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(MOEOCA)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 anesthesized 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 ligation 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 ligation 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/cm^2.

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

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

Polymer	HEMA:MMA:MA (w/w/w)	Azo compound*	Is% pH 6	Is% pH 7	Is% pH 8
S1	6:1:0	B(MOEOCA)AB	36	36	35
P11	4:1:0.05	B(MOEOCA)AB	26	47	67
P12	9:2:0.10	B(MOEOCA)AB	29	64	100

* 0.7 mol % of B(MOEOCA)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 × 0.4cm column was packed with LiChrospher 100 RP-18 (5 µm) (Merck, Darmstadt, Germany). A guard column (0.4 × 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 theofylline 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:

$$F = \frac{\left(\sum \frac{AUC_{test}}{DOSE_{test}}\right) * \frac{1}{n_{test}}}{\left(\sum \frac{AUC_{iv}}{DOSE_{iv}}\right) * \frac{1}{n_{iv}}} * 100$$

where AUC_{test}, $DOSE_{test}$, n_{test}, and AUC_{iv}, $DOSE_{iv}$, n_{iv} are AUC, dose and number of rats used following 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 (ΣAUC/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

Intravenous administration
ΣAUC/6 DOSE = 7.9 (±0.8) Correlation between DOSE and AUC = 0.979
Small intestinal administration of uncoated theophylline capsule
ΣAUC/6 DOSE = 6.8 (±0.8) F = 87%
Cecal administration of uncoated theophylline capsule
ΣAUC/5 DOSE = 6.2 (±0.4) F = .79%

79%, and the difference in (ΣAUC/dose)*1/n for intravenous and cecal administration was statistically very significant ($p < 0.003$). A prolonged absorption time was observed in the cecum since the mean maximal plasma concentration was reached after 5.3h (±0.8). These results show that theophylline is a suitable model drug for testing a colonic delivery system. In our experimental setup, it exhibits good uptake from the cecum.

Absorption of theophylline from coated capsules in the small intestine.

Theophylline plasma concentrations remained low (Figure 1), although rats are coprophagic and hence contain bacteria in their small intestine. No sign of biodegradation of the polymers was evident. Colour changes (decrease in chromophores as a result of azo reduction) of the coatings were not seen at the end of the experiment, as expected because the bacterial concentration in the proximal part of the small intestine is considerably lower than in the colon (18).

The appearance of the drug in the plasma can be attributed to simple diffusion through the polymer coating. The increase in theophylline plasma concentration is uniform for the duration of the experiment, but a statistically significant difference ($p < .001$; ANOVA) can be detected in the time of appearance of the drug in the plasma. When the capsules were coated with P12, theophylline appears on average after 4.4h (±1.5) in the plasma, whereas 9.0h (±1.7) and 18.3h (±4.6) are necessary to detect the drug when P11 and S1 are used, respectively. The difference between the average dose in the three groups, and the average amount of coating on the capsules in the three groups were not statistically significant (ANOVA, $p > 0.05$).

The difference between the three groups is attributable to the polymer composition (Table 1). The results of the *in vivo* experiments concur with the data of the swelling and permeability experiments (15). In the environment of the small intestine (pH 7–8) (19) of the rat, the carboxylic acid groups of P11 and P12 are ionized, and the polymers will be more swollen than S1. This explains why the concentration of theophylline in the plasma is the highest when using P12 as a coating.

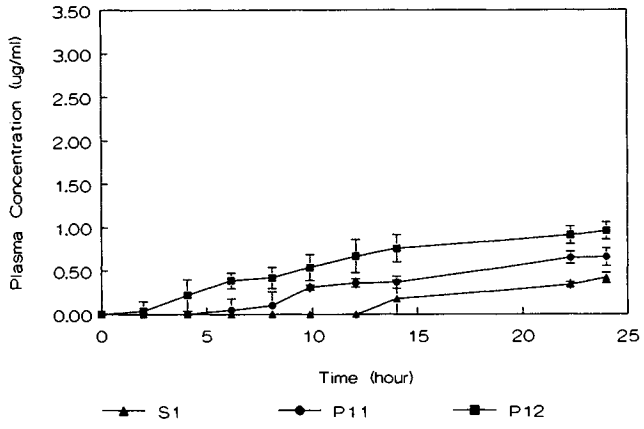

Figure 1: Theophylline plasma concentration after small intestinal administration of capsules coated with S1, P11, and P12. Error bars indicate SD, n = 4–6, except for S1 after 8.1h and 9.9h, where n = 2.

Absorption of theophylline from coated capsules in the cecum

Figure 2 shows the results of theophylline absorption in the cecum. The bioavailability of theophylline from these dosage forms could not be calculated since the experiment had to be stopped after 27 to 28 hours for ethical reasons. During this period, maximal plasma concentration (C_{max}) was not always reached, or insufficient data were collected in the time period after C_{max}. However, one can easily see that the obtained plasma concentrations were much higher than those in the small intestine (compare with Figure 1). This difference is attributable to the bacterial degradation of the azo polymer coatings. Anaerobic azo bond cleavage probably represents a non-enzymatic reduction by enzymatically generated reduced flavins. The flavins act then as an electron carrier from NADPH-dependent flavoproteins to the azo bond (20). Visual inspection of the coatings at the end of the experiment showed pale yellow coatings due to azo reduction (except for one animal).

A comparison of the results of the three groups (S1, P11, and P12) indicates that in the group of S1, none of the animals reach C_{max} before the end of the experiment. Surprisingly, in one rat no evidence of bacterial degradation of the azo coating could be detected. The colour change of the coating due to azo reduction could not be noticed. In the group of P11, one rat reaches C_{max} after 23h, three rats after approximately 25h, and two rats at the end of the experiment, i.e. approximately 27h. When P12 is used as the coating material, four rats reach C_{max} after approximately 23h, and two rats at the end of the experiment.

From these data, it can be concluded that there is a difference between rats treated with S1 and rats treated with P11 or P12. In the group of S1, the onset of azo degradation does not seem to start before approximately 16 hours. On the other hand, the difference between animals in the same group is considerable. It must be noted that the difference between the average dose in the three groups, and the average amount of coating on the capsules in the three groups were not statistically significant (ANOVA, $p > 0.05$).

The difference in the onset of azo degradation can be explained by the higher degree of swelling of P12 and P11 as

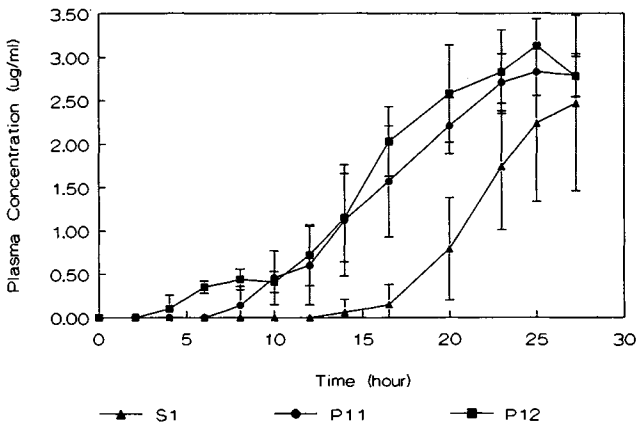

Figure 2: Theophylline plasma concentration after cecal administration of capsules coated with S1, P11, and P12. Error bars indicate SD, n = 5–6, except for P12 after 25h, where n = 3.

compared to S1. Measurement of the pH in the cecum at the end of the experiment showed a mean value of 6.9 (±0.2; n = 6), which concurs with the data from Ward and Coates (1987) (19). At this pH, The accessibility of azo bonds in S1 is lower than in P11 or P12.

Although the results of *in vitro* release experiments with ibuprofen in sonicated rat cecal content (cell-free extract) (21) showed a significantly faster release of the model drug from capsules coated with P12 than with P11, *in vivo*, the use of P12 does not seem to yield any benefit. From the obtained results, it cannot be inferred that the onset of azo degradation starts earlier for P12 than for P11. This may be surprising since, at the observed pH, P12 exhibits a higher degree of swelling, and hence a higher accessibility to bacterial azo reductase activity would be expected.

The results of the *in vivo* experiments with P11 and P12 show that at least 10 to 12 hours are needed before the azo coatings start to degrade, and it takes at least 23 to 25 hours to reach C_{max}. In our experimental set-up, the delivery system is kept artificially in the cecum, where the conditions for absorption are still favourable. However, under therapeutic conditions it is unlikely that the dosage form remains in the same region of the colon for more than 24 hours, and the conditions for drug absorption will worsen when passing from the proximal to the distal part of the colon. The degradation of the coatings is slow, and the intersubject variation is large. Therefore, these colonic dosage forms are still insufficient to deliver peptides, hormones, or other drugs with a narrow therapeutic concentration range systemically. However, to exert a local action in the colon, these azo polymers may prove useful.

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