J. Pharm. Pharmacol. 1991, 43: 353–355
Communicated November 30, 1990

COMMUNICATIONS

Relative anti-inflammatory effect of oral dexamethasone-β-D-glucoside and dexamethasone in experimental inflammatory bowel disease in guinea-pigs

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Abstract—The relative anti-inflammatory effect of dexamethasone and a prodrug, dexamethasone-β-D-glucoside, has been assessed in guinea-pigs with experimentally-induced inflammatory bowel disease (IBD). The glucoside prodrug is designed to reach the large intestine following oral administration. The active agent is liberated when the prodrug is hydrolysed by glycosidases of colonic bacteria. Guinea-pigs were administered degraded carrageenan in their drinking water to produce experimental IBD. Starting on day 15, dexamethasone (1·3 μmol kg^{-1}) or dexamethasone-β-D-glucoside (1·3 or 0·65 μmol kg^{-1}) was administered by gastric intubation once daily for 5 days. Relative to control animals, the drug and prodrug treatments significantly ($P<0·05$) reduced the total number of caecal ulcers. While there was no difference statistically between the drug and prodrug treatments, the data suggest that a lower dose of dexamethasone, administered as its glucoside prodrug, could reduce side-effects without reduced efficacy. These results support the hypothesis that localized delivery of dexamethasone to the large bowel can improve pharmacotherapy of IBD by reducing the side-effects associated with corticosteroids.

Inflammatory bowel disease (IBD) has traditionally been treated by sulphasalazine (salicylazasulphapyridine), topical corticosteroids, and in some cases systemic corticosteroids (Hanauer & Kirsner 1988). In the case of topical steroids, administered in the form of enemas, beneficial effects have usually been found only in left-side colitis (Hanauer & Kirsner 1988). The terminal ileum and ascending colon are thus considered inaccessible to topical corticosteroid enemas. The oral route offers the potential for delivery of corticosteroids using either enterically coated delivery systems (Dew et al 1982; Bogentoft et al 1983; Thomas et al 1985) or prodrugs (Friend & Chang 1984, 1985; Tozer et al 1991). An example of the latter system is the use of drug glycosides (e.g. dexamethasone-β-D-glucoside) which are poorly absorbed from the gastrointestinal tract; release of the active agent is mediated by glycosidases of gut microflora residing primarily in the colon.

It has been known for many years that bacteria residing in the large intestine of most mammals produce a variety of enyzmes capable of hydrolysing glycosides (Hawskworth et al 1971; Renwick 1982; Goldman 1983; Brown 1988). Occasionally, toxic substances are released in the large intestine through the liberation of aglycones from certain plant glycosides, such as amygdalin and cycasin (Spatz et al 1966; Goldman 1983); bacterial glycosidases have also been implicated in the activation of sennosides and related compounds in the large intestine (Hardcastle & Wilkens 1970). The use of glycosides to selectively deliver corticosteroids to the large bowel has apparently not been exploited until recently (Friend & Chang 1984, 1985; Tozer et al 1991). There have been a number of steroid-glycosides synthesized (Lange & Amundson 1962; Hirschmann et al 1964); however, these compounds were apparently not prepared as prodrugs for colon-specific drug delivery.

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Some aspects of the pharmacokinetics of colonic delivery of dexamethasone have recently been studied in the guinea-pig (Tozer et al 1991). To complement these studies, we have examined the relative efficacy of dexamethasone in treating IBD when given orally in prodrug and drug solutions. The animal model chosen to examine the relative efficacy of the glycoside based delivery system was carrageenan-induced experimental IBD in guinea-pigs (Watt & Marcus 1971, 1975; Grasso et al 1973; Anver & Cohen 1976).

Materials and methods

Animals. All guinea-pigs (10–11 weeks old, male Hartley, 350–400 g), obtained from Simonsen Laboratories, Gilroy, CA, were housed individually in hanging polycarbonate cages containing hardwood-chip bedding (Sanichips, P. J. Murphy Forest products, Rochelle Park, NJ) in an environmentally controlled animal room on a 12 h light/dark cycle. The animals were fed commercial chow (Purina Guinea Pig Chow #5025) and UV purified drinking water before initiation of the experimentally-induced IBD. The room temperature was maintained at 22°C ± 1°C with a relative humidity of 40 to 48%. Body weights and water consumption were measured throughout the experiment.

Carrageenan and drug treatment. The animals had free access to drinking water containing 5·0% (w/v) of degraded carrageenan (Polygeenan C-16; Sanofi Bioindustries, Moulins Premiers B.P.23, 84800 Isle Sur La Sorgue, France). The carrageenan is supplied as an acid-degraded product from the red seaweed *Eucheuma spinosum*. It has a molecular weight of between 20 000 and 40 000. The carrageenan treatment was continued for 14 days. On day 15, the animals were divided randomly into four groups (n = 8) and the following doses of prodrug or drug were administered via gastric intubation: 1·3 μmol kg^{-1} (0·71 mg kg^{-1}) or 0·65 μmol kg^{-1} (0·36 mg kg^{-1}) of dexamethasone-β-D-glucoside (prepared according to the procedure of Friend & Chang 1984) or 1·3 μmol kg^{-1} (0·50 mg kg^{-1}) of dexamethasone (Sigma Chemical Co., St. Louis, MO). The animals were fasted overnight before drug or prodrug administration; drinking water (containing 5% wt degraded carrageenan) was removed approximately 30 min before drug or prodrug administration. Following intubations, the animals were allowed free access to food and water. The prodrug and drug were each administered in approximately 1·0 mL of H_2O/95% EtOH (0·95:0·05) once daily for 5 days in the morning. A control group of animals received the intubation solution (1·0 mL of H_2O/95% EtOH (0·95:0·05)) once daily for 5 days. All animals continued to receive carrageenan in the drinking water until they were killed by oxygen deprivation using CO_2 approximately 24 h after the last dose of prodrug or drug.

The gastrointestinal tract was removed and the contents were evaluated for consistency and then washed with saline. The

caecal and colonic intestinal mucosa was examined for gross ulceration using a direct light dissecting microscope. Severity of ulceration was assessed arbitrarily according to the number of ulcers found in the caecum (the colon was free of ulcers in all animals) as follows: 0 = 0, 1+ = 1–5, 2+ = 6–10, 3+ = 11–15, 4+ = 16–20, 5+ = 21–30, 6+ = 31–100, 7+ = over 100 ulcers. The total number of ulcers was also used to evaluate the data. The extent of ulceration (number of ulcers) in each group was evaluated using the Student-Newman-Keuls test (multiple comparison), which requires analysis of variance (ANOVA) on all the data to test the global hypothesis that all the samples were drawn from a single population. ANOVA revealed that there was a significant value of F ($P < 0.004$), hence validating the use of the multiple comparison test.

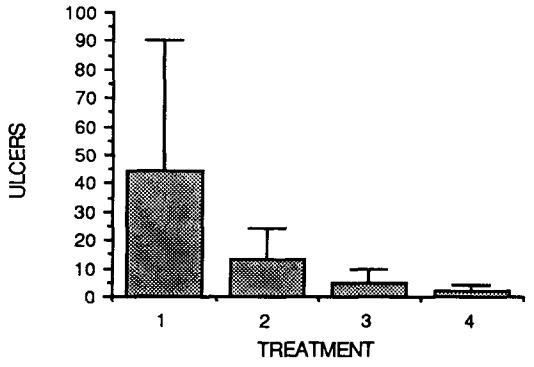

FIG. 1. Mean number of ulcers (±s.d., n = 8) of the large bowel in guinea-pigs given 5% degraded carrageenan in their drinking water. The animals were divided into four groups which received the following treatments from day 15 to 20: 1, H_2O/EtOH (v/v, 95:05); 2, dexamethasone (1·3 μmol kg^{-1}); 3, dexamethasone-β-D-glucoside (0·65 μmol kg^{-1}); and 4, dexamethasone-β-D-glucoside (1·3 μmol kg^{-1}).

Results and discussion

The animals' daily intake of water containing degraded carrageenan was relatively constant during the course of the study; however, water consumption decreased toward the end of the second week of carrageenan treatment. The animals lost weight in all cases over the two week carrageenan treatment as shown in Table 1. However, all animals, regardless of treatment, gained weight from days 15 to 20. There were no differences statistically between any of the groups (control and treatment group) with respect to weight gain or loss over the course of the study.

The severity of damage was assessed in each specimen by counting the number of ulcers (Table 2). An arbitrary scale from 0 to 7, based on the number of ulcers, was used to evalute the relative effectiveness of the treatments. This data treatment is similar to that used by Watt et al (1980). Relative to the control animals with experimentally induced IBD, all the drug treatment groups showed improvement. Qualitatively, the prodrug form of dexamethasone at both doses appeared to be more effective than was dexamethasone in controlling the extent of ulceration.

Comparisons between groups, based on the total number of ulcers, were made using the Student-Newman-Keuls test. The mean number of ulcers in each treatment group is shown in Fig. 1. The multiple comparison test indicated that all three drug treatments (dexamethasone and the two doses of dexamethasone-β-D-glucoside) were significantly different from the control animals ($P < 0.05$). However, within the three drug treatments, there was no significant difference. The lack of statistical significance could be due to the limited number of animals in each group. There was a high degree of variance in the control group (see Fig. 1). Also, the fact that the 0·65 μmol kg^{-1} dose of dexamethasone-β-D-glucoside was equally as effective as 1·3 μmol kg^{-1} dexamethasone indicates that side-effects could be reduced without compromising efficacy. The bioavailability of dexamethasone from its glucoside prodrug is near unity in the guinea-pig (Tozer et al 1991). Therefore, the primary advantage of this delivery system is to localize delivery of the drug to the large intestine thereby allowing the use of lower doses without sacrificing efficacy.

We have shown in normal guinea-pigs that there is a selective advantage (defined as ratio of bioavailability of dexamethasone in the caecal/colonic tissues after oral prodrug administration to that of dexamethasone in the caecal/colonic tissues following a reference i.v. dose (Tozer et al 1991)) of dexamethasone delivery in the guinea-pig large intestine. Assuming a selective advantage in delivery of dexamethasone was obtained in the present study,

Table 1. Average (±s.d.) body weight gain/loss (g) of guinea-pigs during induction of experimental IBD while treated concurrently (days 15 to 20) with dexamethasone, dexamethasone-β-D-glucoside, or the dosing vehicle (control).

Treatment[a]	Day 7[b]	Day 14	Day 20
Control	−65±27	−44±48	−8±39
Dexamethasone, 1·3 μmol kg^{-1}	−55±35	−31±21	+17±25
Dexamethasone-β-D-glucoside, 0·65 μmol kg^{-1}	−53±38	−14±26	+24±23
Dexamethasone-β-D-glucoside, 1·3 μmol kg^{-1}	−58±26	−30±34	+18±27

[a] Animals were administered the dosing vehicle (ca. 1·0 mL; H_2O/EtOH, 95:05) once daily for 5 days by gastric intubation without (control) or with the drug or prodrug beginning on day 15 of the carrageenan treatment. Weight gain/loss was calculated from the difference in weight from the previous 7 day period. [b] Day after starting carrageenan treatment.

Table 2. Effect of dexamethasone (1·3 μmol kg^{-1}), dexamethasone-β-D-glucoside (1·3 or 0·65 μmol kg^{-1}) and intubation solution alone on the incidence and severity of ulceration of the caecum of the guinea-pigs given 5% degraded carrageenan in their drinking water.

	No. of animals with graded severity[a]							
Severity of ulceration:[a]	0	1+	2+	3+	4+	5+	6+	7+
Treatment								
Control	0	0	1	1	2	0	2	2
Dexamethasone, 1·3 μmol kg^{-1}	1	1	2	0	2	1	1	0
Dexamethasone-β-D-glucoside, 0·65 μmol kg^{-1}	2	3	1	2	0	0	0	0
Dexamethasone-β-D-glucoside, 1·3 μmol kg^{-1}	4	4	0	0	0	0	0	0

[a] Severity assessed arbitrarily according to the number of ulcers found in the caecum (the colon was free of ulcers in all animals) as follows: 0 = 0, 1+ = 1–5, 2+ = 6–10, 3+ = 11–15, 4+ = 16–20, 5+ = 21–30, 6+ = 31–100, 7+ = over 100 ulcers.

the data indicate that local delivery of corticosteroids is important in controlling IBD. Such a result is consistent with the proposed mechanism of action of anti-inflammatory steroids (localized response in target cells) in inflammatory conditions (Swartz & Dluhy 1979; Schleimer 1985) including IBD (Lee et al 1980; Hawkey & Truelove 1981).

The results are also consistent with a number of reports concerning the use of dexamethasone to control IBD (Gallone et al 1980; Horntrich et al 1980; Del Soldato et al 1985; Hashizume et al 1989). In one case (Hashizume et al 1989), dexamethasone was administered directly into the ascending colon via an artificial caecal fistula. Although the report was limited to treatment of one patient, and sulphasalazine (4 g daily) was continued throughout the dexamethasone treatment, the results suggest that local delivery of dexamethasone to the caecal and colonic tissues may be useful in controlling IBD of the caecum and ascending colon. While the artificial caecal fistula technique of Hashizume et al is novel, the same effect can be derived from an oral delivery system and it would therefore probably gain wider acceptance.

While the experimental IBD guinea-pig model is useful in the initial screening of new pharmacotherapeutic treatments, the distribution of gut microflora and associated metabolic activity differ considerably compared with man. Ideally, there should be little or no β-glucosidase activity in the stomach and small bowel and markedly high concentrations of β-glucosidase activity in the large bowel. However, the guinea-pig has relatively high bacterial β-glucosidase activity in its small intestine (Hawksworth et al 1971). In man, the bacterial β-glucosidase activity in the small intestine is less than one-hundredth that of guinea-pigs, while activity in the colon is comparable (Hawskworth et al 1971). Thus, the potential effectiveness of the glucoside prodrug approach for selective local delivery of corticosteroids to the large bowel mucosa is probably much greater in man than can be demonstrated in most laboratory animals, including the guinea-pig (Tozer et al 1991).

In summary, the data from this study support the hypothesis that corticosteroids can be delivered to, and released in, the large intestine of mammals and as a result, higher caecal and colonic tissue levels of the drug can be obtained than is possible when the compound is delivered systemically (Tozer et al 1991). These studies need to be extended to other animal models and the variables associated with colonic delivery via prodrugs (dose level, dosing frequency, solid dosage form, effect of fasting and non-fasting) also need to be examined.

This work was supported by United States Public Health Service Grant GM 35147, awarded by the National Institute of General Medical Sciences. We would also like to acknowledge a scholarship from the New Zealand Pharmacy Education and Research Foundation and the technical assistance of Jaqueline Tefft.

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