Mechanism of Absorption Enhancement in Humans After Rectal Administration of Ampicillin in Suppositories Containing Sodium Caprate

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Purpose. The medium chain fatty acid sodium caprate (C10) is approved as an absorption enhancer but its mechanism of action has not been studied in humans. The aim of this study was to investigate the mechanism of action of C10 in human subjects after rectal administration.

Methods. Twelve healthy human subjects were randomised to receive ampicillin suppositories with (AM-C10) or without (AM) C10. Serum and urine samples were collected and analysed for ampicillin by HPLC. Rectal biopsies were taken before and 25 min (approximate time of maximum serum concentration, C_{max}, for ampicillin) and 185 min (during the final part of the elimination phase) after rectal administration of the suppositories. The osmolality of the rectal fluid was also measured.

Results. AM-C10 administration increased C_{max}, area under the serum concentration-time curve (AUC) and urinary recovery of ampicillin 2.6-, 2.3- and 1.8-fold, respectively, compared to AM. Histological examination of the biopsies showed that AM-C10 exposure resulted in reversible mucosal damage that occurred at the same time as the C_{max} for ampicillin while AM prolonged mucosal damage. A reversible increase in rectal fluid osmolality was observed with both treatments.

Conclusions. AM-C10-enhanced absorption of ampicillin coincides with non-specific damage to the rectal mucosa. C10 itself as well as the suppository base and the hyperosmolality of the rectal fluid contributed to this effect. However, the histological damage was reversible with AM-C10, suggesting that C10 also has a protective effect on the rectal mucosa.

KEY WORDS: absorption enhancement; medium chain fatty acid; sodium caprate; rectal drug absorption; tight junction; suppository; epithelial permeability; human rectal morphology.

INTRODUCTION

There are no studies on the mechanism of action of absorption enhancers in vivo in humans, even though that this class of pharmaceutical additives has been extensively studied for almost two decades (1). Animal studies suggest that various classes of absorption enhancers may have damaging effects on mucosal tissues (2–4). The potential toxicity of these compounds has restricted their use in humans (5,6). Naturally occurring substances with absorption enhancing properties could provide safe alternatives. For instance, the sodium salt of the medium chain fatty acid capric acid, sodium caprate (C10), modifies the tight junction barrier of various intestinal epithelia (7,8). C10 is used in an approved rectal drug product in Sweden, Doktacillin™, which comprises a triglyceride base containing sodium ampicillin and C10, and Japan (9). To our knowledge the Doktacillin™ suppository is the only example of an approved drug product that contains an absorption enhancer.

In this study, we investigated the mechanism of action of C10 in Doktacillin™ suppositories in healthy volunteers. Matching suppositories without C10 were used as controls. The serum concentrations of ampicillin in the two groups were investigated, as was the rectal morphology at light and electron microscopic levels. Formulation-induced effects on the rectal fluid osmolality were also investigated.

MATERIALS AND METHODS

Subjects

The study subjects comprised 12 healthy volunteers (5 men, 7 women) aged 21–36 (median 25) years. They had no signs or symptoms of gastrointestinal disease and had normal dietary habits. Blood tests showed values of haemoglobin, C-reactive protein, creatinine and liver enzymes within the normal range in all subjects. Ten were free of medication, one woman had dihydroergotamine on demand for hypotension and one woman had contraceptives. Two weeks prior to the study the subjects underwent a rigid sigmoidoscopy without preceding enema, and a rectal biopsy was collected for the histological examination.

Pharmaceutical Preparations

Ampicillin suppositories containing C10 (AM-C10; Doktacillin™; ASTRA, Södertälje, Sweden) were obtained from the local hospital pharmacy, and had the following composition: 250 mg ampicillin, 25 mg C10 and 950 mg Pharmasol B-105 (10). The corresponding ampicillin suppositories without C10 (AM) were produced ex tempore by Centrallaboratoriet, Apoteksbolaget, Stockholm, Sweden (composition: 250 mg ampicillin, 950 mg Pharmasol B-105). Pharmasol B-105 was a generous gift from Dr. Tsukasa Hamasaki at NOF Corporation, Tokyo, Japan and ampicillin for AM was obtained from Doktacillin™ powder for injection (ASTRA, Södertälje, Sweden). The sodium salt of ampicillin was used throughout.

Study Design

The subjects were randomised to receive either AM-C10 or AM. An IV cannula was inserted and blood samples for routine blood tests and samples for serum analysis of ampicillin were drawn. The suppositories were then administered in a double-blind manner. The subjects were given two suppositories each, since the Doktacillin™ suppositories are intended for children and the study comprised of adults. One subject receiving AM suppositories did not complete the study.

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NOTATIONS: AM; ampicillin suppositories without sodium caprate, AM-C10; Doktacillin™ suppositories (ampicillin suppositories containing sodium caprate) C10; sodium caprate.

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Blood and Urine Samples

IV blood samples were drawn 0, 20, 60, 90, 120, 180 and 240 min after administration of the suppositories. The blood was allowed to coagulate for 30 min before the samples were centrifuged for 10 min. Serum samples were frozen at −20°C pending analysis for ampicillin. Urine was collected for 240 min after administration of suppositories. Urine volume was measured and samples were frozen at −20°C pending analysis for ampicillin. Urinary recovery was not obtained from two of the placebo subjects due to sampling errors.

Ampicillin Analysis

Ampicillin levels in serum and urine were analysed within 6 months from the time of sampling using a reversed phase HPLC method as described previously, with minor modifications (11).

Pharmacokinetics

The areas under the serum concentration-time curves (AUCs) were calculated using the log trapezoidal rule with extrapolation to infinity. The area from the last point to infinity was estimated by dividing the last measured serum concentration by the terminal rate constant.

Biopsies and Microscopy

Rectal biopsies (8 cm from the anal verge in the dorsal rectal wall) were taken two weeks prior to the study ("time 0") and at 25 and 185 min after administration of the suppositories. The biopsies were fixed in 1.5% glutaraldehyde/1.5% formaldehyde in 0.1 M phosphate buffered saline (Karnovsky's fixative). Two biopsies were taken, one for examination with light microscopy and one for transmission electron microscopy.

Light Microscopy

After fixation, the biopsies were dehydrated and embedded in paraffin. Five μm sections were stained with hematoxylin/eosin. For each biopsy (0, 25 and 185 min) two sections were randomly picked for examination. The sections (a total of 72) were examined by four independent blinded examiners. The biopsies were scored according to the degree of damage to the mucosa: see figure legend in fig. 2. Microphotographs were taken using a Zeiss Axioskop fitted with a microscope camera MC 100, Zeiss, Oberkochen, Germany and Kodak Gold 100 film.

Transmission Electron Microscopy

After fixation, biopsies were postfixed in 1% osmium tetroxide, dehydrated in ethanol and embedded in Epon. Seventy-five nm sections were contrasted with uranyl acetate and lead citrate. One section was made for each biopsy (0, 25 and 185 min). The sections (a total of 36) were examined in a Philips 420 or Philips EM 301 electron microscope operated at 60 kV.

Osmolality

Rectal fluid samples for osmolality measurements were drawn through a plastic catheter via the sigmoidoscope before the biopsies were taken. At time 0, there was not enough rectal fluid present in the rectum to sample. At 25 and 185 min after administration of the suppositories, small samples of rectal fluid, generally less than 300μl, were collected. The samples were mixed with 0.1% sodium azide (1:1) to avoid bacterial growth, and kept refrigerated until they were analysed. Osmolality was measured using a Vapor Pressure Osmometer (Wescor Inc., Logan UT, USA). Four samples were too small in volume or too solid to allow determination of osmolality, resulting in exclusions of one AM-C10 and one AM recipient at 25 min and two AM recipients at 185 min.

Statistics

The difference between two independent samples was investigated using the Mann Whitney test and the Wilcoxon signed rank test was used to investigate the difference between paired samples. A three way ANOVA, partially nested design, was used to investigate the differences between the biopsies.

Ethics

The study procedure was approved by the Ethical Committee at the Faculty of Health Sciences, University of Linköping, Sweden.

RESULTS

Rectal Absorption of Ampicillin

The serum concentration-time curves for ampicillin after administration of AM or AM-C10 are shown in Fig. 1. AM-C10 increased the bioavailability of ampicillin, as determined by urinary recovery, from 13.2% in AM recipients to 23.3% (1.8-fold; $p < 0.05$, Tab. 1). Similarly, C_{max} and AUC of ampicillin were increased 2.6- and 2.3-fold, respectively ($p < 0.05$, Tab. 1).

Rectal Morphology

At the control examinations two weeks prior to the study all subjects had normal rectal mucosa on rigid sigmoidoscopy,

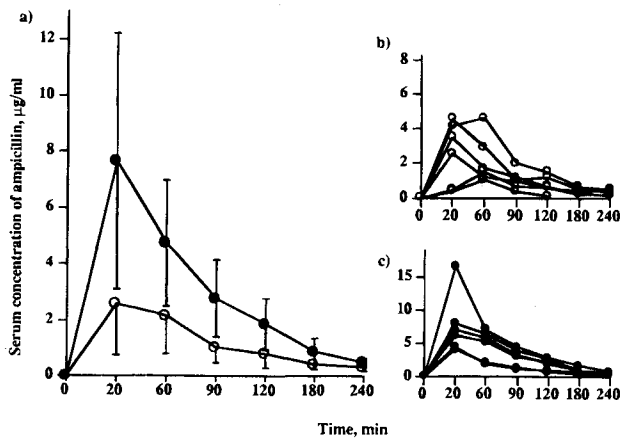

Fig. 1. a) Mean serum concentrations ±S.D. after administration of AM (○) and AM-C10 (●), n = 5–6. Individual serum concentration curves for subjects receiving b) AM and c) AM-C10.

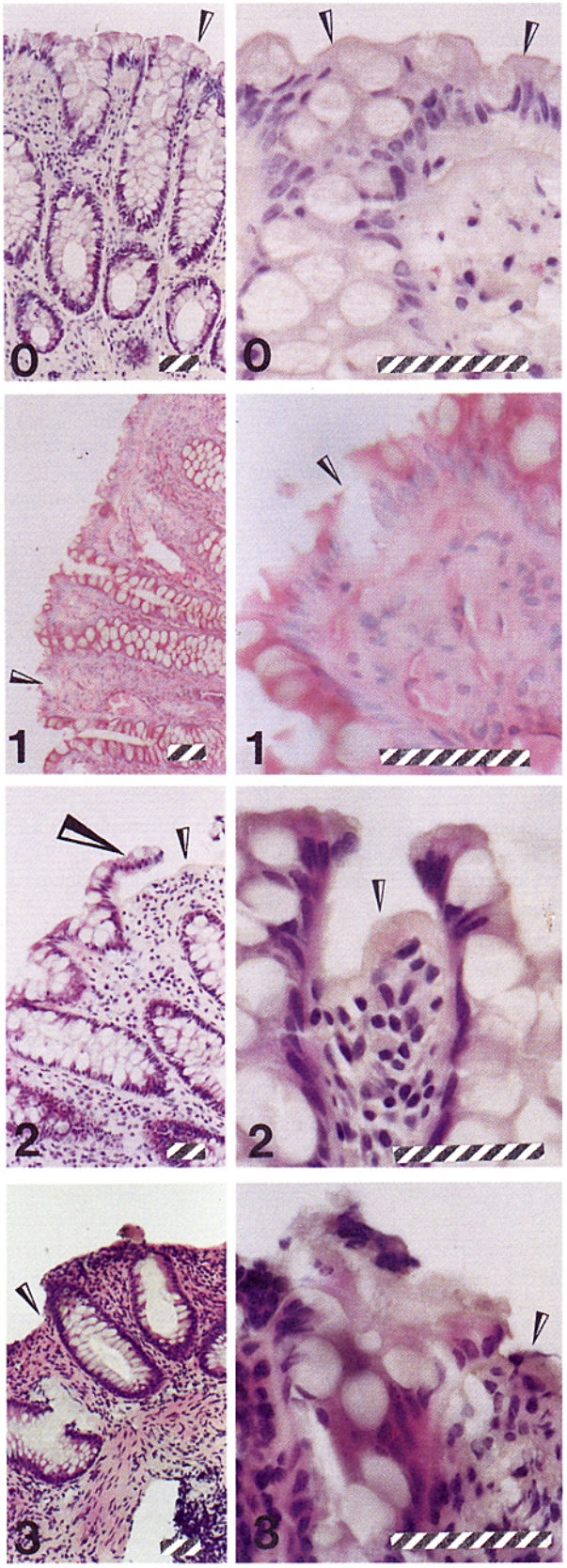

Fig. 2. Light micrographs of biopsies, illustrating the four different types of injuries. The scoring of the injuries (0–3) was based on the severity of damage to the epithelial cells and underlying tissue. 0) shows undamaged, intact tissue with a continous epithelium (arrowheads), 1) injured epithelial cells (arrowheads) with intact basal lamina, 2) detached epithelial cells (large arrowheads) and bare, but intact, basal lamina (small arrowheads), 3) total loss of the epithelial cells and damaged basal lamina (arrowheads). The bar indicates 50 μm.

Table 1. Pharmacokinetics of Ampicillin After Rectal Administration of Suppositories Containing 500 mg Ampicillin, with or Without 50 mg Sodium Caprate (C10) as Absorption Enhancer, to Human Subjects

Suppository	C-max μg/ml	AUC μgxh/ml	Urinary recovery (0-240 min) % of dose
Without sodium caprate (AM)	2.96 ± 1.52	361 ± 144[a]	13.2 ± 3.4[b]
With sodium caprate (AM-C10)	7.69 ± 4.57[c]	824 ± 334[c]	23.3 ± 10.5[c]

Note: All values are means ±S.D, n = 6.
[a] n = 5.
[b] n = 4.
[c] Significantly different from control, $p < 0.05$.

and routine light microscopic examination showed no signs of mucosal disease. After administration of the suppositories, sigmoidoscopy revealed moderately hyperaemic rectal mucosa, and mucus discharge was observed in both AM and AM-C10 groups. The severity of epithelial damage, as scored after examination by light microscopy, is summarised in Tab. 2, and photomicrographs showing the different degrees of damage are shown in Fig. 2. In general, control sections (time 0) had an intact epithelium (Tab. 2).

Twenty-five min after the administration of AM, an increase in epithelial damage was observed ($p < 0.05$, Tab. 2). The increase was mainly a result of a slight increase in type 3 scores, i.e. loss of the epithelial cells and damaged basal lamina, Fig. 2. Epithelial damage 25 min after AM-C10 was more severe than after AM ($p < 0.001$). Marked increases in both detached epithelial cells (type 2 score) and in loss of epithelial cells together with damaged basal lamina (type 3 score) occurred in the AM-C10 group (Tab. 2). In some cases the bare basal lamina was observed as crypts extending above the basal lamina, due to the lack of epithelial cells (Fig. 2).

At 185 min, epithelial damage had progressed in the AM group. In contrast, the epithelial damage in the AM-C10 group was reduced ($p < 0.001$, vs 25 min), indicating that the epithelial damage induced by AM-C10 was reversible. The reduction was mainly a result of an increase in the number of biopsies with an intact epithelium at this time point versus that at 25 min.

The results of the qualitative transmission electron microscope recordings supported the findings from the light microscope. In general, the control preparations had an intact epithelium (Fig. 3a). After exposure to AM or AM-C10, various degrees of damage to the epithelial cell layer and lamina propria were observed. At the epithelial level the damage ranged from mild, with mucus discharge and loss of microvilli, to more severe, with discontinuities in the epithelium and cell death (Fig. 3b).

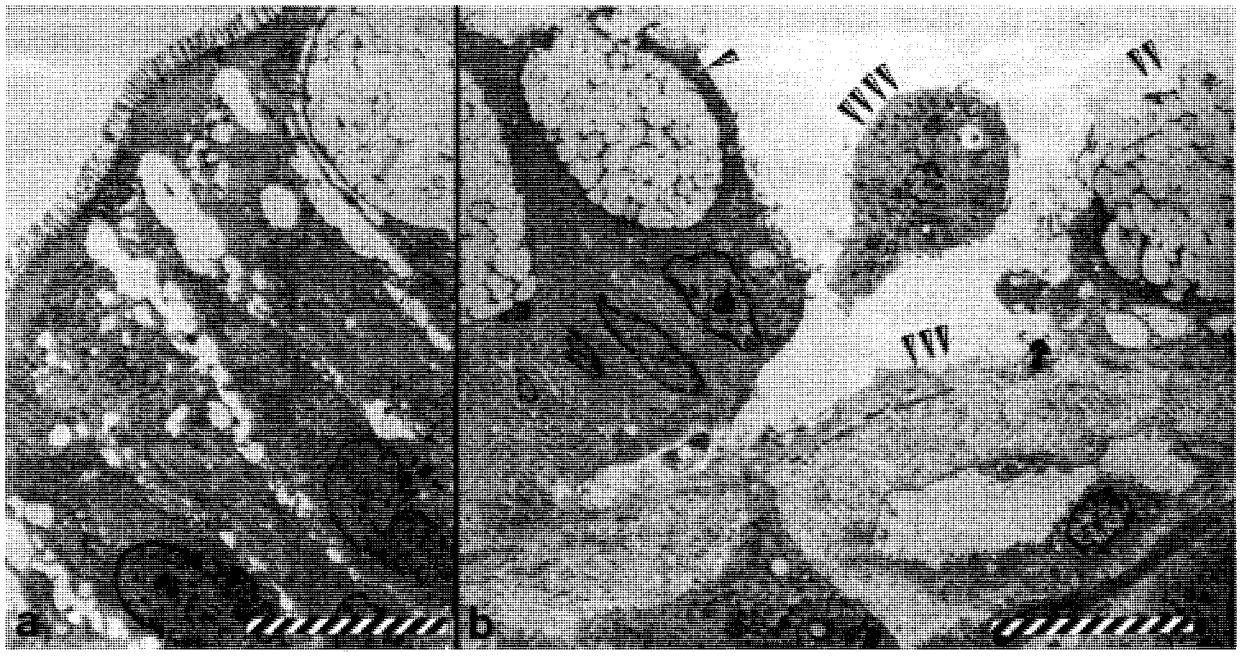

Fig. 3. Transmission electron micrographs of biopsies. (a) shows an intact epithelium before administration of the suppositories, (b) an example of injury 25 min after administration of AM suppositories, with cells detaching from the basal lamina (one arrowhead), mucus discharge (two arrowheads), exposed basal lamina (three arrowheads) and cellular debris (four arrowheads). The bar indicates 10 μm.

Osmolality of the Rectal Fluid

In the control situation, too little fluid was present in the rectal lumen to allow sampling. However, previous investigations indicate that rectal fluid (faeces) is slightly hypertonic (12). At 25 min after administration of the suppositories, samples from the rectal fluid were hypertonic to a variable degree, giving mean values of 682 ± 194 mmole/kg and 721 ± 474 mmole/kg for volunteers receiving AM and AM-C10, respectively (Fig. 4). At 185 min the osmolality had decreased to less variable tonicities of 346 ± 63 mmole/kg and 330 ± 24 mmole/kg for AM and AM-C10, respectively ($p < 0.05$). No significant differences in osmolality were observed between the AM and AM-C10 groups at any time.

Table 2. Histological Scoring of Rectal Biopsies Examined Under Light Microscopy[a]

	Time (min)	Score[b] 0	1	2	3	Average score
AM	0 (control)	29	7	11	1	0.67
	25	24	5	10	9	1.08
	185	15	10	13	9	1.38
AM-C10	0 (control)	27	11	8	1	0.62
	25	6	4	25	13	1.94
	185	23	7	15	3	0.96

Note: Statistics: Within AM group: 25 vs 0 min: $p < 0.05$ and 185 vs 0 min: $p < 0.001$. Within AM-C10 group: 25 vs 0 min: $p < 0.001$, 185 vs 0 min: $p < 0.05$ and 185 vs 25 min: $p < 0.001$. Between groups: 25 min: $p < 0.001$ and 185 min: $p < 0.05$.

[a] Each group (AM and AM-C10) comprised 6 subjects. For each subject, two sections were examined at each time point.

[b] The scoring was performed as illustrated in figure 2 by four independent examiners. The figures in the table denote the number of sections scored 0, 1, 2 or 3 at each time point.

DISCUSSION

This is the first study to investigate the mechanism of rectal absorption enhancement in human subjects. The results show that C10 is active as an absorption enhancer after rectal

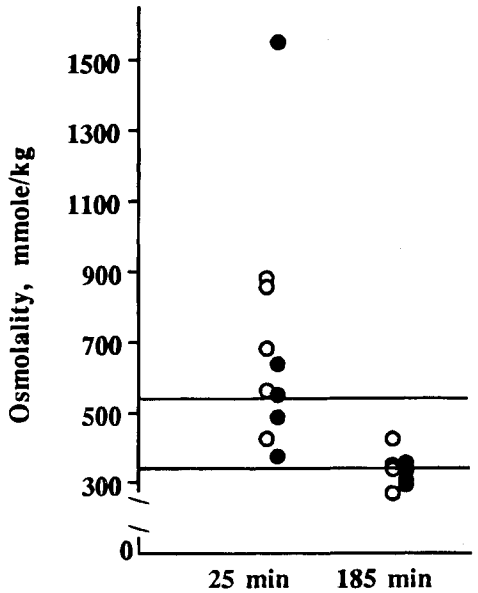

Fig. 4. Osmolality in rectal fluid samples taken at 25 and 185 min after administration of AM (○) or AM-C10 (●). The area between the lines shows previously reported rectal osmolality values in controls (12).

administration in suppositories and that absorption enhancement coincides with non-specific damage to the rectal mucosa. The results also suggest that C10 in the suppositories induces rapid recovery of the rectal mucosal damage, while damage caused by corresponding suppositories without C10 is prolonged. These findings should be taken into account in the formulation and use of conventional suppositories in humans.

The rectal bioavailability of ampicillin has not been reported previously in humans but is very low in rats (4–8%) and in rabbits (3%) (13–15). Although extrapolation of these data may not be reliable, in this study, the rectal bioavailability of ampicillin after administration in conventional triglyceride suppositories (AM) was somewhat higher than in animals (13%), suggesting that the suppository base in AM may have increased the permeability of the human rectal epithelium to ampicillin. This is supported by studies in the rat, where the absorption of ampicillin in a suppository formulation without the addition of an absorption enhancer was 11% (16). However, a much higher rectal bioavailability was observed in humans after administration of AM-C10, indicating that C10 is a more potent absorption enhancer than the suppository base.

The effects of C10 on the intestinal epithelium in cell culture and in rat colon and human ileum in vitro are related to a reversible increase in the permeability of the paracellular pathway (7,8,17,18). In contrast, the effect of AM-C10 in the human rectum was related to non-specific epithelial damage with loss of the epithelial cell layer and an injured lamina propria as the most severe effect. Several factors could contribute to this discrepancy.

Firstly, as with other surface active agents, C10 displays a steep concentration-effect relationship (19,20,21). Within a limited concentration interval (10–13 mM), the effect of C10 is specific (7,8,22). In the in vivo situation this concentration will be exceeded if the drug delivery system does not control the release of the enhancer. If we assume that C10 is instantly released from the suppository base and that the volume of the rectal fluid is 3 ml (23), a maximal concentration of 86 mM of C10 is obtained. This concentration would be sufficient to cause non-specific damage to the rectal epithelium. However, studies on the in vitro release of C10 from the triglyceride base indicate that only a fraction of the total dose of C10 is released at the time of C_{max} for ampicillin (24). This fact together with the rapid absorption of C10 across the intestinal epithelium (25), indicate that the concentration of C10 in the rectal lumen in the present study was much lower than 86 mM at any given time. This suggests that, in addition to C10, other factors also contributed to the non-specific effects on the rectal epithelium.

Secondly, the damaging effects of the conventional triglyceride suppository (AM) in the present study indicate that this suppository base also acts as a non-specific absorption enhancer. This is supported by similar studies in experimental animals, indicating that a variety of tri-, di- and monoglycerides damage mucosal tissues (2,26,27). Unfortunately, more detailed comparisons with previous studies are not possible, since the specific composition of the triglyceride base was not disclosed.

Thirdly, the increased osmolality of the rectal fluid may have contributed to the absorption enhancement synergistically. Thus, non-specific morphological changes coinciding with a dramatic increase in epithelial permeability were observed in Caco-2 monolayers after exposure to C10 in a hypertonic buffer (21). Similar synergistic effects between absorption enhancer and hyperosmolality have been observed in rat and rabbit rectum (28). It is likely that ampicillin released from the suppository base contributed to the increased osmolality.

The finding that the mucosal damage induced by AM-C10 was reversible is supported by studies in rabbits showing that addition of fatty acids to a suppository formulation limited the mucosal damage (27). A possible explanation for this result is provided by preliminary mechanistic studies of C10 in Caco-2 monolayers, suggesting that C10 influences both the opening and closure of the tight junctions (29).

Our data has implications for the development of rectal drug formulations. The fact that the triglyceride suppository base caused non-specific and prolonged damage to the human rectal mucosa indicate that such suppositories should be used for limited time periods only. Since suppositions containing triglyceride bases are the most commonly used rectal drug products in Sweden,[5] there is an obvious need for improvement in this class of drug products. A strategy to reduce this damage could involve the addition of C10 or other free fatty acids to the suppository formulation.

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[5] The majority of the 90 registered rectal drug products in Sweden are suppositories, of which more than 75% have a triglyceride base as a vehicle (10).

REFERENCES

1. T. Nishihata, J. H. Rytting, and T. Higuchi. *J. Pharm. Sci.* **70**:71–75 (1981).
2. E. J. van Hoogdalem, C. Vermeij-Kerrs, A. G. de Boer, and D. D. Breimer. *J. Pharm. Sci.* **79**:866–870 (1990).
3. K. Nakanishi, M. Masada, and T. Nadai. *Chem. Pharm. Bull.* **31**:4161–4166 (1983).
4. E. S. Swenson, W. B. Milisen, and W. Curatolo. *Pharm. Res.* **11**:1132–1142 (1994).
5. E. S. Swenson and W. J. Curatolo. *Adv. Drug Delivery Rev.* **8**:39–92 (1992).
6. B. J. Aungst, H. Saitoh, D. L. Burcham, S.-M. Huang, S. A. Mousa, and M. A. Hussain. *J. Controll. Release* **41**:19–31 (1996).
7. E. K. Anderberg, T. Lindmark, and P. Artursson. *Pharm. Res.* **10**:857–864 (1993).
8. T. Sawada, T. Ogawa, M. Tomita, M. Hayashi, and S. Awazu. *Pharm. Res.* **8**:1365–1371 (1991).
9. K. Takahashi, T. Murakami, R. Yumoto, T. Hattori, Y. Higashi, and N. Yata. *Pharm. Res.* **11**:1401–1404 (1994).
10. Swedis. Data obtained from Swedis data base; Medical Products Agency, Uppsala, Sweden.
11. J. Lal, J. K. Paliwal, P. K. Grover, and R. C. Gupta. *J. Chromatogr. B* **655**:142–146 (1994).
12. I. Nordgaard-Andersen, M. Rye Clausen, and P. Brobech Mortensen. *J. Parenter. Enteral Nutr.* **34**:324–331 (1993).

13. M. Mishima, A. Nagatomi, T. Yamakita, Y. Miura, and O. Tsuzuki. *Biol. Pharm. Bull.* **18**:566–570 (1995).
14. E. J. van Hoogdalem, A. G. de Boer, and D. D. Breimer. *Pharm. Weekbl. (Sci.)* **10**:76–79 (1988).
15. H. Yaginuma, Y. Isoda, Y. Wada, S. Itoh, M. Yamazaki, A. Kamada, H. Shimazu, and I. Makita. *Chem. Pharm. Bull.* **30**:1073–1076 (1982).
16. K. I. Nishimura, Y. Nozaki, A. Yoshimi, S. Nakamura, M. Kitagawa, N. Kakeya, and K. Kitao. *Chem. Pharm. Bull.* **33**:282–291 (1985).
17. M. Tomita, M. Shiga, M. Hayashi, and S. Awazu. *Pharm. Res.* **5**:341–346 (1988).
18. J. D. Söderholm, L. Hedman, and G. Olaison. Tight junctional permeability in human ileal mucosa—modulation with sodium caprate and cytochalasin B. *Gut.* **37 (suppl 2)**:A39 (1995).
19. E. K. Anderberg and P. Artursson. *J. Pharm. Sci.* **82**:392–398 (1993).
20. E. K. Anderberg, C. Nyström, and P. Artursson. *J. Pharm. Sci.* **81**:879–887 (1992).
21. T. Lindmark, T. Nikkilä, and P. Artursson. *J. Pharmacol. Exp. Ther.* **275**:958–964 (1995).
22. M. Tomita, M. Hayashi, and S. Awazu. *J. Pharmacol. Exp. Ther.* **272**:739–743 (1995).
23. A. G. de Boer, E. J. van Hoogdalem, and D. D. Breimer. *Adv. Drug Delivery Rev.* **8**:237–251 (1992).
24. Dr. C. Graffner. Astra Läkemedel AB, Sweden. (personal communication).
25. K. Takahashi, T. Murakami, A. Kamata, R. Yumoto, Y. Higashi, and N. Yata. *Pharm. Res.* **11**:388–392 (1994).
26. L. Lohikangas, M. Wilen, M. Einarsson, and P. Artursson. *Eur. J. Pharm. Sci.* **1**:307–312 (1994).
27. C. De Muynck, C. Cuvelier, D. Van Steenkiste, L. Bonnarens, and J. P. Remon. *Pharm. Res.* **8**:945–950 (1991).
28. N. Yata, W. M. Wu, R. Yamajo, T. Murakami, Y. Higashi, and T. Higuchi. *J. Pharm. Sci.* **74**:1058–1061 (1985).
29. Y. Kimura, T. Lindmark, and P. Artursson. Regulation of immediate and long term effects of the absorption enhancer sodium caprate in human intestinal epithelial (Caco-2) cells. *Proc. 23rd Int. Symp. Control. Rel. Bioact. Mater.* 423–424 (1996).