In Vivo Evaluation of a Colon-Specific Drug Delivery System: An Absorption Study of Theophylline from Capsules Coated with Azo Polymers in Rats

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Azo polymers based upon 2-hydroxyethyl methacrylate, methyl methacrylate, and methacrylic acid, and containing N,N'-bis[(methacryloyloxyethyl)oxy(carbonylamino)]azobenzene as azo aromatic agent were evaluated in vivo as coatings for colon-specific drug delivery. The gastrointestinal absorption of theophylline from capsules coated with the azo polymers was examined in the proximal part of the small intestine and the cecum of male Wistar rats. The capsules were surgically inserted in the region of interest. The plasma concentration of the drug was higher when the capsules were inserted in the cecum as compared to the small intestine. The appearance of theophylline in the plasma when capsules were administered in the small intestine can be attributed to simple diffusion of the drug through the swollen polymer coating. Release and absorption from the cecum is the combined result of diffusion and degradation of the azo polymer coatings by bacterial azo reductase.

KEY WORDS: theophylline; azo polymers; colon-specific drug delivery; biodegradable coatings.

INTRODUCTION

During the last decade, different colon-specific drug delivery systems based on pH-sensitive polymers (1,2), bacterial degradable polymers (3-7), or prodrugs (8-11) have been investigated. In previous papers, we reported the bacterial degradation of azo polymers in vitro (12-13). In this paper, the biodegradation of the azo polymers in vivo is presented. In order to study the biodegradation, experiments were performed in male Wistar rats because of the similarity with human intestinal microflora. The predominant species in the rat colon are Bifidobacteria, Bacteroides, and Lactobacilli. Rats are coprophagic and have a large number of bacteria in the whole gastrointestinal tract. Coprophagy was minimized by using cages with bottom screens having openings larger than the feces size. Because of the size of the animals, oral administration of non-disintegrating solid dosage forms is difficult. Therefore, capsules were surgically inserted directly into the region of interest, instead of peroral administration.

The degradation of the polymers was studied by following the plasma concentration of theophylline from capsules coated with the azo polymers. Theophylline was used as the model drug because of its good absorption from the large intestine in humans (14), and the availability of a sensitive assay by high performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Chemicals

Theophylline and diprophylline (internal standard) were from Boehringer (Ingelheim, Germany), and were of pharmacopoea quality. All other reagents and organic solvents were of analytical or HPLC grade. The water used for HPLC was purified with a Milli-Q system (Millipore, Brussels, Belgium).

The composition of the azo polymers and their degree of swelling is given in Table 1. S1 is a copolymer of 2-hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA), and P11 and P12 are terpolymers of HEMA, MMA, and methacrylic acid (MA). N,N'-bis[(methacryloyloxyethyl)oxy(carbonylamino)]azobenzene (B(MOECA)AB) was the incorporated azo compound. The purity of the monomers was more than 99%. The synthesis of the azo polymers is described elsewhere (12,13,15).

Animal studies

Male Wistar rats (250–300 g) were fasted for 16 hours with free access to water before experimentation. Anesthesia was induced with intraperitoneal sodium pentobarbital (Nembutal®, 60 mg/kg).

The intravenous solution of theophylline was injected by means of a catheter into the vena femoralis. Theophylline capsules, both uncoated and coated with azo polymers, were directly inserted into the proximal part of the small intestine or cecum of anesthetized rats. A small incision was made in the region of interest, and the capsule was inserted distal to the incision. The intestine was then carefully ligated with polyester suture before and after the incision.

To study the absorption of theophylline from capsules in the small intestine, a ligature was also made 15 cm distal to the incision to prevent the capsule from moving into the cecum. To examine the absorption from the cecum, an additional ligature was made at the end of the cecum to keep the capsule in the cecum. Care was taken not to interrupt the mesenteric blood flow. The rats were placed in a restraining box for the whole duration of the experiment.

Blood was sampled from the vena jugularis by means of a catheter. Plasma was harvested by centrifugation at 15000 rpm for 6 min. and stored at −40°C until assayed by HPLC. At the end of the experiment, the rats were killed.

Preparation of capsules

Mini capsules (Nr.9, Elanco Lilly) were filled with a mixture of theophylline and lactose (10:40). The capsules were coated by dipping them in an alcoholic solution (20% w/w) of the azo polymers. The amount of polymer coating on the capsules was approximately 6mg/cm².

No plasticizer was used in the coating formulation. The coated capsules were microscopically examined for cracking.

Table I. Composition of Azo Polymers and Their Swelling Index (Is%) as a Function of pH

<table>
<thead>
<tr>
<th>Polymer</th>
<th>HEMA:MMA:MA (w/w/w)</th>
<th>Azo compound*</th>
<th>Is%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>pH 6</td>
</tr>
<tr>
<td>S1</td>
<td>6:1:0</td>
<td>B(MOECA)AB</td>
<td>36</td>
</tr>
<tr>
<td>P11</td>
<td>4:1:0.05</td>
<td>B(MOECA)AB</td>
<td>26</td>
</tr>
<tr>
<td>P12</td>
<td>9:2:0.10</td>
<td>B(MOECA)AB</td>
<td>29</td>
</tr>
</tbody>
</table>

* 0.7 mol % of B(MOECA)AB was added to the feed monomer mixture. The swelling index (Is%) was calculated as follows: Is% = 100 * (Ws - Wd)/Wd; where Wd is the dry polymer weight, and Ws is the swollen polymer weight after uptake of water (15).

and homogeneity before use. Due to the presence of azo bonds in the polymers, the coatings are orange-colored.

Analysis of theophylline

Determination of theophylline in rat plasma was based upon the method described by Augustijns and Verbeke (1992) (16).

Isocratic HPLC was performed using a LiChroGraph L-6000 HPLC pump (Merck-Hitachi, Darmstadt, Germany); a Rheodyne Model 7125 Syringe Loading Sample Injector (Rheodyne Inc., Cotati, CA, USA) equipped with a 20 µl loop; a LiChroGraph L-4000 UV detector (Merck-Hitachi, Darmstadt, Germany), set at 272 nm; and a Merck-Hitachi Model D-2500 Chromato-Integrator (Darmstadt, Germany). The 24.4 x 0.4cm column was packed with LiChrospher 100 RP-18 (5µm) (Merck, Darmstadt, Germany). A guard column (0.4 x 0.4 cm) with the same packing material was used to protect the analytical column. The mobile phase, which consisted of potassium dihydrogen phosphate solution (0.01 M): methanol: acetonitrile (900:200:13; v/v/v), was filtered through a nylon membrane filter (0.45µm) and degassed by ultrasonication before use. The flow rate was 1.0 ml/min.

The relationship between peak area ratio and theophylline concentration was found to be linear (r > 0.999) in the concentration range 0.25–20 µg/ml. The detection limit for theophylline was 0.25 µg/ml. The relative standard deviation of both the intra- and interday variability was 7% or less. The recovery of theophylline from rat plasma was between 91.9 and 98.6% in the concentration range from 0.71–19.60 µg/ml.

Bioavailability of theophylline

In order to calculate the bioavailability of theophylline from uncoated capsules in the cecum and the small intestine of rats, and to examine the suitability of this drug for testing a colonic delivery system in vivo, plasma concentrations were followed until a value was reached which was less than 10% of the maximal plasma concentration.

The area under the plasma concentration vs time curve (AUC) of each rat was calculated using Topfit (version 2.0) data analysis system (17). The mean bioavailability parameter F for the uncoated theophylline capsules in the cecum and the small intestine was calculated as follows:

\[
F = \frac{\left( \sum \frac{\text{AUC}_{\text{test}}}{\text{DOSE}_{\text{test}}} \right) \times \frac{1}{n_{\text{test}}}}{\left( \sum \frac{\text{AUC}_{\text{i.v.}}}{\text{DOSE}_{\text{i.v.}}} \right) \times \frac{1}{n_{\text{i.v.}}}} \times 100
\]

where \(\text{AUC}_{\text{test}}, \text{DOSE}_{\text{test}}, n_{\text{test}}, \text{AUC}_{\text{i.v.}}, \text{DOSE}_{\text{i.v.}}, n_{\text{i.v.}}\) are AUC, dose and number of rats used for administration in the cecum or small intestine and intravenous administration, respectively. Comparisons were made with Student’s T-test, and differences were considered to be significant when \(p < 0.05\).

RESULTS AND DISCUSSION

Absorption of theophylline from uncoated capsules in the cecum and small intestine.

The results of the bioavailability studies are summarized in Table 2. A linear relation was found between AUC and dose, indicating linear kinetics in the examined concentration range. The mean bioavailability of theophylline from a capsule in the small intestine was 87%, but the difference in \(\Sigma\text{AUC/dose}\)^*1/n for intravenous and small intestinal administration was only marginally significant (\(p = 0.05\)). The mean maximal plasma concentration was reached after 1.4h (±0.5).

The bioavailability in the rat cecum was found to be

Table II. Bioavailability of Theophylline from Uncoated Capsules in the Small Intestine and Cecum of Male Wistar Rats

<table>
<thead>
<tr>
<th>Method</th>
<th>AUC/DOSE (%)</th>
<th>Correlation</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous administration</td>
<td>7.9 (±0.8)</td>
<td>0.979</td>
<td></td>
</tr>
<tr>
<td>Small intestinal administration of uncoated theophylline capsule</td>
<td>6.8 (±0.8)</td>
<td>F = 87%</td>
<td></td>
</tr>
<tr>
<td>Cecal administration of uncoated theophylline capsule</td>
<td>6.2 (±0.4)</td>
<td>F = .79%</td>
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