 DOI: 10.1002/masy.200750860

Polyurethane-Based Extended Release Form of Piroxicam

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Summary: The anti-inflammatory effect of piroxicam (Px) immobilized on a polyurethane (PU) matrix was studied *in vivo* by measuring levels of t-methylimidazole acetic acid (t-MeImAA) in urine of experimental animals. Released into body from the polymeric matrix, Px affected inflammation process, significantly reducing the t-MeImAA levels. The retention of Px within the PU/drug system – largely due to physical modification of the polymer – resulted in durable therapeutic action of the system.

Keywords: high performance liquid chromatography (HPLC); inflammation; piroxicam; polyurethanes; t-methylimidazole acetic acid

Introduction

Biocompatibility and physicochemical properties of polyurethanes have long been drawing attention of developers of composite materials.

Among the variety of their applications, polyurethanes have also performed well as drug carriers, especially when continuous drug delivery into a body was required. Such extended-release, or depot, forms are essentially a polymeric base (matrix) into which low-molecular bioactive substances are introduced. Besides the extended drug delivery, advantages of the depot forms also include reduced side effects.

Due to the higher structural heterogeneity and contact area of drug-filled polyurethane (PU) composites vs. non-filled ones, the former are known to degrade much faster both *in vitro* and *in vivo*.[1] An increase in the wetting ability of a PU matrix, resulting from its modification with drug, also has a significant effect on the polymeric destruction progress and the pattern of drug release from PU depot form.

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Earlier works[2–4] described the synthesis of biocompatible PU composites, showed how the drug release process depended on the drug nature and the PU matrix structure and properties, and demonstrated that a drug did maintain its activity when incorporated in a PU composite.

The present work aimed to study the effect of piroxicam – a nonsteroidal anti-inflammatory drug – immobilized on a PU matrix on level of t-methylimidazole acetic acid (t-MeImAA), the end metabolite of histamine, in urea of rats with an inflammatory process. According to ^{14}C histamine analysis, t-MeImAA is accumulated in urea.[5]

As a cyclooxygenase inhibitor, piroxicam blocks the synthesis of histamine, as well as prostaglandins, heparin, and kinins – biologically active substances that are involved in inflammation induction and are inflammation mediators.[6]

Materials and Methods

Piroxicam (Px) was obtained from Sopharma (Bulgaria); t-Methylimidazole acetic acid (t-MeImAA) and cellulose phosphate, from Sigma-Aldrich (Germany); isopropanol, acetyl chloride, dichloromethane, diethyl ether, and triethylamine, from Merck,

(Germany); methanol, from Lab-Scan (Germany); and sodium dodecyl sulfate (SDS), from Fluka (Germany).

The PU matrix was a macrodiisocyanate synthesized from oligoglycol (Mw 2,002) and toluylene diisocyanate (a mixture of 2,4 and 2,6 isomers, 80:20). Sponge-like PU/Px composites were produced by adding Px to the prepolymer at 3 percent by weight, 2,4,6-tris(dimethylamino)methane as catalyst, and distilled water.

Acute inflammation in experimental rats was induced by injecting subcutaneously into an animal's left hind leg a phlogistic substance that provoked edema. The injection procedure was done in aseptic conditions using a fine needle. The progress of the inflammatory process was monitored by measuring the edema volume, as described earlier.[6]

Twenty-five white non-purebred male rats, weighting 180–250 g each, were used in the experiment. The animals were divided into five equal groups: a control group of intact animals and four groups with inflammation induced through injection of the phlogistic substance – 0.1 mL of 6.0% aqueous dextran solution. One of these four groups received no other treatment; another one received a therapeutic dose of Px through tube, and the other two were treated as follows: subcutaneous implantation of PU matrix with or without Px plus injection of dextran solution once a day for five days. Table 1 summarizes treatment data for the four groups (2 to 5).

Urine from the Animals was Collected in Tubes and Kept on Ice Until HPLC Analysis.

t-MeImAA determination in biological samples was done in three steps – sample preparation, t-MeImAA extraction, and reversed-phase high performance liquid chromatography – as described earlier.[6]

HPLC Analysis

The HPLC analysis was carried out at ambient temperature using chromatographic system LKB (Sweden) including controller 2152, UV detector 2151, λ 214 nm, pump 2150, and recorder 2210. Monolithic column Chromolith RP-18 (Merck, Germany), 100×4.6 mm ID, with bimodal pores was used for analytic separation.

The mobile phase's aqueous part contained sodium dodecyl sulfate (3.0 g/L); 800 μL triethylamine was added to 1 L of this solution, and pH 3.35 was adjusted with 0.5% solution of H_3PO_4.[7] The aqueous part was mixed with methanol (65:35 v/v), and the mobile phase was filtered and degassed.

The flow rate was 1.0 mL/min, and sample volume was 10.0 μL. t-MeImAA detectability threshold was 0.01 μg/mL. The measurement consistency was checked by doing three to five injections of each sample. The external standard method was used to calculate results. The relative standard deviation (RSD) was 2.29% for the t-MeImAA standard and 3.37% for t-MeImAA in samples.

Table 1.
Treatment of experimental animals.

Group 2	Group 3	Group 4	Group 5
Injection of 0.1 mL 6% dextran solution	Injection of 0.1 mL 6% dextran solution Introduction of 0.28 mg/kg Px (therapeutic dose) through tube	Injection of 0.1 mL 6% dextran solution Subcutaneous implantation of PU matrix w/o Px into abdominal region under ethanol anesthetic (1.5–2.0 g/kg) Injection of 0.1 mL 6% dextran solution once a day for five days	Injection of 0.1 mL 6% dextran solution Subcutaneous implantation of PU matrix with Px into abdominal region under ethanol anesthetic (1.5–2.0 g/kg) Injection of 0.1 mL 6% dextran solution once a day for five days

Results and Discussion

Earlier studies[1,2,4] with disulphiram, cephasolin, and naltrexone as filler drugs showed that the drug immobilization on polymeric matrix did not change the polymer's chemical structure but led to formation of hydrogen bonds between functional groups of the drug and reactive groups of the polymer. It was also shown that this physical polymer modification was the main factor of drug retention within the PU/drug system; the drugs were released into body through diffusion and polymer surface hydrolysis. It will be safe to assume that the same is the case with Px, too.

Figures 1 and 2 show typical chromatograms of urea samples from animal groups 2 and 3, which had developed dextran-induced inflammation edemas. After the edemas became visible, group 3 (Figure 2) received a therapeutic dose of Px (0.28 mg/kg). Comparison of the two chromatograms demonstrates a 50 percent decrease in the t-MeImAA peak height of group 3 (retention time 11.81 ± 0.35 min; $n = 5$) against group 2 (retention time 12.47 ± 0.17 min; $n = 5$).

The *in vivo* experiments established that the t-MeImAA level in rats with inflammation was higher than that in intact animals (0.04 ± 0.005 mg/mL vs. 0.02 ± 0.004 mg/mL, $n = 5$) (Figure 3). This consists with works

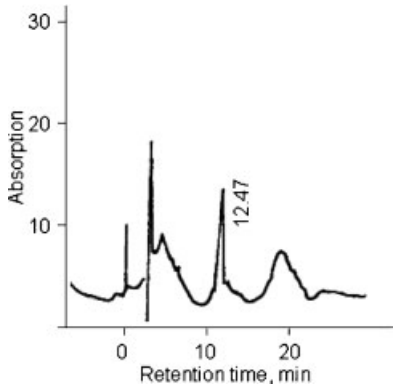

Figure 1.
A typical chromatogram of urine samples from rats with inflammation (6% dextran solution). t-MeImAA retention time 12.47 min; column Chromolith RP-18; λ 214 nm; room temperature.

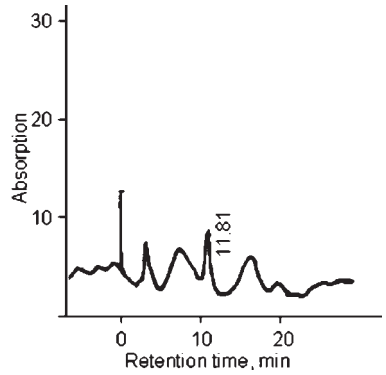

Figure 2.
A typical chromatogram of urine samples from rats with inflammation (6% dextran solution) that received a therapeutic dose of Px (0.28 mg/kg). t-MeImAA retention time 11.81 min; column Chromolith RP-18; λ 214 nm; room temperature.

describing an increase in t-MeImAA excretion in case of inflammation[7–9] and certain diseases (e.g., mastocytosis, atopic dermatitis, and asthma).[10,11] What is interesting, the forth group of animals, which was treated with dextran solution and implantation of a PU sponge *without* Px, had the same t-MeImAA level as the control, intact, group (0.02 ± 0.003, $n = 3$).

The anti-inflammatory effect of Px-containing PU composites was studied by comparing it with that of the drug's conventional dosage form. The change in the t-MeImAA level in murine urine measured in five days after implantation was used as an indicator of Px release from the depot form. This time span was chosen taking into account findings of an earlier study on similar PU depot forms with Antaxon®,[2] which had shown that the drug was actively released for five days with a sharp slowdown in the process thereafter.

As can be seen on Figure 3, the t-MeImAA level in the animals with PU/Px implants declined to 0.004 ± 0.001 mg/mL ($n = 3$), and in the animals that received the drug through tube, to 0.01 ± 0.002 mg/mL ($n = 3$). This is by 90 percent and 75 percent, respectively, lower then the t-MeImAA level detected in five days in the rats with inflammation that had not been treated

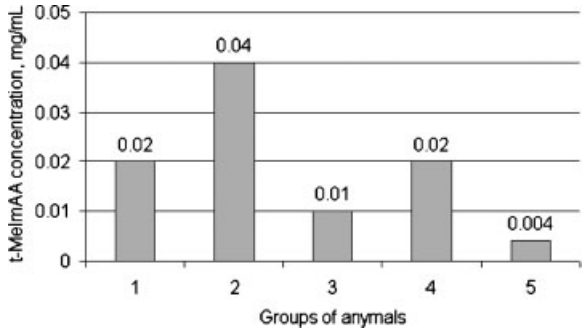

Figure 3.
t-MeImAA concentration in different groups of animals: (1) intact animals (control); (2) animals with inflammation; (3) animals with inflammation + Px (therapeutic dose through tube); (4) animals with inflammation + PU matrix without Px; (5) animals with inflammation + PU matrix with Px.

with Px in any form. It can be presumed that the introduction of Px suppressed manifestations of the inflammation process (redness, edema, dysfunction of injured organ, etc.) through painkilling, resulting in reduced levels of histamine and, accordingly, its end metabolite – t-MeImAA.

Two interesting facts draw attention on Figure 3. First, the t-MeImAA level in rats treated with Px, both in the drug's conventional dosage form and immobilized on a PU matrix, dropped well below that in intact animals – two to five times, respectively. Second, the implantation of a PU matrix alone, without Px, resulted in reduction in the t-MeImAA concentration down to the level in intact animals.

Since the objective of the experiments was to study the effect of the PU/Px system on t-MeImAA level, these facts were not investigated specially. A possible explanation for the drastic t-MeImAA decrease in animals treated with Px can be that the therapeutic dose of the drug for rats had been calculated based on that for humans in proportion with the body weight. Although satisfactory to the requirements of the study, that might be incorrect from the medical point of view. If so, neither the appearance nor the behavior of the animals thus treated showed any signs of harm done by the possible overdose.

As for the "healing effect" of PU matrix alone, the reduction in the t-MeImAA concentration was most probably organism's response to synthetic polymeric implant. The same was observed repeatedly earlier, and is well described.[1]

Conclusions

The retention of Px within the PU/drug system – largely due to physical modification of the polymer – results in durable therapeutic action of the system.

Released into body from the polymeric matrix, Px affects inflammation process. This was demonstrated by the significantly reduced levels of t-MeImAA in urea of experimental animals with inflammation and implanted PU/Px composite.

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