Conclusions. Important parameters and processes such as edema, deformation of the tablet, microviscosity inside the tablet and encapsulation can be monitored in real time by the combined use of the noninvasive techniques MRI and EPR leading to better understanding of the differences between the in vitro and in vivo situation.

KEY WORDS: biodegradable polymers; EPR; MRI; drug delivery; in vitro/in vivo correlation.

INTRODUCTION

Although biodegradable polymers are now used clinically for the treatment of various diseases, there is only limited knowledge about the processes involved in the degradation and erosion of implants in vivo, as well as the in vivo mechanisms of drug release. A critical point in the design of delivery devices is understanding the mechanisms of polymer degradation and drug delivery and whether there are any differences between these processes in vivo and in vitro. To answer this question, appropriate techniques are needed which can monitor the key processes and key parameters of drug release and polymer erosion in vivo in real time, without disturbing the sample. Unfortunately, the most frequently applied analytical methods such as chromatography, microscopy, calorimetry or infrared spectroscopy require surgical extraction and do not meet these criteria. Radioactive labeled molecules as model drugs provide only information concerning the concentration of the labeled compound and require a special synthesis.

The noninvasive method of Magnetic Resonance Imaging (MRI) is widely used clinically as a diagnostic tool. MRI monitors the local concentration and the physical state of protons. Therefore, MRI can potentially be used to characterize the penetration of water into the polymer implant, which is a key event in the release of the drug and degradation of the polymer. It has been shown in vitro that MRI can be applied to follow the hydration process of tablets and to characterize the state of water in terms of proton relaxation times or self diffusion coefficients (3,4). MRI has been also been used in vivo to detect the presence of a biodegradable implant in the monkey brain (5) and to follow the release of gadolinium compounds from implants (6,7). Recently it has been demonstrated that MRI may also be used to characterize the biodegradable drug delivery system itself in vivo (8).

EPR spectroscopy is a noninvasive analytical technique which can provide unique information in pharmacy (9). Recently, low frequency EPR spectrometers have been developed which make studies in vivo feasible (10,11). The sensitivity of the equipment is sufficient to detect drug-derived free radicals directly in vivo and to study the impact of antioxidants on their formation (12). Unique information can be obtained by EPR in the field of drug delivery (13,14) using nitroxide radicals as model drugs. The EPR spectra are sensitive to the nitroxide mobility as illustrated in Fig. 1. In addition to the measurement of nitroxide mobility, pH measurements inside the delivery systems are possible using specially designed nitroxides. A polymer degradation induced pH drop from 4 to 2 inside subcutaneously implanted PLGA-tablets has been reported recently (15). It has been demonstrated that MRI and EPR can give complementary information regarding the processes of polymer erosion and drug release (8). However, the MRI and EPR experi-
In Vitro Studies

The tablets were placed in 20 ml 0.1 M phosphate buffer, pH 7.4 at 37°C. The buffer was changed daily. The polymer samples were removed and handled without mechanical damage. For EPR measurements, the tablet were rinsed carefully with fresh buffer solution to remove nitroxide molecules from the surface. The water film at the surface was removed by blotting carefully with an absorbent paper. The tablets were put in airtight plastic boxes during the measurements to avoid drying. In the case of the in vitro MRI evaluation of P(FAD-SA) 50:50 it was necessary to put the tablets in a hydrogel (Aerosil in water 10% m/m), because otherwise no image contrast was obtained by the tablet itself. All studies were carried out in triplicate.

In Vivo Studies

The in vivo studies were performed according to an approved protocol and adhered to the principles of laboratory animal care (NIH publication #85-23, revised 1985). PCA loaded tablets were implanted subcutaneously into the neck (dorsal side) of anesthetized (Ketamine/Xylazine 100/20 mg/kg) male Wistar rats (200–300 g). EPR and MRI measurements were performed on the same anesthetized animals. Each group had three animals.

EPR-Spectroscopy

EPR spectra were recorded using a home built 1.1 GHz spectrometer equipped with a surface coil using the following parameters: central magnetic field B0 = 42 mT, scan range = 10 mT, incident microwave power = 40 mW, amplitude of modulation = 0.1 mT, scan time = 300 s, time constant = 0.3 sec.

Magnetic Resonance Imaging

MR imaging was performed with a 7.0 T horizontal bore Magnex magnet and a SMIS system. The rats were anesthetized and placed supine over a home designed “butterfly” shaped RF surface coil. Axial spin-echo T1 and T2 weighted sequences (T1: repetition time = 500 ms, echo time = 16 ms; T2: repetition time = 1800 ms, echo time = 50 ms) were used. Other imaging parameters were 4 acquisitions, number of slices = 7, slice thickness = 2mm, field of view = 40 mm, and image matrix = 128 x 128. The signal intensity of muscle tissue was used as an internal standard to quantify the signal intensity of the tablet core.

RESULTS

Magnetic Resonance Imaging

In Vivo

No MRI image was attainable with dry tablets. MRI Signal enhancement from outside to inside as well as a decrease of the tablet thickness was observed for P(FAD-SA) 20:80 after exposure to 0.1M phosphate buffer (Fig. 2, top). In contrast, exposure to buffer did not result in MRI contrast enhancement for P(FAD-SA) 50:50 tablets. To be able to obtain MRI images under these circumstances we placed the P(FAD-SA) tablets in

MATERIALS AND METHODS

Chemicals

The spin probe PCA (3-Carboxy-2,2,5,5-tetramethylpyrroloidine-1-oxyl) was purchased from Aldrich Chemicals. Poly(unsaturated acid dimer—sebacic acid) (P(FAD-SA)) polymers were synthesized as previously described (17). The fatty acid dimer (FAD) 13,14-diocetyl-octacosane-1,28-dioic acid is a dimer derivative of erucic acid. Hard fat (Witepsol® H15) was obtained from Hüls GmbH, Germany and Methocell from FLUKA AG, Switzerland.

Sample Preparation

PCA-loaded tablets (3 mmol/kg polymer) tablets of 8.6 mm in diameter and 2 mm thickness were prepared by melt molding (90°C, 2 min) in a home made teflon device. The tablets were allowed to cool down slowly for 30 minutes at room temperature. Thereafter, the samples were stored under nitrogen atmosphere for 5 days at −15°C.