Soft Drugs 18. Oral and Rectal Delivery of Loteprednol Etabonate, a Novel Soft Corticosteroid, in Rats—for Safer Treatment of Gastrointestinal Inflammation

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Purpose. As a safe anti-inflammatory corticosteroid, the utility of loteprednol etabonate (LE) for the treatment of gastrointestinal inflammation, via oral and rectal administration, was investigated in rats. Methods. In vivo, LE solution and suspension were orally administered (20 mg/kg), and various LE preparations (solution, suspension & suppository) were applied in rectal loops (0.2 mg per loop). In vitro, various GI tissues were used to study the stability and partition of LE. Results. After oral administration of LE solution, LE reached the upper GI tract effectively, but not the colon, due to absorption and/or decomposition. In suspension, LE reached most of the GI tract (except rectum) in 8 hr and showed little absorption. After rectal applications, LE remained intact in the rectal loop for more than five hours with a slow rate of disappearance, however, LE distributed in the rectal membrane to some extent. The concentrations of LE and its inactive metabolites in plasma after both oral and rectal administrations were lower than the detection limit (0.1 µg/ml) at anytime during the experiments. In vitro, LE in solution was stable in stomach, but not in cecum, due to the hydrolysis by the cecal resident micro flora. In solution, LE distributed into the mucosal membranes efficiently (about 2.5 – 4.0 µg/g tissue). Conclusions. The results suggest that LE can be orally or rectally delivered in the GI tract for the topical treatment of the inflammatory bowel disease.

KEY WORDS: soft corticosteroid; loteprednol etabonate; oral delivery; rectal delivery; inflammatory bowel disease.

INTRODUCTION

Increasing drug potency by the structural modification frequently leads to a parallel increase in toxicity, especially in drugs that show multiple activities, such as corticosteroid. Drug design must therefore take into account the compound’s therapeutic index, the ratio of its efficacy to toxicity. “Soft drug” concept was introduced by means of designing pharmaceutical agents of reduced toxicity with structural modification to achieve a satisfactory therapeutic index (1–7). In this concept, a lead compound is modified so that the active new drug undergoes a predictable and controllable metabolism in vivo to non toxic moieties after it achieves its therapeutic role.

Loteprednol etabonate (LE), chloromethyl 17α-ethoxy-carboxyloxy-11β-hydroxy-3-o xoandrosta-1,4-diene-17β-carboxylate, one of the most promising soft corticosteroids, was synthesized from an inactive metabolite of prednisolone, Δ4-corticenic acid (A), based on the “inactive metabolite approach” (3). In vivo, LE undergoes a facile, systemic two-step metabolism into first an inactive acid etabonate analog, Δ4-corticenic acid etabonate (AE), and then into the lead compound, A, in the body. Therefore, LE, although possessing potent topical anti-inflammatory activity, causes much less systemic side effects than other corticosteroids (3). The in vitro studies using rat blood have confirmed that LE is mainly hydrolyzed into the inactive metabolite, AE (8). The topical anti-inflammatory activities of LE have been shown to be similar to that of betamethasone, so that the ophthalmic trial in human is currently undergoing (3).

Present studies were carried out to expand the application of LE to the mucosal membranes such as gastrointestinal and colorectal membranes. For this topical therapy in the GI tract, the soft steroid must distribute into targeted mucosal membranes at clinically effective concentrations, then be rapidly detoxicated after entering into the systemic circulation. Therefore, the oral and rectal deliveries of LE were evaluated in rats from the following points of view: 1. Stability of LE in GI tract, 2. Distribution of LE into the mucosal membranes along the GI tract, and 3. Concentration of LE in the systemic circulation. Two different dosage preparations, LE in 20% dimethyl β-cyclolextrem (DMCD) solution and LE in 5% sodium carboxymethylcellulose (CMC Na) suspension, were administered orally for the comparative distribution studies, and various LE preparations, solution, suspension and suppository, were prepared to investigate the the usefulness of LE for rectal application. The blood level of LE was monitored after oral and rectal administration. The in vitro studies, such as the stability of LE in the GI membranes, and the partitions of LE into GI membranes were also performed to demonstrate the feasibility of GI application of LE.

MATERIALS AND METHODS

Materials

The soft steroid, Loteprednol etabonate (LE), was kindly supplied by Otsuka Pharmaceutical Co. Ltd. (Tokushima, Japan). Δ4-corticenic acid etabonate (AE), and Δ4-corticenic acid (A) were obtained from Xenon Vision Inc. (Alachua, FL). Heptakis(2,6-di-O-methyl)-β-cyclolextrem (DMCD) and Hydroxypropyl-β-cyclolextrem (HPCD) were obtained from Pharmatec Inc. (Alachua, FL). Low density CMC Na, propylene glycol (PG) and polyethylene glycol (PEG, MW = 1450) were obtained from Sigma Chemical Company (St. Louis, MO). Witepsol H-15 was obtained from Dynamit Nobel Chemicals (Troisdorf-Oberlat, West Germany). All other chemicals were commercially available products of special reagent grade.
Animals

Male Sprague Dawley rats weighing 200 g to 250 g were obtained from Charles Rivers (Wilmington, MA). All the animal studies were conducted according to the guidelines set forth in the declaration of Helsinki and the Guiding principals in the Care and Use of Animals (DHEW Publication, NIH-80-23).

Oral Administration of LE in Rats

Dosage preparations of LE: LE solution was prepared by dissolving the compound (4 mg/ml) in a 50 mM phosphate buffer (pH 7.4) containing 20% DMCD. For the suspension, micronized LE (5–6 μ) was suspended in the previously mentioned buffer containing 5% CMC Na at a concentration of 20 mg/ml. The suspension was further sonicated in a Branson ultrasonicator (Smithkline Company) for 30 min. The osmolarity of the vehicle for the solution and suspension was adjusted to 280 mOsm/kg (μ OSMETTE micro osmometer, Precision Systems) by adding NaCl, if necessary.

Oral delivery of LE: Animals were fasted overnight (about 16 hr) prior to the experiment, but water was given freely. LE was administered orally in solution or suspension by a stomach intubation at a dose of 20 mg/kg (5 ml/kg of solution or 1 ml/kg of suspension). For LE suspension, 4 ml/kg water was also administered orally immediately after the administration of the suspension. At designated time intervals (1, 3, 5, or 8 hr after the administration of LE), the rats were sacrificed by lethal overdose of pentobarbital and the GI tract including the stomach, small intestine, cecum and colon were removed carefully so as not to disturb the luminal contents. The isolated small intestine was further divided into four regions of the same length from the stomach side (S-1, S-2, S-3 and S-4). The rectum was isolated by cutting at the sigmoid flexure, and the rest of the colon (downward of cecum) was divided into two regions with equal length from the cecum side (L-1 and L-2). For the determination of LE and AE remaining in each divided lumina after oral administration, each segment of the isolated GI tract was prepared as follows: The inner luminal contents were washed out with 10 ml of 50% acetonitrile in aqueous solution and then with 15 ml of 100% acetonitrile. The membrane tissue was homogenized in 100% acetonitrile with a Tekmar Tissumizer, and centrifuged. The washings of luminal contents and the supernatant of the tissue homogenate were combined, and pure acetonitrile was added to the combined mixture so that the total volume was 40 ml. The combined mixture was then vigorously shaken with a vortex mixer and centrifuged at 3000 rpm for 10 min. The supernatant was removed and analyzed by high performance liquid chromatography (HPLC). In separate experiments, the recovery of LE and AE was determined by spiking different concentrations of LE or AE to the intestinal lumen, and the samples were prepared by the same extraction method mentioned before. The results indicated that the recovery of LE and AE was 100 ± 3%.

Rectal Application of LE in Rats

Dosage preparations of LE: Five LE formulations were prepared as follows. a. LE suspended in pH 7.4, 50 mM phosphate buffer solution containing 5% CMC Na (isotonic); b. LE dissolved in a pH 7.4, 10 mM phosphate buffer containing 20% DMCD (isotonic); c. LE dissolved in PG; d. LE dissolved in PEG 1450, water soluble suppository base; and e. LE suspended in Witcosol H-15, oleaginous suppository base. Micronized LE (5–6 μ) was used for preparing suspensions. The suppository was prepared by dispersing LE into the fused suppository base (PEG or Witcosol H-15) and solidifying the suspension in a glass tube (0.55 cm inner diameter) at room temperature. LE concentration in all preparations was 1 mg/g or ml.

Rectal Application of LE: Animals were fasted for 16 hours before the experiment, but water was given freely. Sodium pentobarbital (Nembutal, Abbott Laboratories) was injected intraperitoneally in the animals at a dose of 30 mg/kg. After the anesthesia, body temperature of the rats was kept above 36°C by lamps during the experiments. A midline incision was made to expose the peritoneal cavity. A rectal loop, approximately 3 cm, was made by ligating the rectal tract at the sigmoid flexure and by closing the basement of the anus with a drop of surgical cement (Aron Alpha A "Sankyo," Sankyo Co. Ltd., Tokyo, Japan). LE solution or liquid suspension was administered through polyethylene tubing (PE 50, Clay Adams) cannulated into the rectal lumen. LE suppository (LE in PEG or Witcosol H-15) was administered in the rectum and the basement of the anus was sealed with surgical cement. The LE dose administered was 0.2 mg in 0.2 ml or 0.2 g of vehicle per loop. At designated times (1, 3 or 5 hr) after rectal application of LE, blood was withdrawn from jugular vein, and then rats were sacrificed by lethal overdose of pentobarbital. Subsequently, the rectal loop containing luminal contents was isolated and the inner contents of the rectal lumen were washed with 5 ml of 50% acetonitrile in aqueous solution and then 10 ml of 100% acetonitrile. The rectal membrane was homogenized with a Tekmar Tissumizer in 100% acetonitrile and centrifuged. The washing of luminal contents and the supernatant of the tissue homogenate were combined, and pure acetonitrile was added to the combined mixture so that the total volume was 40 ml. The combined mixture was then vigorously shaken with a vortex mixer and centrifuged at 3000 rpm for 10 min. The supernatant was analyzed for LE concentrations by HPLC to determine the total amount of LE remained in the rectal loop and tissue membrane. For the determination of LE in blood samples, 0.2 ml of 5% dimethylsulfoxide (DMSO) in acetonitrile solution was added to 0.1 ml of blood to halt metabolism and precipitate the blood protein. The samples were then centrifuged at 3000 rpm for 10 min and the supernatant was analyzed by HPLC. The recovery of LE and AE from the rectal loop homogenizes and blood samples, 100 ± 3%, were determined as described in the previous section.

Stability of LE in Stomach and Cecum in Vitro

LE was dissolved in an isotonic, pH 7.4 phosphate buffer solution containing 20% DMCD at a concentration of 0.1 mg/ml. The stomach and cecum of overnight fasted rats were freshly isolated. After the insertion of a polyethylene