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A comparison of prednisolone absorption and the effect of sodium salicylate and mannitol in normal and damaged rat bowel

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Summary

A model of ulcerated bowel was produced in the rat rectum by treatment with Brij 35 suppositories. Prednisolone absorption from enemas in normal and damaged bowel was assessed using an in situ rectal loop. In animals 20 h after damage drug absorption was reduced but returned to normal levels after one week. Co-administration of equiosmolar sodium salicylate (1 and 2% w/v) and mannitol (2.5 and 4.1% w/v) solutions in normal bowel induced a significant water flux into the lumen and changed the plasma tritium profile compared to the control. In damaged bowel plasma tritium levels remained low following co-administration of mannitol (2.5% w/v) but were restored to normal control levels by co-administration of salicylate (1% w/v). The altered absorption in normal bowel can be attributed in part to osmotic stress but in damaged bowel the difference between the effect of equiosmotic solutions of salicylate and mannitol suggests that a different mechanism is operating.

Introduction

In the diseased bowel, drug absorption may be delayed, depressed, enhanced or normal (Parsons, 1977) and there appears to be no clear relationship between the disease state and drug bioavailability. Prednisolone is an example of a drug for which a variety of changes in bioavailability have been reported in patients with intestinal pathology (Bergrem and Opedal, 1983; Elliot et al., 1980; Milsap et al., 1983; Tanner et al., 1981). The factors responsible for changes in drug absorption and the role of excipients in the damaged bowel are poorly understood.

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The investigation of drug absorption in the diseased bowel requires the use of appropriate animal models. In most studies intestinal pathology in laboratory animals has been induced by either irradiation or administration of antimitotic drugs (Mattila and Venho, 1978). These treatments not only reduce the absorptive surface area throughout the bowel but also affect the proliferating compartments in other body tissues. Holyhead et al. (1983) induced localized rectal ulceration and mucosal inflammation in the rat by administration of suppositories of Brij 35 (polyoxyethylene 23 lauryl ether). The extent of tissue damage was shown to be reproducible and it was demonstrated that following insult rectal regeneration was rapid and by one week complete. Using this model the absorption of [^{3}H]prednisolone was assessed in normal tissue and in tissue 20 h and 1 week after treatment with Brij 35 suppositories using the in situ rat rectal loop procedure described by Thomas et al. (1984). The effect of sodium salicylate, which has been reported to enhance drug absorption non-specifically (Nishihata et al., 1982), and equiosmotic solutions of mannitol on prednisolone absorption in normal and damaged tissue was also investigated.

Materials and Methods

Solutions and suppositories

All the test solutions were prepared in modified Krebs buffer (NaCl 107 mM, KCl 4 mM, $NaHCO_3$ 5 mM, $CaCl_2$ 1 mM, $MgCl_2 \cdot 6H_2O$ 0.98 mM, KH_2PO_4 1.03 mM and glucose 20 mM; all reagents were Analar grade) containing [^{3}H]prednisolone (15 μCi/ml Amersham Int., Slough, Berks), prednisolone 0.1 mg/ml (MSD, Hoddesdon, Herts) and [^{14}C]polyethylene glycol 4000, a non-absorbable marker ([^{14}C]PEG 4000, 0.5 μCi/ml, Amersham Int., Slough, Berks). Test solutions containing sodium salicylate (2% w/v and 1% w/v, BDH Chemicals, Poole, Dorset) and mannitol (4.1% w/v and 2.5 w/v, BDH Chemicals, Poole, Dorset) were prepared and the osmotic pressure determined to be 443 and 576 mOsm, respectively, for each pair of solutions, using an Osmet osmometer. Suppositories of polyoxyethylene 23 lauryl ether (Brij 35, Sigma (London) Chemicals, Poole, Dorset) mean weight 78 mg were prepared.

Normal tissue

Male Wistar rats (190–210 g) were fasted overnight with water ad libitum, and anaesthetized with pentobarbitone (i.p. Sagatal 90 mg/kg) immediately prior to the preparation of a rectal in situ loop as described by Thomas et al. (1984). 1 ml test solution was introduced into the loop and luminal and tail tip plasma samples collected at intervals for 40 min.

Damaged tissue

Fasted rats were anaesthetized with pentobarbitone (i.p. Sagatal 66 mg/kg) and a Brij 35 suppository inserted into the distal portion of the rectum. A wax-covered cotton wool bud was inserted into the anus to prevent leakage. The cotton wool bud was removed after 60 min, and the animals allowed to recover with food and water

ad libitum. After 20 h or one week the animals were anaesthetized and the rectal absorption of prednisolone from the test solutions determined as described above.

Histology

At the end of each experiment the rectal tissue was fixed in either Carnoys or Bouin Hollande fluid and then processed by standard procedures for histological examination.

Results

Water flux in the rectal loop was calculated from the change in [^{14}C]PEG 4000 activity during the experiment and expressed as the percentage change in the luminal fluid volume from time zero. Over the experimental period there was no net water flux following the administration of the drug solution to normal animals and to animals one week after damage. However, there was a significant increase in luminal fluid volume (32.3 ± 10.2% at t = 40 min, mean ± S.D.) in animals 20 h after damage ($P < 0.05$, Student's unpaired t-test). The hypertonic salicylate (2 and 1% w/v) and mannitol (2.5 and 4.1% w/v) solutions induced a net water flux into the lumen compared with the control in both normal and damaged tissue but there was no significant difference in water flux produced by the individual solutions.

At each time interval the measured concentration of [^{3}H]prednisolone remaining in the loop was corrected for the changes in fluid flux and expressed as a percentage of the original dose. The semi-logarithmic plot of the percentage of the original dose remaining against time showed a linear relationship indicating that the luminal disappearance of prednisolone followed first-order kinetics. The disappearance rate constant was calculated for each solution (Table 1).

The concentration of tritium in the plasma samples was calculated as (dpm/mg)

TABLE 1

THE DISAPPEARANCE RATE CONSTANT FOR THE UPTAKE OF [^{3}H]PREDNISOLONE FROM RECTAL LOOPS (MEAN ± S.D., n = 6)

Loop tissue	Microenema	K (min^{-1})
Normal	prednisolone alone	0.012 ± 0.004
*20 h after damage	prednisolone alone	0.008 ± 0.002
1 week after damage	prednisolone alone	0.012 ± 0.004
Normal	prednisolone + 2.5% mannitol	0.010 ± 0.002
Normal	prednisolone + 1% salicylate	0.010 ± 0.002
Normal	prednisolone + 4.1% mannitol	0.010 ± 0.003
Normal	prednisolone + 2% salicylate	0.011 ± 0.003
*20 h after damage	prednisolone + 2.5% mannitol	0.004 ± 0.001
20 h after damage	prednisolone + 1% salicylate	0.010 ± 0.001

*Significantly different to the other groups ($P < 0.05$ unpaired Student's t-test).

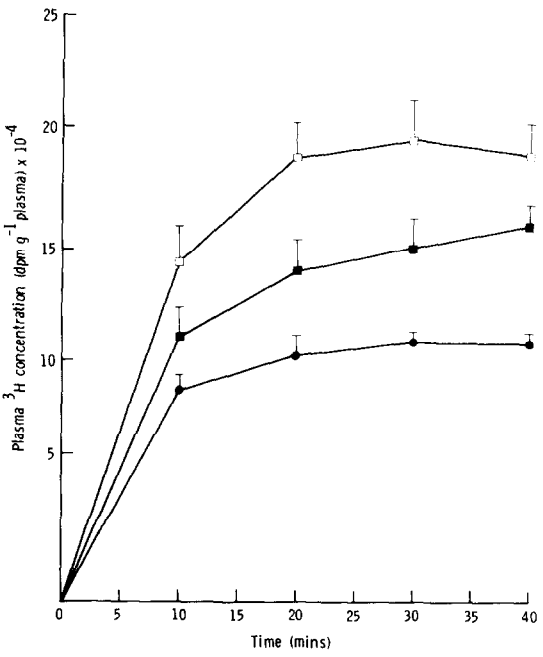

Fig. 1. Plasma tritium concentration with time following rectal administration of [^{3}H]prednisolone solution. □, normal rats; ●, rats 20 h after damage; ■, rats one week after damage. Each value is the mean + S.E.M. of 6 animals.

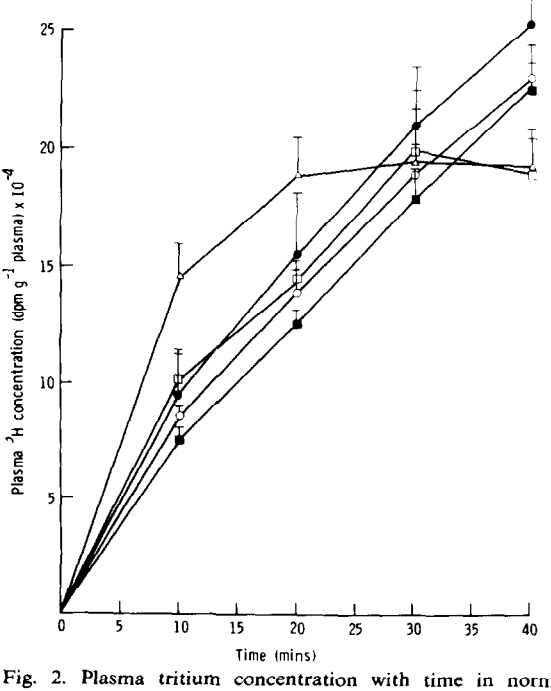

Fig. 2. Plasma tritium concentration with time in normal rats following rectal administration of [^{3}H]prednisolone enemas. △, prednisolone alone; □, prednisolone + 2.5% mannitol; ●, prednisolone + 1% salicylate; ○, prednisolone + 4.1% mannitol; ■, prednisolone + 2% salicylate. Each value is the mean + S.E.M. of 6 animals.

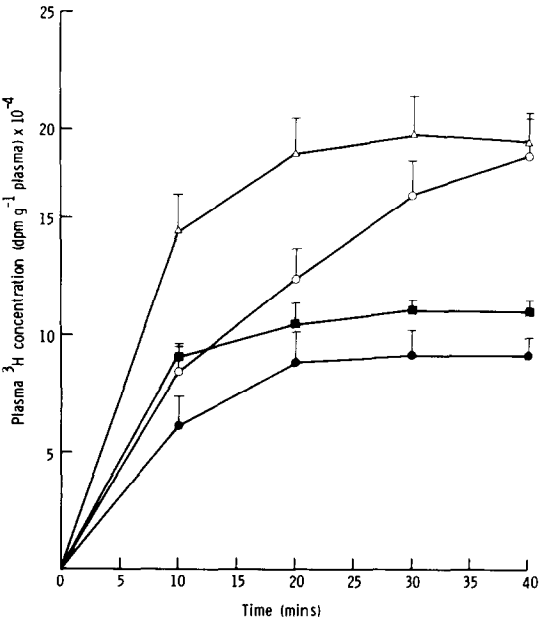

Fig. 3. Plasma tritium concentration with time following administration to [^{3}H]prednisolone enemas to normal rats and rats 20 h after damage with a Brij 35 suppository. △, prednisolone alone in normal rats; ■, prednisolone alone in rats 20 h after damage; ○, prednisolone + 1% salicylate in rats 20 h after damage; ●, prednisolone + 2.5% mannitol in rats 20 h after damage. Each value is the mean + S.E.M. of 6 animals.

and then corrected to a standard dose of 10 μCi. Plasma activity against time curves were then plotted for each solution. Plasma tritium levels were significantly reduced ($P < 0.05$) in animals 20 h after treatment with a Brij 35 suppository compared with control animals and with animals one week after treatment (Fig. 1). In normal rats at 10 min, the mean plasma tritium level was higher ($P < 0.05$) than that measured following co-administration of salicylate or mannitol. At 40 min, the absorption of [^{3}H]prednisolone was depressed compared to the 1% and 2% salicylate-treated groups (Fig. 2). In the damaged rats, co-administration of 2.5% mannitol had no effect on prednisolone absorption, but 1% salicylate significantly enhanced absorption (Fig. 3).

Histological examination of the tissue exposed to Brij 35 confirmed the published descriptions of rectal ulceration, and the rapid regeneration of the tissue during the following 7 days. The prednisolone solution and the test solutions containing 2% sodium salicylate or 4.1% mannitol induced no detectable histological damage in normal tissue (Fig. 4A, B and C). In the tissue 20 h after damage examination of the site of maximum damage revealed no detectable changes to the squamous regenerating epithelium, nor the regenerating glands, following exposure to the prednisolone solution and the drug solution containing 1% sodium salicylate or 2.5% mannitol

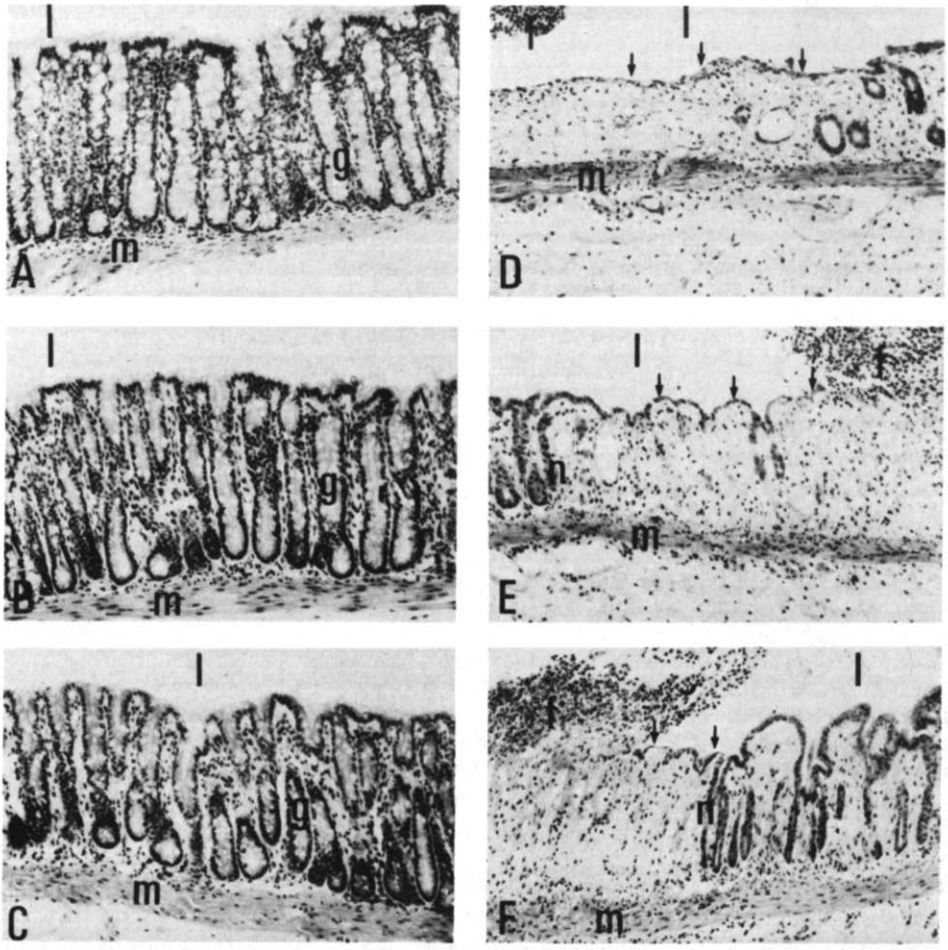

Fig. 4. Photomicrographs of rectal mucosae from normal rats (A, B and C) and rats 20 h after damage with suppositories of Brij 35 (D, E and F). Tissue in A and D are from loops exposed to the prednisolone solution alone. Tissue in B and E are from loops exposed to prednisolone solution with co-administration of 2% (B) and 1% (E) sodium salicylate. Tissue in C and F are from a loop exposed to a prednisolone solution with co-administration of 4.1% (C) and 2.5% (F) mannitol. Note the addition of salicylate (B and E) or mannitol (C and F) to the prednisolone solution induces no additional damage compared with the respective controls (A and D). l = lumen; m = muscularis mucosa; g = glands; arrow = squamous regenerating epithelium; C = fibrin clot; n = new glands. All micrographs at ×130.

(Fig. 4D, E and F). The picture of damage conformed to that produced by the initial insult.

Discussion

The reduced rectal absorption of prednisolone 20 h after damage and the recovery of absorption to normal levels after one week closely parallel the tissue regeneration

quantified by Holyhead et al. (1983). From this it is concluded that the reduction in prednisolone absorption is a consequence of the ulceration and inflammation following administration of the Brij 35 suppository. While the tissue damage in this study was induced experimentally and therefore does not follow the natural history of large bowel disease, the model is clearly of value for the investigation of drug bioavailability across ulcerated and inflammed mucosae.

The phenomenon of enhanced drug absorption when administered with salicylate is well documented (Nishihata et al., 1982). In the present study drug absorption from normal bowel was initially reduced in the presence of salicylate but at 40 min the plasma tritium profile continued to rise whilst the profile for the normal group had reached a plateau. A significant water flux into the lumen occurred in the presence of the hypertonic salicylate solutions which may account for the delayed absorption of prednisolone (Mattila and Venho, 1978). The similarity in prednisolone absorption and water flux following co-administration of equiosmotic salicylate and mannitol solutions also suggests that water flux delays absorption. In addition enhanced absorption may result from osmotic stress which can produce a change in the integrity of the epithelium, possibly through the transient disruption of the intercellular junctional complexes, with concomitant absorption via the paracellular pathway. Delayed absorption from hyperosmotic enemas was not observed in other studies of salicylate-enhanced absorption (Yoshioka et al., 1982) and osmotic effects have been discounted in salicylate-enhanced absorption (Fix et al., 1983). Nishihata et al. (1984) have proposed that the enhancing effect of salicylate is due to a purely cell-mediated mechanism although it was observed that salicylate enhanced absorption of cefoxitin into isolated red cells varied inversely with media osmolarity.

In the damaged bowel the less concentrated solutions of mannitol and salicylate were used in order to minimize any damage additional to that of the initial insult. Enhanced drug absorption in this tissue was observed only following co-administration of salicylate, in contrast to the observations on the normal rectum. Mucosal damage changes significantly the barrier across which these compounds promote absorption. Breschi et al. (1981) concluded from in vitro studies of intestinal absorption that with epithelial degeneration the limiting step to absorption became situated in the deeper layers of the gut. In the present study the oedema and inflammation of the lamina propria may represent the elements of a deeply placed limiting barrier to absorption across which salicylate alone can act, possibly via the expression of a cell-mediated interaction.

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