A budesonide prodrug accelerates treatment of colitis in rats

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Abstract

Although oral glucocorticoids are the treatment of choice for moderate to severe ulcerative pancolitis, their systemic side effects and adrenal suppression account for considerable morbidity. An oral glucocorticoid-conjugate (prodrug), budesonide-β-D-glucuronide, which is not absorbed in the small intestine but is hydrolysed by colonic bacterial and mucosal β-glucuronidase to release free budesonide into the colon was synthesised. The objective of this study was to compare treatment with budesonide-β-D-glucuronide with treatment with free budesonide by examining: (1) the healing of experimental colitis and (2) the extent of adrenal suppression. Pancolitis was induced with 4% acetic acid. Animals were then randomised to receive oral therapy for 72 hours with (1) budesonide-β-D-glucuronide, (2) free budesonide, or (3) vehicle. Drug efficacy and colitic healing was determined by measuring gross colonic ulceration, myeloperoxidase activity, and in vivo colonic fluid absorption. Adrenal suppression was determined by measuring plasma adrenocorticotrophic hormone and serum corticosterone. Vehicle-treated colitis animals had gross ulceration, increased myeloperoxidase activity, and net colonic fluid secretion. Treatment with oral budesonide-β-D-glucuronide accelerated all measures of colitis healing at a fourfold lower dose than did free budesonide. Furthermore, treatment with budesonide-β-D-glucuronide did not result in adrenal suppression whereas free budesonide treatment did. A newly synthesised orally administered glucocorticoid-conjugate accelerates colitis healing with limited adrenal suppression. Development of an orally administered colon-specific steroid delivery system represents a novel approach to inflammatory bowel disease treatment.

(*Gut* 1994; 35: 1439–1446)

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Accepted for publication 12 January 1994

Colonic inflammatory bowel disease is currently treated with both steroidal and non-steroidal anti-inflammatory drugs which are administered via oral, rectal, and intravenous routes.[1] Systemic side effects associated with the administration of glucocorticoids by the oral and intravenous routes ensure that these are generally reserved for the treatment of severe acute disease.[2] Rectal administration of glucocorticoids is a route patients often choose not to use. In addition, this method is only partially effective because drug distribution is largely confined to the distal region of the colon.[3]

A new group of corticosteroids has recently been developed. In comparison with conventional corticosteroids, these drugs display a high degree of topical anti-inflammatory activity but do not show appreciable systemic activity. This unique activity ratio is achieved because a very high potency is coupled with rapid metabolism of the drug to products that have minimal or no biological activity. This rapid metabolism, also referred to as high first-pass liver metabolism, allows for a high therapeutic efficacy and high systemic tolerability.

Budesonide is a member of this group of steroids which display high first-pass liver metabolism. Budesonide is a non-halogenated glucocorticoid structurally related to 16-alpha-hydroxyprednisolone.[4] Administration of this drug via inhalation has proved effective in the treatment of asthma and rhinitis. It also presented a low adverse drug event profile.[4] Budesonide, given as an enema, has also proved superior to other glucocorticoids in the treatment of distal ulcerative colitis.[5]

Budesonide has now been formulated into an enterocapsule preparation which allows the drug to bypass the upper gastrointestinal tract, thus facilitating delivery of the active compound to the terminal ileum.[6] Unfortunately, it was found that less than 5% of the compound was available beyond the ileum and caecum.[6] Therefore, while this compound would be useful for the treatment of small intestinal Crohn's disease,[7] it is not likely to have an appreciable effect on colonic inflammatory bowel disease. In addition, a recent dose ranging study showed that effective doses of budesonide (9 mg/d) cause significant adrenal suppression.[8]

Because of these problems, we designed and synthesised an orally administered, colon-specific budesonide prodrug (budesonide-β-D-glucuronide) with a delivery mechanism that would increase budesonide concentrations along the colon relative to systemic drug concentrations, thereby increasing colonic drug efficacy and decreasing systemic side effects.[9] The advantage of colon-specific delivery has been already documented in the case of the non-steroidal anti-inflammatory agent 5-aminosalicylic acid. In that instance, the prodrugs sulphasalazine and olsalazine, from which the active drug is released in the colon by the action of microbial azoreductases, were used.[10–12]

The objectives of the present study

were: (1) to test the efficacy of budesonide prodrugs in treating experimentally induced colitis in rats and (2) to determine whether adrenocortical axis suppression occurred with administration of the budesonide prodrug.

Methods

Glacial acetic acid, purchased from Fisher Scientific (Nepean, ON, Canada) was used to prepare a 4% solution in water (pH 2·4). All remaining reagent grade chemicals were purchased from Sigma Chemical Company (St Louis, MO, USA).

SYNTHESIS OF BUDESONIDE PRODRUG

A detailed description of synthesis methods used in our experiment has already been published.[13] Briefly, budesonide (Sigma Chemical Co, St Louis, MO, USA; MW 430.5) was reacted with triacetyl-1-bromo-alpha-D-methyl glucuronic acid in the presence of an acid scavenger, silver carbonate. The protecting groups on the sugar were removed by mild basic hydrolysis. The final product (budesonide-β-D-glucuronide, sodium salt; MW 606.6) was isolated after purification on a reverse phase C-18 flash column. The structure of the prodrug was confirmed by 1H-NMR<IR and elemental analysis. In addition, the prodrug was converted to the parent compound (a mixture of R and S epimers) quantitatively by incubation with B-glucuronidase (type IX from *Escherichia coli*, Sigma Chemical Co, St Louis, MO, USA). Release of budesonide from its prodrug was measured by high performance liquid chromatography (HPLC)>. The system used consisted of the following instruments (all from Waters Associates Inc, Milford, MA, USA): high pressure pumps (model 6000A), automatic sample injector (WISP710B), UV absorption detector (model 481), and chromatography data station (model 840). The separation was performed on a Whatman partisil ODS-3 column (10 μm, 3·9×300 mm). A mobile phase flow rate of 1·0 ml/min was used, and the eluent was monitored at 246 nm. The mobile phase consisted of 0·02 M sodium acetate buffer (pH 4·8)/acetonitrile (68:32, v/v). All separations were performed at ambient temperature. The retention times observed under these conditions were 22 minutes for budesonide and 18 minutes for triamcinolone acetonide (internal standard).

INDUCTION OF COLITIS

Colitis was induced as described in detail elsewhere.[14 15] Briefly, non-fasting male Sprague-Dawley rats (250–275 g; Biotron, University of Alberta, Edmonton, AB, Canada) were anaesthetised via an intraperitoneal injection of pentobarbital (55 mg/kg) and atropine (0·5 mg/kg). Through a sterile midline abdominal incision, the colon was isolated and the junction of the caecum and ascending colon was occluded with a reversible ligature; care was taken to avoid compromising neural or vascular integrity. The colon was cleansed of its luminal contents with 154 mM sodium chloride solution at 37°C, and the residual fluid was expressed manually through the rectum. Acetic acid (4%, 2 ml) was injected into the lumen of the colon through a 26 gauge needle passed obliquely through the colonic wall just distal to the occluding ligature. After 20 seconds 10 ml of air were injected to clear the acetic acid from the colon. The occluding ligature was removed and the midline incision closed. The animals were allowed to recover from the anaesthesia in a light-cycled room that provided free access to standard rat chow pellets (5001, Purina Mills Inc, St Louis, MO, USA) and water. All in vivo studies outlined below were carried out 72 hours after induction of colitis.

ADMINISTRATION OF BUDESONIDE PRODRUG

Budesonide-β-D-glucuronide was administered by oral gavage 24 and 48 hours after the induction of colitis. The budesonide conjugate solutions were prepared immediately before their oral administration; the appropriate volume of stock solution was mixed with 154 mM sodium chloride to a total volume of 1 ml (pH 7·4). Rats were administered doses of free budesonide and equivalent doses of the respective conjugate ranging from 0·0137 to 0·44 μmol/kg/d.

IN VIVO INTESTINAL FLUID ABSORPTION

Intestinal absorption of fluid was assessed as described previously.[15] Seventy two hours after the induction of colitis, rats were anaesthetised with pentobarbital (55 mg/kg) and atropine (0·5 mg/kg) and maintained at 37°C using a thermostatic heat lamp. The intestinal tract was exposed through a midline abdominal incision. An occluding ligature was placed at the caecal-ascending colon junction. Sodium chloride solution (154 mM, 37°C) was instilled into the proximal colon via a cannula inserted through an incision just distal to the proximal occluding ligature in order to flush out the luminal contents of the colon. Residual saline was emptied by gentle manual expression. A 12 cm long intestinal loop, beginning 2 cm below the caecal-colonic junction and extending distally to the peritoneal reflection, was created with ligatures. In isolating the loop, care was taken not to compromise mesenteric, vascular, or neural integrity. A 26 gauge needle was inserted obliquely through the outer muscle layer along the antimesenteric border, and 2 ml of sodium chloride solution (37°C, 154 mM) were instilled into the empty loop. In no case was fluid leakage detected, and the loop was only mildly distended. Similar loops were formed in the jejunum, beginning 2 cm distal to the ligament of Trietz, and in the ileum, beginning 2 cm proximal to the ileocecal valve. The viscera were returned to the abdominal cavity, and the incision was closed. Sixty minutes after abdominal closure, the animals were given a pentobarbital overdose (240 mg/kg), and the intestinal loops

were removed. The length of each loop was recorded. The intestinal loops were weighed, both full and empty, to determine residual intraluminal volume. Results were expressed as the difference between initial and residual loop volumes over the hour per centimetre of intestine.

MACROSCOPIC ULCERATION

The colon was rapidly excised, opened along its mesenteric border, and gently rinsed of its luminal contents with an iced 154 mM solution of sodium chloride. The colon was then placed flat, mucosal surface upwards, on a glass plate chilled to 4°C. A transparent acetate was placed 5 mm above the mucosal surface, and the area of ulceration and total surface area were traced by a single observer (NC). Areas in square centimetres were then calculated using a Zeiss computerised videoscope (Videoplan, Carl Zeiss Co, Toronto, ON, Canada). These data were also transformed into percentage-based terms expressing improvement in macroscopic ulceration; control animals scored 0%.

MYELOPEROXIDASE ACTIVITY

An assay of colonic myeloperoxidase activity was used to quantify neutrophil infiltration. The entire length of colon from the ascending-caecal junction to the rectum was homogenised in 5 ml of 0·5% hexadecyltrimethylammonium bromide in 50 mM phosphate buffer (pH 6) using a polytron homogenizer (Brinkman Instruments, Rexdale, ON, Canada) three times for 30 seconds each at 4°C. The homogenate was then sonicated for 10 seconds and centrifuged. The supernate (0·1 ml) was combined with 2·9 ml of a 50 mM phosphate buffer (pH 6) containing 1·05 mM *o*-danisidine hydrochloride and 0·15 mM hydrogen peroxide.[16] The change in absorbency at 460 nm was measured with a Beckman DU-6 spectrophotometer (Beckman Instruments Inc, Irvine, CA, USA). One unit of myeloperoxidase activity was defined as that degrading 1 μM of peroxide per minute at 25°C.

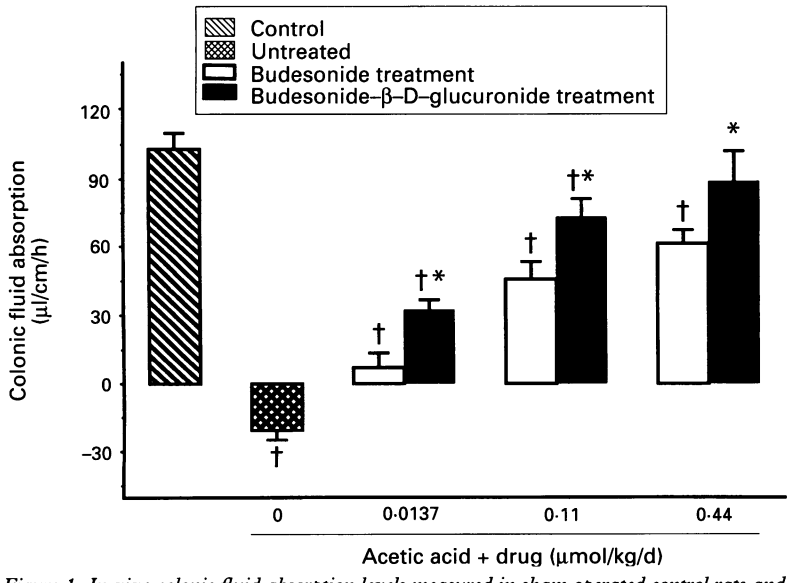

*Figure 1: In vivo colonic fluid absorption levels measured in sham operated control rats and in those with untreated acetic acid induced colitis. Values were also measured in rats treated with budesonide and budesonide-β-D-glucuronide. Both drugs were administered via oral gavage to animals with colitis 24 and 48 hours after the induction of colitis, and doses ranged from 0 to 0·44 μmol/kg/d. Data represent the mean (SEM) for six animals per group. *p<0·02 compared with free budesonide; †p<0·01 compared with control.*

SERUM CORTICOSTERONE

Blood (2 ml) was collected into evacuated blood collection tubes (Becton Dickinson Vacutainer Systems, Rutherford, NJ, USA) between the hours of 0800 and 1000 by intracardiac puncture. It was then centrifuged at 4°C for 10 minutes at 4000 rpm (Centra-7, International Equipment Co, Needham Heights, MA, USA). The serum was removed and stored at −70°C before analysis. Stock anti-corticosterone antiserum (B3-163) was obtained from Endocrine Sciences RIA Reagents (Tarzana, CA, USA). Diluted antiserum was prepared by adding together 10 ml borate buffer (0·05 M, pH 8), 400 000 dpm of 1,2-^{3}H-corticosterone, 0·2 ml of 10% bovine serum albumin in a borate buffer, 0·2 ml of 0·25% bovine gamma globulin in a borate buffer, and 100 μl of stock antiserum. The serum sample for analysis was thawed and placed in 12×75 mm test tube; it was then diluted with a borate buffer and incubated at 60°C for 30 minutes. The solution was transferred to a 2 ml conical tube (Kimax #45150, Fisher Scientific, Nepean, ON, Canada) and incubated with diluted antiserum at 37°C for 45 minutes and then at room temperature for two hours. Saturated ammonium sulphate solution (0·25 ml) was added to the tube, and the mixture was centrifuged at 3000 rpm for 10 minutes to separate free and bound steroids. The supernate was decanted into a microvial (Simport Ltd, Quebec City, QB, Canada), 4 ml of a scintillation cocktail was added (Scintiverse, bio-HP, Fisher Scientific, Nepean, ON, Canada), and radioactivity was counted using a liquid scintillation counter (LKB Wallace, 1219 Rack Beta, Turka, Finland).

PLASMA ADRENOCORTICOTROPIC HORMONE

Adrenocorticotropic hormone (ACTH) concentrations were determined using blood (3 ml) collected by intracardiac puncture between 0800 and 1000 hours after pentobarbital overdose of the animal. Blood was drawn into a chilled siliconised tube. The blood was then centrifuged at 4°C for 10 minutes at 4000 rpm. The plasma was transferred to a polypropylene tube and stored at −70°C until the time of analysis. Samples did not come into contact with glass during the sample collection procedure. ACTH concentrations were measured using a commercial immunoassay kit (Nichols Institute, San Juan, Capistrano, CA, USA). The ACTH immunoassay uses a monoclonal antibody and a polyclonal antibody, both of which leave a high affinity and specificity for defined amino acid regions of the ACTH molecule. The

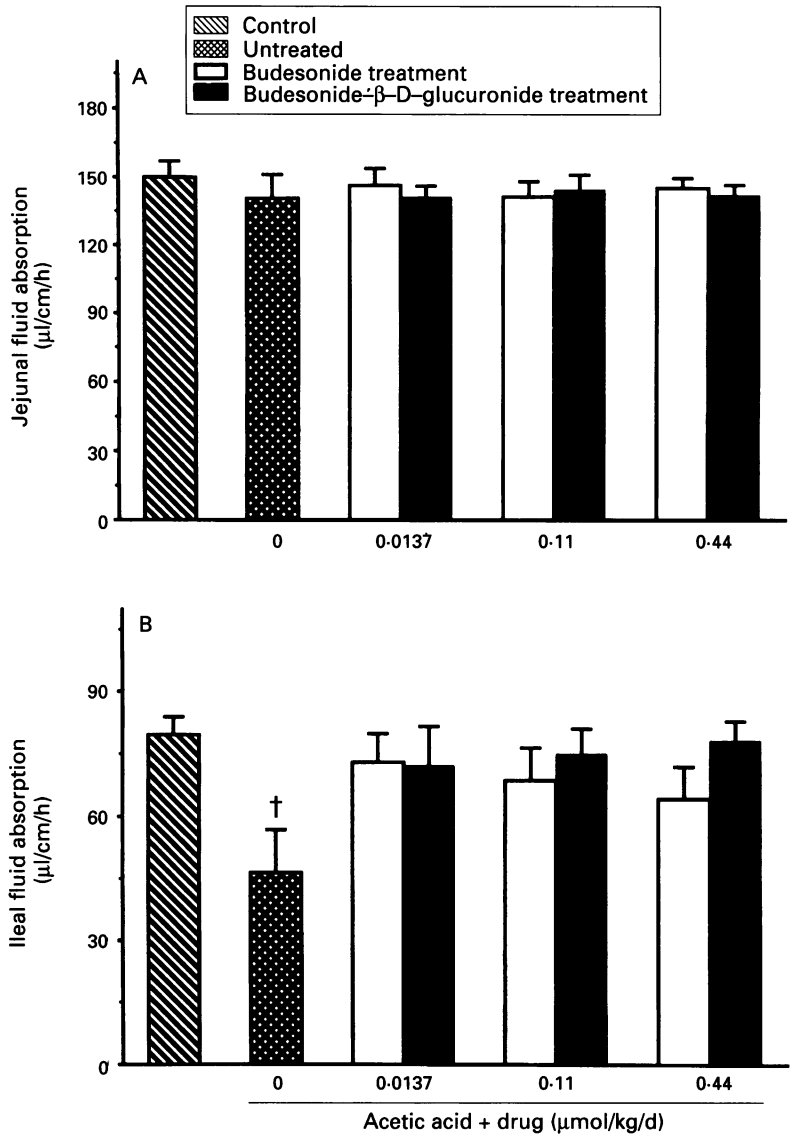

Figure 2: In vivo jejunal (A) and ileal (B) fluid absorption levels measured in sham operated control rats and in those with untreated acetic acid induced colitis. Levels were also measured in rats treated with budesonide and budesonide-β-D-glucuronide. Both drugs were administered by oral gavage to animals with colitis 24 and 48 hours after the induction of colitis, and doses ranged from 0 to 0·44 μmol/kg/d. Data represent the mean (SEM) for six animals per group. †$p<0·02$ compared with control.

polyclonal antibody is conditioned by affinity chromatography to bind only to the C-terminal region of ACTH molecules. The monoclonal antibody binds only to the N-terminal region of ACTH molecules. Both antibodies bind ACTH molecules without competition or steric interference to form a soluble sandwich complex. The monoclonal antibody is radio labelled for detection, while the polyclonal antibody is coupled to biotin. As, there is a high affinity reaction between biotin and avidin,[18] addition of an avidin-coated plastic bead to the reaction mixture allows for a specific and efficient means of binding the sandwich complex. In the assay process, standards, controls, and samples were incubated with a solution containing the radiolabelled antibody, the biotin-coupled antibody, and an avidin-coated plastic bead. At the end of the assay incubation period, the bead was washed to remove unbound components and the radioactivity levels of remaining bound solid phase products were measured in a gamma counter (Packard, Fisher Scientific, Nepean, ON, Canada). Since the complex occurs only in the presence of an intact ACTH molecule containing both N-terminal and C-terminal regions, the radioactivity of the bead bound complex is directly proportional to the amount of intact ACTH in the sample.

STATISTICS

Statistical analysis of the data was performed using repeated measure analysis of variance. When the overall analysis showed significance, the Student's *t* test was used to examine the location and significance of differences.

Results

IN VIVO COLONIC FLUID ABSORPTION

In vivo net colonic fluid absorption was used as one measure of the rate of repair of mucosal integrity taking place. Net colonic fluid absorption in sham operated controls (102·5 (9·9) μl/cm/h) was unaffected (113·0 (11·2) μl/cm/h) by the highest dose of budesonide studied (0·44 μmol/kg/d). This result suggests that the budesonide we tested did not merely enhance existing intestinal fluid and electrolyte transport; changes in colonic fluid absorption were therefore a measure of injury and/or repair.

Figure 1 shows the colonic fluid absorption data for colitic animals treated with free budesonide and its conjugate. Upon induction of colitis, colonic fluid absorption became negative (−21·8 (4·2) μl/cm/h); there was net secretion of fluid into the colonic lumen. Treatment with budesonide-β-D-glucuronide significantly accelerated mucosal repair and improved in vivo fluid absorption more rapidly than did free budesonide at each dose tested. Fluid absorption data were fitted to the modified Hill equation to calculate the ED_{50} (effective daily dose providing half maximal effect) for budesonide-β-D-glucuronide and free budesonide. The mean (SD) ED_{50} for budesonide-β-D-glucuronide was 0·0132 (0·0034) μmol/kg/d, and for budesonide it was 0·0514 (0·0128) μmol/kg/d. The fourfold difference between these two values is highly significant ($p<0·001$).

IN VIVO JEJUNAL AND ILEAL FLUID ABSORPTION

Jejunal fluid absorption (149·6 (9·7) μl/cm/h) was unaffected by either the induction of colitis or treatment with budesonide or its conjugate (Fig 2(A)). In contrast, Figure 2(B) shows that 4% acetic acid colitis caused a significant decrease in ileal in vivo fluid absorption. Both budesonide and budesonide-β-D-glucuronide improved ileal fluid absorption equally (Fig 2(B)). Basal fluid absorption in the small intestine was not stimulated in control animals given even the highest dose of budesonide tested (0·44 μmol/kg/d), indicating that fluid absorption was a sensitive measure of functional mucosal injury for the entire intestine.

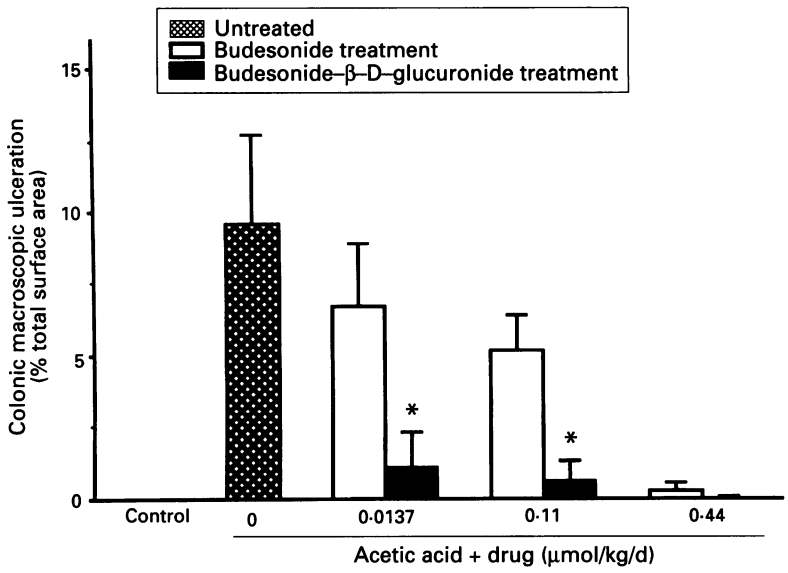

*Figure 3: Colonic macroscopic ulceration measured in sham operated control rats and in those with untreated acetic acid induced colitis. The area of ulceration is expressed as a percentage of the total colonic area. Control rats scored 0%. Scores were recorded for those rats treated with budesonide and budesonide-β-D-glucuronide. Both drugs were administered via oral gavage to animals with colitis 24 and 48 hours after the induction of colitis, and doses ranged from 0 to 0·44 μmol/kg/d. Data represent the mean (SEM) for six animals per group. *p<0·02 compared with free budesonide.*

COLONIC MACROSCOPIC ULCERATION

Figure 3 shows the degree of macroscopic colonic ulceration seen in each treatment group. Sham operation (control) and/or treatment with budesonide (data not shown) did not induce macroscopic ulceration of the colon. Induction of colitis using 4% acetic acid produced gross macroscopic ulceration in approximately 10% of the total colonic surface area. In findings analogous to the results seen with in vivo colonic fluid absorption measurement, treatment with budesonide-β-D-glucuronide (0·0137 and 0·11 μmol/kg/d) improved macroscopic colonic ulceration at a faster rate than did free budesonide at the same strength. Treatment with the highest dose of budesonide-β-D-glucuronide, 0·44 μmol/kg/d, however, produced improvements in gross mucosal ulceration similar to those seen with free budesonide given at the same strength.

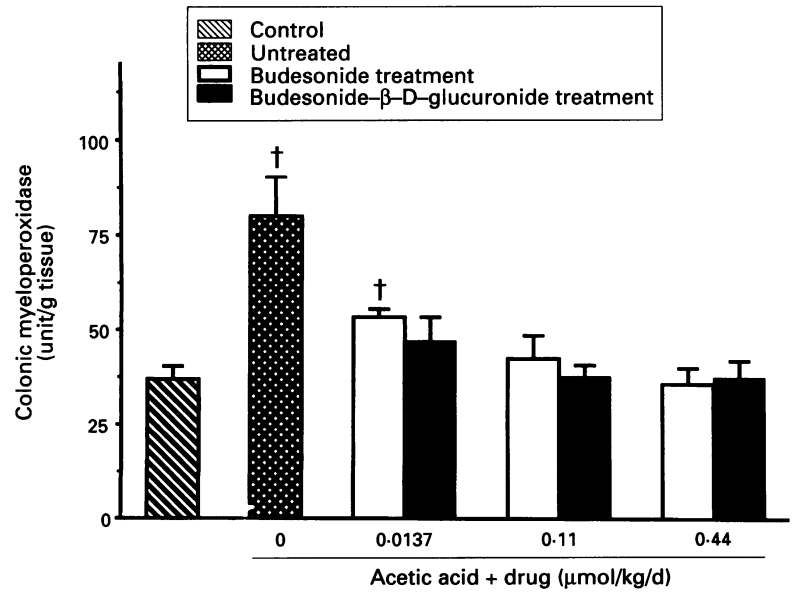

Figure 4: Colonic myeloperoxidase activities measured in sham operated control rats and in those with untreated acetic acid induced colitis. Activities were also recorded for rats treated with budesonide and budesonide-β-D-glucuronide. Both drugs were administered by oral gavage to animals with colitis 24 and 48 hours after induction of colitis, and doses ranged from 0 to 0·44 μmol/kg/d. Data represent the mean (SEM) for six animals per group. †p<0·05 compared with control.

COLONIC MYELOPEROXIDASE ACTIVITY

As shown in Figure 4, the induction of colitis significantly increased myeloperoxidase activities relative to those of sham operated control animals. Histological assessment of this colitis model has confirmed that neutrophils are the predominant cellular infiltrate.[15] Increasing doses of budesonide and budesonide-β-D-glucuronide caused a reduction of myeloperoxidase activity to normal values at the highest doses administered. No significant difference in improvement of myeloperoxidase activity existed between budesonide-β-D-glucuronide and free budesonide.

SERUM CORTICOSTERONE CONCENTRATIONS

Figure 5 shows the serum corticosterone concentrations of budesonide treated animals. Colitic animals which received no treatment showed a significant increase in serum corticosterone concentrations over those of sham operated controls. Treatment with free budesonide significantly reduced corticosterone values to below control levels at all doses tested. In contrast, treatment with budesonide-β-D-glucuronide retained corticosterone at normal values; no evidence of adrenosuppression was seen even at the highest dose (0·44 μmol/kg/d) used.

PLASMA ACTH VALUES

The data shown in Figure 6 show the effect of budesonide on plasma ACTH concentrations. Values were comparable in sham operated controls and in animals with acetic acid induced colitis. Similar to the results with corticosterone, treatment with free budesonide reduced plasma ACTH values, while budesonide-β-D-glucuronide treatment did not.

Discussion

Glucocorticoids remain the treatment of choice for human inflammatory bowel disease, although their oral administration often results in systemic side effects. In an attempt to reduce these side effects, corticosteroids which incorporate first-pass metabolism kinetics are being designed. Recently, the corticosteroid, budesonide, has been shown to have a high topical anti-inflammatory activity and a 90% first-pass hepatic metabolic conversion to inactive metabolites.[4]

When budesonide was administered orally as a controlled ileal release capsule to patients with active ileal Crohn's disease, budesonide (9 mg/d) was as effective as prednisolone (40 mg/d) at inducing disease remission by 10 weeks.[7] Unfortunately, despite its reported 90% first-pass metabolism, budesonide 9 mg/d caused a 40% depression of plasma cortisol

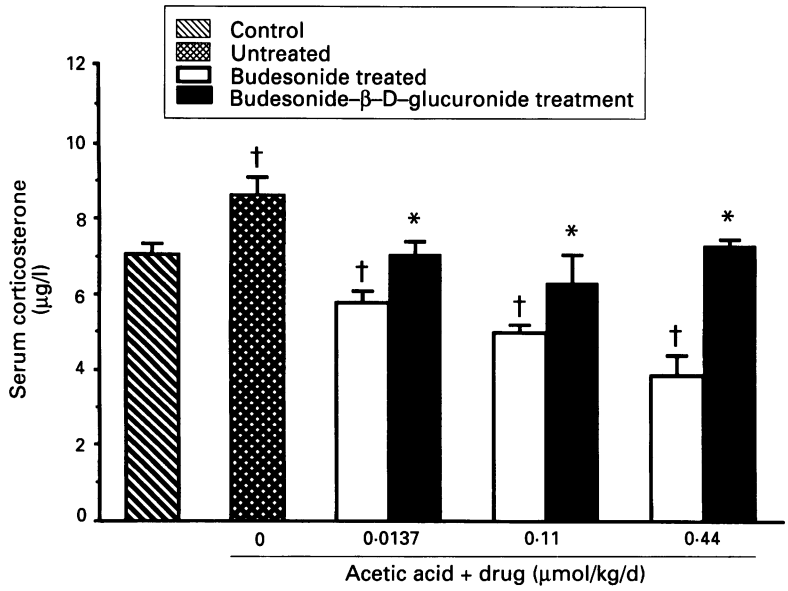

*Figure 5: Serum corticosterone concentrations measured in sham operated control rats and in those with untreated acetic acid induced colitis. Values were also measured for animals treated with budesonide and budesonide-β-D-glucuronide. Both drugs were administered via oral gavage to animals with colitis 24 and 48 hours after induction of colitis, and in doses ranging from 0 to 0·44 μmol/kg/d. The data represent the mean (SEM) for six animals per group. *p<0·05 compared with free budesonide; †p<0·02 compared with control.*

and a short ACTH test ([8], personal communication: G Greenberg). These findings are somewhat surprising and suggest that free budesonide may actually have less than a 90% first-pass metabolism or that it may be metabolised to active metabolites. The presently available budesonide controlled ileal release capsules contain small pellets designed to resist the action of gastric contents and to begin releasing free budesonide only during passage through the small intestine. The results of pharmacokinetic and gastrointestinal transit studies of orally administered budesonide controlled ileal release capsules indicate that approximately 11% of the oral dose

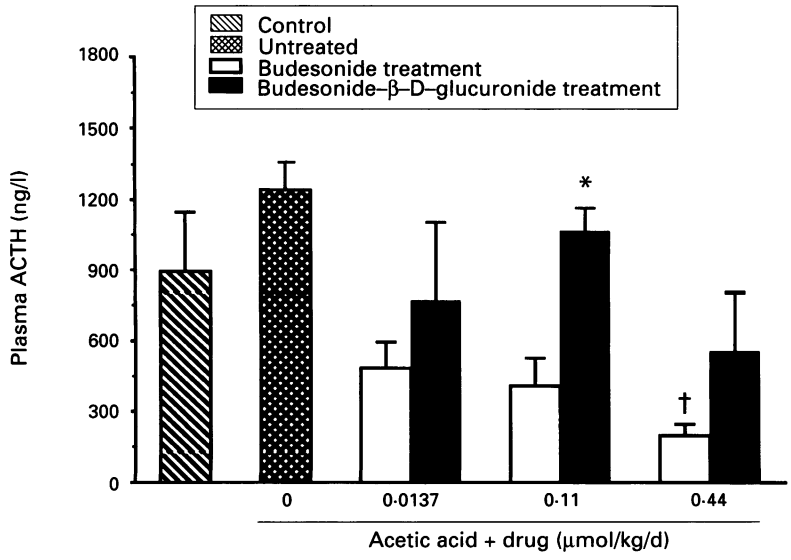

*Figure 6: Plasma adrenocorticotrophic hormone (ACTH) values measured in sham operated control rats and in those with untreated acetic acid induced colitis. Values were also measured for rats treated with budesonide and budesonide-β-D-glucuronide. Both drugs were administered by oral gavage to animals with colitis 24 and 48 hours after induction of colitis, and in doses ranging from 0 to 0·44 μmol/kg/d. Data represent the mean (SEM) for six or eight animals per group. *p<0·02 compared with free budesonide; †p<0·05 compared with control.*

becomes available systemically and, when administered with food, only 4% of the drug reaches beyond the caecum and into the colon.[6] It is therefore unlikely that the present formulation of budesonide, orally administered controlled ileal release capsule, will be delivered to the colonic lumen in sufficient concentrations to be effective in treating colonic inflammatory bowel disease.

In animal studies, budesonide administered by subcutaneous injection healed experimentally induced colitis.[18] Furthermore, administration of budesonide directly to colonic mucosa in the form of an enema has been shown to promote the healing of experimentally induced rat colitis[18–20] and human pouchitis.[21] These results suggest that budesonide, if dispensed through the appropriate drug delivery system to achieve adequate colonic concentrations, may be effective in healing colitis.

We therefore synthesised a prodrug conjugate of budesonide, budesonide-β-D-glucuronide, which would readily deliver free active budesonide to the colonic lumen with minimal absorption of budesonide in the stomach and small intestine. The prodrug conjugate is increased size and is extremely hydrophobic relative to free budesonide. Because these properties render it poorly absorbed in the proximal intestine, the conjugate reaches the large intestine where it is hydrolysed to free budesonide and sodium glucuronate. This delivery of high local concentrations of free budesonide to the colonic lumen causes raised mucosal concentrations of budesonide: lower effective oral doses of the prodrug conjugate can therefore be used to reach these therapeutic levels in the colon and achieve colitis healing through the local effects of budesonide. As a result of the lower oral dose required for therapeutic effect, and the limited small intestinal absorption of the conjugate, a reduction in systemic corticosteroid side effects should be observed. A further reduction in systemic exposure should result from hepatic metabolism of any free budesonide once it has been delivered to the colon and absorbed into the portal venous system. Assuming a bioavailability of 0·1, a large selective advantage should be noted when colonic delivery is evaluated using a kinetic model.[9] This very effective colonic prodrug delivery system of budesonide will allow its use in the treatment of colitis, but by the nature of its design will not allow it to be used for small intestinal disease therapy.

In vivo, colonic fluid absorption is a sensitive measure of mucosal functional absorptive injury.[15] As shown in Figure 1, induction of colitis with acetic acid significantly impaired net colonic fluid absorption to such an extent that it induced a net secretory state. These results are similar to those previously documented.[15] Oral administration of the conjugate budesonide-β-D-glucuronide facilitated the healing of the colon and restored net colonic fluid absorption more effectively than did free budesonide. It probably did so by providing higher intraluminal, and subsequently

intramucosal, colonic concentrations of budesonide. While an increased systemic absorption of budesonide could have also given similar results, this is an unlikely event, as the conjugate did not cause adrenosuppression (Figs 5 and 6).

ED_{50} data were used to compare the relative potencies of the conjugate and the free drug. The fourfold increase in relative potency of the conjugate is similar to that of several other prodrugs examined using the same experimental colitis animal model. A glucuronide prodrug of dexamethasone had a 16 fold increase in potency over free dexamethasone.[22] Orally administered macromolecular dextran prodrugs of dexamethasone show a three to ninefold increase in potency over similar doses of oral dexamethasone.[23] A dextran prodrug of methylprednisolone showed a fourfold increase in potency over free methylprednisolone.[23] The ED_{50} of budesonide-β-D-glucuronide found in this study (0·0125 μmol/kg/d) was similar to that documented for dexamethasone-β-D-glucuronide (0·0125 μmol/kg/d).[22] The ED_{50} of free dexamethasone was calculated at 0·18 μmol/kg/d,[22] while that of free budesonide was calculated to be 0·0514 μmol/kg/d. These results suggest that budesonide has an oral bioavailability greater than 0·15.[24]

Neither free budesonide nor its conjugate had any effect on fluid absorption in the jejunum of rats with colitis. Furthermore, neither drug had any effect on fluid absorption in the jejunum, ileum, or colon of sham operated control rats. These results suggest that free budesonide and its conjugate do not simply stimulate intestinal fluid transport, but instead improved absorption in the ileum and colon by facilitating the repair of the mucosal injury.

The healing of the fluid absorptive injury observed in the ileum of colitic animals (Fig 2(B)) may have occurred as a consequence of systemic budesonide released from the conjugate either in the small intestine or colon. Pharmacokinetic experiments indicate that a small fraction of a prodrug released in the large intestine can be subsequently absorbed and distributed throughout the body.[9] A similar drug distribution in the current experiment may explain the fact that ileal fluid absorption was affected equally by budesonide and budesonide-β-D-glucuronide. Alternatively, Empey *et al* have shown that in this animal model of colitis reduction in ileal fluid absorption is likely a bystander dysfunction which improves in parallel with the healing of colitis.[25]

Colonic myeloperoxidase activities reflect the number of neutrophils in the tissue.[16] While myeloperoxidase activity returned to near normal values at the highest doses of drug administration, no difference existed between responses to budesonide-β-D-glucuronide and free budesonide (Fig 4). It is therefore unlikely that budesonide-β-D-glucuronide's beneficial effects occur as a consequence of neutrophil migration inhibition: these are more probably the result of actions exerted at another step in the inflammatory process. Indeed, Jacobson *et al* have recently shown that in the trinitrobenzene sulphonic acid model of colitis, administration of budesonide prevents intestinal injury but does not alter myeloperoxidase activity.[20]

Since adrenosuppression is directly related to the levels of exogenous free glucocorticoid circulating in the blood stream, we hypothesised that budesonide-β-D-glucuronide treatment should cause less adrenosuppression than same dose treatment with free budesonide. In humans, ACTH directly regulates the production of cortisone, while in the rat it regulates the production of corticosterone. Measurement of ACTH and corticosterone values is a sensitive means of determining the degree of adrenosuppression in rats. Figures 5 and 6 show that at every dose tested budesonide-β-D-glucuronide had a limited effect on ACTH and corticosterone values. This is in contrast to free budesonide administration, which caused a statistically significant adrenosuppression.

The orally administered, colon specific, budesonide prodrug conjugate, budesonide-β-D-glucuronide, improves the healing of experimentally induced colitis with less adrenosuppression than free budesonide. The development of colon specific glucocorticoid prodrugs may thus prove effective as novel therapy in treating human colonic inflammatory bowel disease without causing systemic side effects.

The Budesonide used in this study was a generous gift from Astra Pharma Inc, Lund, Sweden. Dr R N Fedorak is the recipient of a Clinical Investigatorship from the Alberta Heritage Foundation for Medical Research. This work was supported by grants from the Medical Research Council of Canada, the National Institute of Health, and the Crohn's and Colitis Foundation of Canada.

Portions of this work appeared in abstract form: *Gastroenterology* 1993; **104:** A1038.

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