International Journal of Pharmaceutics, 40 (1987) 181–185

Elsevier

IJP 01349

Research Papers

Formulation of Fenbufen suppositories. I. Quantitative histological assessment of the rectal mucosa of rats following treatment with suppository bases

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(Received 25 May 1987)
(Accepted 11 June 1987)

Key words: Suppository; Base; Qualitative histology; Rat

Summary

Quantitative histology was used to assess the interaction of suppositories of hard fats (Suppocire AP and OS1X; Witepsol H12 and H19) and polyethylene glycol (PEG 1500) with the rectal mucosa of rats. All bases significantly increased epithelial cell loss but only following PEG treatment was the natural capacity of the epithelium to resist disruption exceeded, thereby resulting in areas of complete desquamation with a bare basal lamina. On the basis of these data it was possible to rank the interaction of the suppositories with the rectal lining; Suppocire OS1X < Suppocire AP = Witepsol H12 < H19 < PEG 1500.

Introduction

The administration of a suppository exposes a small region of the bowel lining to undiluted excipient and drug. The area of this region, and the duration of the exposure is determined by the liquifaction and spreading characteristics of the base. The nature of the base and the drug will determine whether or not there is an interaction with the rectal mucosa and the depth of the layers involved (Tupper et al., 1982). When epithelial loss is extensive, the bowel wall undergoes an inflammatory response (Holyhead et al., 1983) which can change the nature of the absorption barrier (Whiston et al., 1985). During formulation development it is clearly desirable to select bases with no irritant properties, particularly when repeated dosing will be required. The aim of this study was to use quantitative histology as a tool for comparing the response of the rat rectal mucosa to a range of suppository bases, so that the bases could be ranked with respect to their local interaction. These data would be pertinent to formulation development of Fenbufen suppositories.

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Materials and Methods

Suppositories (6.5 × 4.0 mm) were prepared from the following bases: Witepsol H12 (saturated fatty acid glycerides of chain length C_{10-18}; Dynamit Nobel, Slough, U.K.); Witespol H19 (Witespol H12 plus skin protective agent Softigen

701; Dynamic Nobel, Slough, U.K.); Suppocire AP (glycerol and polyethylene glycol esters of fatty acids C_{12-18}; Alfa Chemicals, Workingham, U.K.); Suppocire OS1X (semi-synthetic saturated glycerides of fatty acids C_{10-18}; Alfa Chemicals, Workingham, U.K.); polyethylene glycol (PEG) 1500 (polymer of ethylene oxide, mol. wt. 1500; Hythe Chemicals, Hythe, U.K.).

Groups ($n = 5$) of 180–200 g male Wistar rats were fasted overnight with water ad libitum. Animals were anaesthetised with sodium pentobarbitone (i.p. 75 mg/kg). A suppository was inserted into the rectum and the animals placed on a heated table at 38°C with their caudal portion raised slightly to prevent leakage. After one hour the animals were either killed, or in the case of one PEG 1500-treated group allowed to recover and killed after 24 h. A control group was treated in a similar way but without suppository treatment. The anal canal and rectum were removed and fixed in Carnoys fluid, dehydrated, treated with chloroform and embedded in wax. Sections of longitudinal profiles were prepared from the rectal tissues and stained with haematoxylin and eosin.

Four sections were selected at random from each animal. A count was made of the following features occurring at the interglandular sites of the surface epithelium: (a) type 1 change – cells detached from the surface or in the process of detachment; (b) type 2 change – epithelium reduced in height being composed of cuboidal and/or squamous cells; and (c) type 3 change – complete epithelial desquamation and bare basal lamina. The counts were made in sequence for each 20 interglandular sites from the anorectal junction to the 100th gland. Data were screened using the Kruskal-Wallis one-way analysis of variance and individual groups were compared using the Mann-Whitney *U*-test for non-parametric data.

Results

The three types of change counted are illustrated in Fig. 1. Cell loss was present at all sites sampled in the untreated control animals and was increased significantly by suppositories of all bases (Table 1). Small counts of type 2 change were present at 3 sites after Suppocire AP and Witepsol H12 but type 2 change was a distinctive feature of all sites following Witepsol H19 and PEG 1500 treatment. The type 3 change was detected only in the PEG 1500-treated tissue where it was present in all regions sampled. However, the epithelial basal lamina was not removed and there was no

TABLE 1

Counts of the 3 types of changes observed in the rectal epithelium between the anorectal junction and the 100th interglandular site of rats following suppository treatment (mean ± S.E.M.; n = 5)

Treatment	Type of change	Interglandular sites				
		1–20	21–40	41–60	61–80	81–100
Untreated control	1	1.4 ± 0.6	1.4 ± 0.3	1.2 ± 0.2	1.6 ± 0.4	0.9 ±0.2
Suppocire OSIX	1	4.3 ± 0.6	6.7 ± 0.8	6.2 ± 0.5	4.3 ± 0.9	5.3 ±0.9
Suppocire AP	1	3.8 ± 0.8	4.5 ± 1.2	6.0 ± 1.3	5.8 ± 0.3	4.9 ±1.4
	2	0.1 ± 0.1	0	0.2 ± 0.1	0.6 ± 0.2	0.3 ±0.1
Witepsol H12	1	3.4 ± 0.5	5.4 ± 0.8	4.2 ± 1.3	4.3 ± 1.0	3.5 ±1.0
	2	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0	0.1 ±0.1
Witepsol H19	1	1.8 ± 0.1	4.8 ± 1.2	8.1 ± 1.9	8.4 ± 2.2	6.35 ± 2.1
	2	1.0 ± 0.4	1.5 ± 0.6	3.7 ± 1.1	3.0 ± 0.9	3.2 ±0.8
Peg 1500	1	2.1 ± 0.3	4.1 ± 0.3	3.9 ± 1.3	4.0 ± 1.0	2.8 ±0.3
	2	1.9 ± 0.7	4.5 ± 1.3	4.0 ± 1.1	3.1 ± 0.6	3.4 ±1.7
	3	0.4 ± 0.4	3.9 ± 2.1	8.1 ± 3.9	6.0 ± 3.2	0.6 ±0.3
Peg 1500 24 h following treatment	1	2.1 ± 0.5	2.1 ± 0.4	1.7 ± 0.7	2.0 ± 0.4	1.6 ±0.4
	2	0.4 ± 0.2	1.1 ± 0.5	2.0 ± 0.8	1.3 ± 0.3	0.9 ±0.6
	3	0	0.1 ± 0.1	0.1 ± 0.1	0	0.1 ±0.1

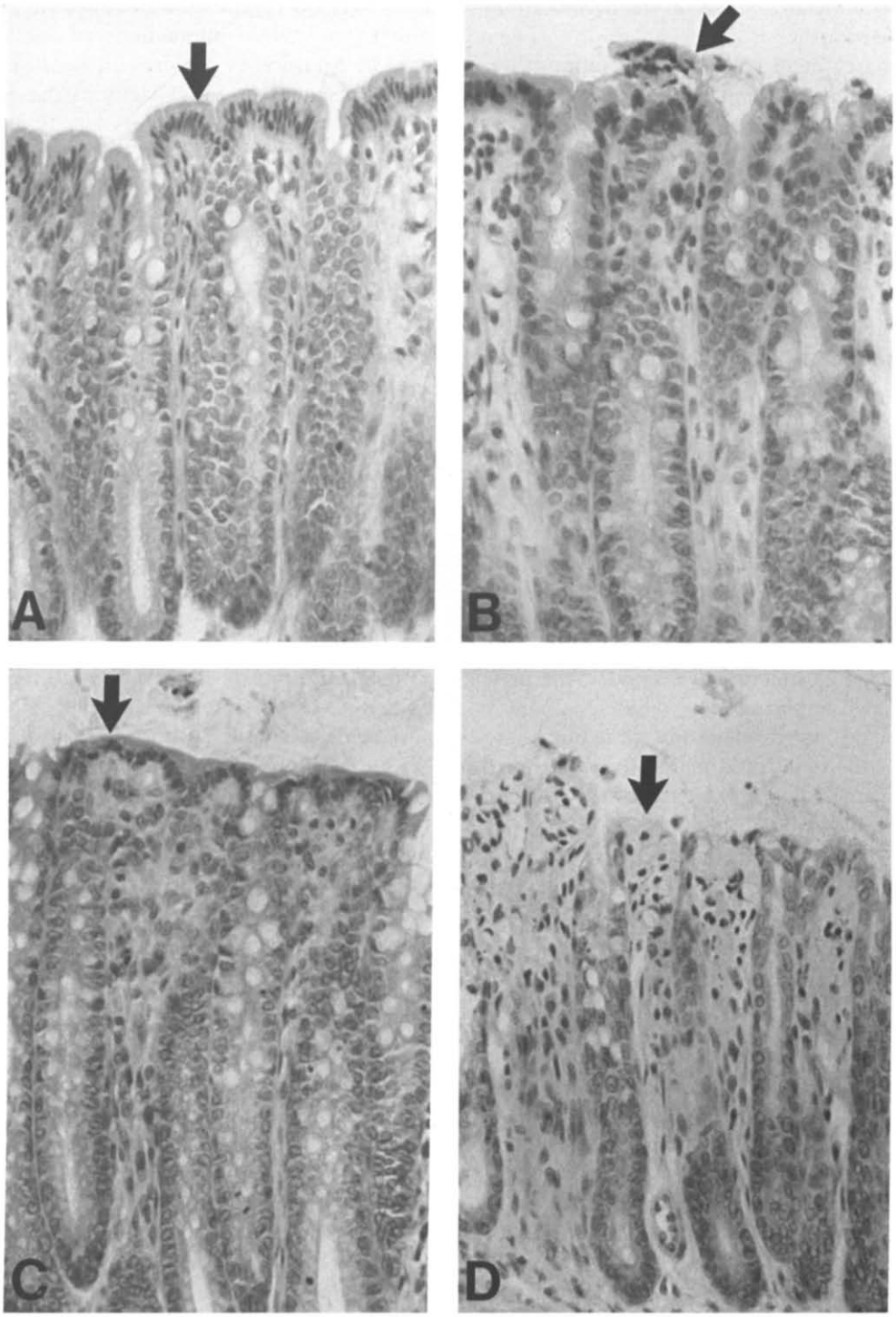

Fig. 1. Photomicrographs of the rectal mucosa of control (A), Suppocire AP- (B), Witepsol H19- (C), and polyethylene glycol 1500

evidence of significant changes in the organisation of the lamina propria.

24 h after treatment with the PEG suppository there was a significant regeneration of the rectal epithelium. It was characterised by the reconstitution of an intact epithelium but it still exhibited some sites of type 2 change (Table 1).

Discussion

The size of the functional compartment within the epithelium of the gastrointestinal tract is determined by the balance between cell loss to the lumen and cell recruitment from the proliferative compartment in the crypts (Wright, 1980). The extent of normal cell loss is shown in the tissue from the untreated control rats. All the experimental tissues showed evidence of significantly increased cell loss. It seems unlikely for two reasons that trauma during administration could have accounted for the changes observed. Firstly, the length of the suppository was less than the length of the rectal lining quantified. Secondly, the profiles of interaction, particularly when type 2 and 3 changes are considered reflect the spreading characteristics of the bases proximal and distal to the site of administration. On the basis of the changes counted, the bases fall into 3 groups. The first, comprising Suppocire OS1X, Suppocire AP and Witepsol H12, induced type 1 change and minimal or no type 2 change. The second typified by Witepsol H19 showed significant type 1 and 2 changes at all sampled sites. Type 2 change here represents the adaptive response of an epithelium to a sudden increase in cell loss. In the small intestine Johnson et al. (1978) described a similar reduction in epithelial cell height as cells changed their shape in order to maximise their luminal surface and maintain an intact barrier. The third group contained PEG alone: this base induced the 3 types of changes with complete epithelial desquamation at many sites. Desquamation resulted because cell loss exceeded the capacity of the remaining cells to maintain an intact barrier by changing their shape.

The distruption of the epithelial barrier by PEG 1500 may be regarded as evidence of an irritant interaction. While interactions of the fat bases may be regarded as non-irritant because they did not exceed the natural capacity of the epithelium to prevent its disruption. It is clear that the modification of Witepsol H12 by the inclusion of Softigen 701 significantly increased the interaction of the base but did not lead to irritancy. This effect might also arise when some classes of drugs are formulated with these bases.

The recovery experiment was undertaken to establish the speed of epithelial regeneration following treatment with the base demonstrating the greatest level of histologically detected interaction. The recovery from PEG 1500 at 24 h was significant but incomplete, for although the barrier was restored all cells had not attained their normal height, Holyhead et al. (1983) described surface cell extending from the borders of ulcers produced by Brij 35 suppositories some 6 h after treatment. This base induced more extensive cell loss both in terms of depth of interaction and area of spreading (Tupper et al., 1982). Ito et al. (1984) described the rapid restoration of an intact surface epithelum in the rat stomach after experimental surface desquamation with a variety of solutions. Within 15 min 75% of the desquamated surface was covered and at 1 h only minor areas remained. It is likely that a similar rapid migration takes place in the rectum to restore the barrier, but a longer time is required before the barrier reaches normal proportions. Thus the type 2 changes at 1 and 24 h are not strictly comparable. At 1 h, as the initial response to insult, the columnar epithelial cells were changing to cuboidal and squamous in an attempt to prevent epithelial disruption. At 24 h type 2 change represented cells returning to normal height as cells are recruited from the responding proliferative compartment.

These studies indicate that local interactions do occur between suppositories and the rectal mucosa. The interactions can be quantified histologically and bases ranked with respect to interaction and irritancy. Such data should be one of the elements used in the selection of bases for suppository formulation.

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