still uncertain but the chemical and physical properties of the implant must be taken into consideration. Nevertheless, the tissue reaction of ABA microparticles and of PLG could be described as similar in nature.

5. Conclusion

The safety and use of biodegradable materials depend on the biocompatibility in the in vivo environment. Microparticles fabricated from different ABA compositions were studied to analyze the inflammatory response and the wound healing process to the implanted material. All implanted microparticles caused an initial acute but localized inflammatory response. Muscle tissue surrounding the injection sites did not show irreversible changes such as necrosis and degeneration. The inflammatory reaction was mainly of the granulation tissue type. The predominant cell type was the macrophage. The implantation sites were gradually replaced by collagenous tissue during the course of degradation. The results of this study in rats suggested that microparticles prepared from ABA-triblock copolymers can be considered as a biocompatible carrier system with properties very similar to PLG.

References

[1] Williams DF, editor. Definitions in Biomaterials. Amsterdam: Elsevier, 1987.

[2] Wise D, Fellman T, Sanderson J, Wentworth R. Lactic/Glycolic acid polymers. In: Gregoiadis G, editor. Drug Carriers in Biology and Medicine. New York: Academic Press, 1979:237–270.

[3] Williams DF. Enzyme hydrolisis of polylactic acid. Eng. Med. 10;1981:5–7.

[4] Vert M, Li S, Garreau H. More about the degradation of LA/GA-derived matrices in aqueous media. J. Control. Release 16;1991:15–24.

[5] Bodmer D, Kissel T, Trächslin E, Factors influencing the release of peptides and proteins from biodegradable parenteral depot systems. J. Control. Release 21;1992:129–138.

[6] Sluzsky V, Tamada J, Klibanov, Langer R. Kinetics of insulin aggregation in aqueous solution upon agitation in the presence of hydrophobic surfaces. Proc. Natl. Acad. Sci. USA 88;1991:9377–9381.

[7] Gilbert D, Kim S. Macromolecular release from collagen monolithic devices. J. Biomed. Mater. Res. 24;1990:1221–1239.

[8] Kissel T, Li Y, Görich S, Volland C, Koneberg R. Parenteral protein delivery systems using biodegradable ABA-block-copolymers. J. Control. Release 39;1996:315–326.

[9] Li Y, Kissel T. Synthesis and properties of biodegradable ABA triblock copolymers consisting of poly(L-lactic acid) or poly(L-lactic-co-glycolic acid) A-blocks attached to central poly(-oxyethylene) B-blocks. J. Control. Release 27;1993:247–257.

[10] Li Y, Volland C, Kissel T. In-vitro degradation and bovine serum albumin release of the ABA triblock copolymers consisting of poly (L(+)-lactic acid) or poly (L(+)-lactic-co-glycolic acid) A-blocks attached to central poly(oxyethylene) B-blocks. J. Control. Release 32;1994: 121–128.

[11] Anderson J. Mechanisms of inflammation and infection with implanted devices. Cardiovasc. Pathol. 2;1993:33S–41S.

[12] Maulding H. Prolonged delivery of peptides by microcapsules. J. Control. Release 6;1987:167–176.

[13] Tabata Y, Ikada Y, Macrophage phagocytosis of biodegradable microspheres composed of L-lactic acid/glycolic acid homo- and copolymers. J. Biomed. Mater. Res. 22;1988:837–858.

[14] Visscher G, Robison R, Maulding H, Fong J, Pearson J, Argentieri G, Biodegradation of and tissue reaction to 50:50 poly(DL-lactide-co-glycolide) microcapsules. J. Biomed. Mater. Res. 19;1985:349–365.

[15] Visscher G, Robison R, Argentierei G. Tissue response to biodegradable injectable microcapsules. J. Biomater. Appl. 2;1987:118–131.

[16] Visscher G, Pearson J, Fong J, Robison R, Argentieri G, Maulding H. Effect of particles size in vitro and in vivo degradation rates of poly(DL-lactide-co-glycolide) microcapsules. J. Biomed. Mater. Res. 22;1988:733–746.

[17] Matlaga B, Yasenchak L, Salthouse T. Tissue Response to implanted polymers: The significance of sample shape. J. Biomed. Mater. Res. 10;1976:391–397.

[18] Schakenraad J, Dijkstra P. Biocompatibility of Poly (DL-lactic Acid/Glycine) Copolymers. Clin. Mater. 7;1991:253–269.

[19] Bowe V. In: Patty FA, editor. Industrial Hygiene and Toxicology, Vol II. New York: Wiley, 1963:1510–1515.

[20] Winer M, Bernstein I. In: Adverse Reactions to Drug Formulation Agents. New York: Marcel Dekker, 1989:101–111.

[21] Grubisic Z, Rempp P, Benoit H. A universal calibration for gel permeation chromatography. J. Polymer Sci. B 5;1967:753–759.

[22] Jeffrey H, Davis S, O'Hagan D. The preparation and characterisation of poly(lactide-co-glycolide) microparticles. I.: Oil-in-water emulsion solvent evaporation. Int. J. Pharm. 77;1991:169–175.

[23] Yamaguchi K, Anderson J. Biocompatibility studies of naltrexone sustained release formulations. J. Control. Release 19;1992:299–314.

[24] Romeis, Mikroskopische Technik. Bück P, editor. Wien: Urban and Schwarzenbeck, 1989:235–236.

[25] Romeis, Mikroskopische Technik. Bück P, editor. Wien: Urban and Schwarzenbeck, 1989:498–499.

[26] Yamaguchi K, Anderson J. In vivo biocompatibility studies of medisorb 65/35 D,L-lactide/glycolide copolymer microspheres. J. Control. Release 24;1993:81–93.

[27] Anderson J. In vivo biocompatibility of implantable delivery systems and biomaterials. Eur. J. Pharm. Biopharm. 40;1994:1–8.

[28] Engelberg I, Kohn J. Physico-mechanical properties of degradable polymers used in medical applications: A comparative study. Biomaterials 12;1991:292–304.

[29] Visscher G, Robison R, Maulding H, Fong J, Pearson J, Argentieri G. Note: Biodegradation of and tissue reaction to poly(DL-lactide-co-glycolide) microcapsules. J. Biomed. Mater. Res. 20;1986:667–676.

[30] Williams D, III. Biodegradability and toxicity of polymers as adjuvants for parenteral drug delivery systems. Control. Drug Delivery 1984:49–55.

[31] Buchhorn G, Willert G. In: Williams DF, editor. Biocompatiblity of orthopaedic implant materials, Vol.I. Boca Raton: CRC Press, 1982:249.

[32] Schakenraad J. et al. In vivo and in vitro degradation of glycine/DL-lactic acid copolymers. J. Biomed. Mat. Res. 23;1989:1271–1288.

[33] Anderson J, Niven H, Pelagalli J, Olanoff L, Jones R. The role of the fibrous capsule in the function of implanted drug-polymer sustained release systems. J. Biomed. Mater. Res. 15;1981:889–902.

[34] Ziats N, Miller K, Anderson J. In vitro and in vivo interactions of cells with biomaterials. Biomaterials 9;1988:5–13.

[35] Behling C, Spector M. Quantitative characterization of cells at the interface of long-term implants of selected polymers. J. Biomed. Mater. Res. 20;1986:653–666.

[36] Papadimitriou J, Robertson T, Walters M. An analysis of the phagocytic potential of mutinucleate foreign body giant cells. Am. J. Pathol. 78;1975:343–355.

[37] Postlewaite A, Kang A. Fibroblasts, In: Gallin J, Goldstein I, Snyderman R, editors. Inflammation: Basic principles and Clinical Correlates. New York: Raven Press, 1988.