Biodistribution of [^{131}I]-5-Aminosalicylic acid loaded chitosan-Ca-alginate microparticles after peroral application to Wistar rats with TNBS colitis

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Introduction

5-ASA is an anti-inflammatory drug commonly used in the treatment of inflammatory bowel diseases and may provide protection against the development of colorectal cancer in these patients (Bernstein, 2002). It inhibits activation of nuclear factor-κB (NF-κB), which is a central regulatory transcription factor included in initiating and development of inflammatory processes (Bantel et al., 2000). However, 5-ASA is rapidly absorbed from the small intestine, and there is little localization of 5-ASA in the colon relative to the small intestine (Zhoy et al., 1999). This has motivated the development of controlled release preparations specifically designed to minimize systemic absorption and to achieve optimum delivery of the biologically active 5-ASA moiety to the distal small intestine and colon. Thus, relatively high concentrations of free 5-ASA can be achieved in the intestinal lumen without producing systemic exposure and subsequent toxicity. This type of target tissue selectivity is a desirable feature for chemopreventive agents and is a significant advantage when considering drug delivery methods to the mucosal surface of the gut. We have designed a new microparticulated system consisting of a chitosan-Ca-alginate matrix with bioadhesive, biodegradable, pH-dependent swelling and dissolution properties. The aim of the work was to test the biodistribution of 5-ASA contained in this new microparticulated system after peroral administration in rats in which colonic inflammation was induced.

Materials and methods

Chemicals

Sodium alginate (LF 10/60, fG 65-75%, viscosity, 20-70 *mPas* for a 1% *w/v* solution), Protanal FMC BioPolymers, Norway; chitosan (150 KDa, $[R^2_G]^{1/2}$ 44±5 nm, viscosity 20-100 *mPas* for a 1% *w/w* solution in acetic acid, 1% *w/w*, minimum 85% deacetylated), France Chitine, France; 5-ASA, Fluka Chemie AG, Switzerland. 2,4,6-trinitrobenzenesulphonic acid, TNBS, sodium salt (Sigma-Aldrich, Inc., Steinheim, Germany). All other reagents were of analytical grade.

Preparation of 5-ASA loaded chitosan-Ca-alginate microparticles

Aqueous dispersion of alginate (3% *w/w*) and 5-ASA (0.5% *w/w*) adjusted to pH 7.0 by 0.2 M NaOH was infused into a spray dryer nozzle unit (Buchi Mini Spray Dryer B-191, Switzerland) and sprayed into solution of chitosan (0.25% *w/w*) and $CaCl_2$ (2.5% w/v) in 1% *w/w* acetic acid under defined conditions (nozzle diameter 0.7 mm, aspirator pressure 100, atomizer pressure 600 Nih^{-1}, flow rate 10 ml, inlet temperature 140^{0}C, outlet temperature 100^{0}C). The microparticles were cured for 24 h, separated and freeze-dried. ^{1}H-NMR, FTIR, DSC and X-ray studies were carried out in order to determine 5-ASA stability during microencapsulation and drug-polymers interactions.

Drug release studies

The drug release studies were carried out in sealed glass vials at 37^{0}C and 75 horizontal strikes/min To compare the drug release under different pH conditions, the experiment were performed in pH

1.2 (fasted stomach) and pH 6.8 (mid jejunum). To simulate the passage through the stomach and the small intestine, all series were additionally tested with a pH gradient method: stomach-120 min (pH 1.2), duodenum-10 min (pH 6), jejunum-120 min (pH 6.8), proximal ileum-30 min (pH 7.2) and distal ileum-30 min (pH 7.5). In separate testing, the PBS (pH 6.8) was replaced by a suspension of 10 % (*w/v*) rat cecal content in bicarbonate buffer pH 7 under CO_2 to maintain an anaerobic environment. The withdrawn samples in different time intervals were analyzed spectrophotometrically (UV Shimadzu 1601 Spectrometer, Japan).

Induction of colonic inflammation

Male Wistar rats (230-250 g, 12-15 weeks old) were used in the experiment. After 24 hours starvation, in anaesthetized rats, 0.5 ml TNBS in 50% ethanol (150 mg/kg body weight) were administered rectally, 8 cm proximally from the anus. The induction and development of inflammation were monitored periodically during 3 weeks. Rats (in groups of 5) were sacrificed every day during the 1^{st} week and the 8^{th}, 14^{th} and 21^{st} day of administration. The development of inflammation was evaluated in respect to the clinical activity (quantified by loss on weight, consistency of faeces and rectal bleeding), macroscopic and pathohistological changes, colon weight/body weight ratio, myeloperoxidase activity and distribution of CD3 T and CD79 B lymphocytes. In control experiments, rats treated with 0.9% saline alone, were used.

Biodistribution studies

Biodistribution of radiolabeled [^{131}I]-5-ASA after peroral administration in a form of chitosan-Ca-alginate microparticles (group I) was followed in comparison with the biodistribution of [^{131}I]-5-ASA administered per orally as an aqueous suspension (group II). Groups of 30 rats were housed in an air-conditioned room at 22 ± 3^0C, 55 ± 5% humidity, 12 h light/dark cycles, and maintained on a normal mouse diet. The 5-ASA formulations were administered in the period of the most intensive inflammatory activity i.e. the 6^{th} day of TNBS administration. The biodistribution was followed periodically within 48 hours. The animals were sacrificed, the blood was collected, and different organs and tissues were removed. The GIT was separated into the stomach, small intestine and colon. The contents of those parts were collected and the remaining gastrointestinal tract sections were thoroughly rinsed. The radioactivity in the stomach, small intestine with PP and mesentery, colon with PP, appendix and mesentery, liver, spleen, kidney, lungs, heart and blood (2 ml per animal) were determined using a scintillation counter (NaI (TL); "well" counter Scaler Type N 529 D, EKCO Electronics, UK). In order to eliminate the radioactivity in the tissues, organs and central circulation due to ^{131}I-solution were performed after oral administration to group of rats. Accumulation of ^{131}I in the thyroid (approx. 99%) at each time point of study was confirmed, so the eventual radioactivity in the tissues, organs and central circulation due to ^{131}I only was eliminated.

Results and discussion

Negatively charged particles (-30.7 ± 1.6 *mV*) were prepared with size of 6.2 μm, drug loading 72% and calcium content 3.6 ± 0.2%. The yield was approximately 60%. Acceptable spherical morphology was observed, but also flattened, disk-shaped particles. The surface appears smooth with low porosity. By imaging with CLSM, the chitosan was localized dominantly in the particle wall, with a low quantity homogeneously distributed throughout the particle matrix. Homogeneous distribution of the alginate throughout the particle was observed with a heterogeneous deposition at the alginate/chitosan interface. ^{1}H-NMR studies confirmed that the formulation and the technology applied ensure 5-ASA stability during microencapsulation. In the FTIR spectra, the characteristic peaks of 5-ASA were not altered indicating no covalent interaction between the drug and the polymers. X-ray and DSC studies indicated molecularly dispersed drug within the particles.

At pH 1.2, alginate is protonated into the insoluble form of alginic acid displaying swelling properties that explain the low amount released (67.57% during 24 h). The release is hindered by chitosan, also; its positively charged groups strongly interact with alginate and 5-ASA, reducing swelling and release, and possibly the protonation of their carboxylated groups. At increasing pH, the increasing deprotonation of chitosan did not result in faster release. In fact, the 5-ASA release in pH 6.8 was non-significantly lower than that in pH 1.2 (65.88% during 24 h). The colonic salts and enzymatic action modified the release-determining factors, probably by increasing the internal matrix pH, porosity and degradation rate. A linear square-root time kinetics of all plots suggests release governed by drug dissolution and diffusion in the aqueous path created in matrix by polymer hydration. The drug release constants were determined and in simulated gastric and upper intestinal conditions they were 6.35 % $h^{-1/2}$ (R=1.00) and 8.61 % $h^{-1/2}$, (R=0.954), respectively, while when gradient and enzymatic methods were used, they were 8.73 % $h^{-1/2}$ (R=0.959) and 13.50 % $h^{-1/2}$, (R= 0.959), respectively. The diffusional exponents according to the general exponential release equation confirm above-mentioned, indicating anomalous (non-Fickian) transport mechanism in 5-ASA release, controlled by polymer relaxation, erosion and degradation.

The TNBS model appears to show high correlation between pathohistological, immunological and clinical features of the inflammation in IBD. In the period of the most intensive inflammatory activity i.e. the 6th day of administration, macroscopically and histologically, marked thickening of the intestinal wall associated with hemorrhages, severe cellular infiltration and ulcers that exceed 2 cm were observed. In the same period, the damage score was the highest, 15$\pm$2 *vs.* 5$\pm$1 on the 1st day and 0 for the control group. The colon/total body weight ratio increased from 8.5$\pm$0.5 mg/mg for the control group to 13$\pm$1 mg/mg, the 1st day, and 30$\pm$6 mg/mg, the 6th day of administration. The maximal activity of MPO on the 6th day of administration was observed and it was 578.48$\pm$10.58 U/g *vs.* 41.23$\pm$11.65 U/g on the 1st day of administration and 34.09$\pm$4.09 U/g in the control group. CD3 T and Cd79 B lymphocytes within the control group were mainly visible along the epithelia, from lamina epithelialis and diffuse in *lamina propria* of the colon. With the development of inflammation, their number significantly decreased and in the areas covered with ulcers, as a result of necrotic and apoptotic changes, they could not be observed.

One hour after peroral administration of 5-ASA loaded chitosan-Ca-alginate microparticles (group I, n = 5), 82.57$\pm$0.03% of the total radioactivity present in all organs, tissues and blood was detected in the stomach, small intestine with PP and mesentery. The percent of radioactivity appearing in the same region in a group of rats treated with 5-ASA suspension (group II, n = 5), 1 hour after, was 40.06$\pm$0.02% (fig. 1a). The total radioactivity present in other organs, separately and in the blood was low and non-significant in the group treated with microparticulated 5-ASA (e.g. 8.13$\pm$0.17% in the upper intestine and 2.95$\pm$0.33% in the colon), while in the group treated with suspension of 5-ASA, it was higher, 18.67$\pm$0.01% and 9.37$\pm$0.21%, respectively. The percent of radioactivity present in the blood and the liver in the group treated with 5-ASA suspension was significantly higher than the one in the group treated with microparticulated 5-ASA (e.g. in the liver, in the group II, it was 14.69$\pm$0.74% *vs.* 0.63$\pm$0.11% in the group I), which can be explained by greater extent of systemically absorbed 5-ASA when as suspension administered. The radioactivity present in the spleen, in the group I (3.77$\pm$0.07%), can be explained by the translocation of the smaller particles also, after being taken up into PP and transported *via* the mesentery lymph supply and lymph nodes to the systemic lymphoid tissues, such as spleen. The radioactivity in the stomach, 3 hours after administration of 5-ASA loaded chitosan-Ca-alginate microparticles, decreased to 42.62$\pm$4.86% and it increased in the upper intestine and colon to 27.84$\pm$4.73% and 10.76$\pm$1.49%, respectively. The percent of radioactivity in the group II, in the same regions was 36.38$\pm$2.16%, 31.74$\pm$3.86% and 3.54$\pm$0.81%, respectively (fig. 1b). The percents of radioactivity in other organs and tissues point to higher extent of 5-ASA absorption (as a parent compound or metabolite), when 5-ASA as a suspension was administered. Five hours after administration of 5-ASA loaded particles, radioactivity in the upper intestine increased to 39.55$\pm$11.23% and in the colon also (18.29$\pm$6.65%),

while in the group treated with 5-ASA suspension, it was 20.71±3.0% and 10.30±0.17%, respectively. In this period of time, again, significantly higher percents of radioactivity in other organs and tissues in the group II were observed (fig. 1c). After 10 hours of administration, radioactivity in the colon in the group I was 45.64±11.06% *vs*. 16.09±5.48% in the group in which 5-ASA suspension was administered (fig. 1d). The tendency for radioactivity to increase in the upper regions of GIT (seen in group II, the 10th hour of administration) and in both groups after 24 hours (data not shown) can be explained also by secretion in the GI lumen of intestinally metabolized 5-ASA, which is supported by the drug release data, also. Significantly higher radioactivity detected in thyroid in group II in all periods of study supports the higher extent of systemically absorbed 5-ASA and subsequent iodolysis when as suspension administered.

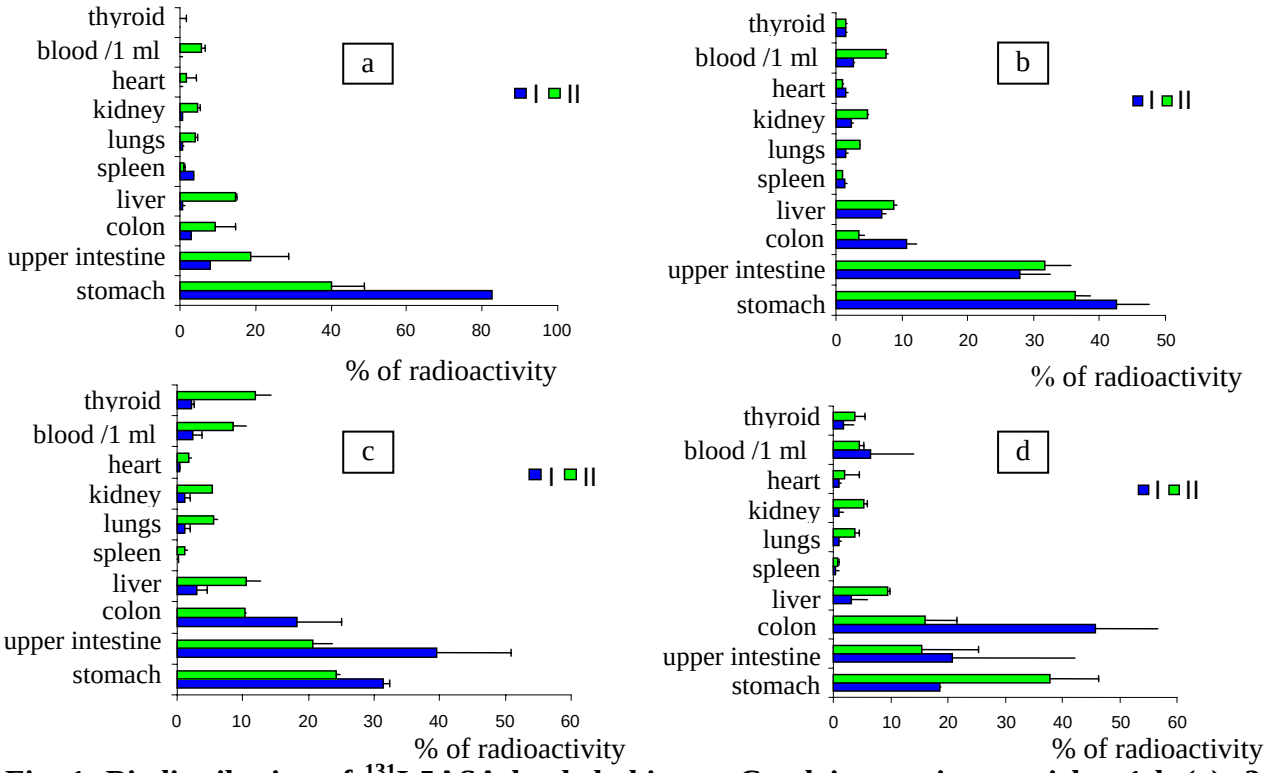

Fig. 1. Biodistribution of ^{131}I-5ASA loaded chitosan-Ca-alginate microparticles, 1 h (a); 3 h (b); 5 h (c) and 10 h (d) after peroral administration to Wistar rats with TNBS induced ulcerative colitis

Conclusion

The biodistribution studies confirm the potential of chitosan-Ca-alginate microparticles for controlled release and colon-specific delivery of 5-ASA. These data and the studies related to the efficacy against colonic inflammation in rats (not presented in this paper) show that described system may be useful for clinical treatment of human colonic inflammatory bowel diseases.

References

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