International Journal of Pharmaceutics, 20 (1984) 163–169
Elsevier

IJP 00688

The application of an in situ loop technique to the study of rectal absorption

N.W. Thomas, K.J. Palin[1] and D.A. Waller

Departments of Human Morphology, and [1] Physiology and Pharmacology, Queen's Medical Centre, University of Nottingham, Nottingham (U.K.)

(Received November 7th, 1983)
(Modified version received February 14th, 1984)
(Accepted February 28th, 1984)

Summary

The application of an in situ loop technique to the determination of rectal water flux and drug absorption in the rat is described. The procedure requires minimal surgical skill and allows rapid sampling of luminal fluid. The method was assessed by studies on: (a) the effect of sodium salicylate solutions (1% and 5% w/v) and equiosmotic solutions of mannitol (2.5% and 11.8% w/v) on rectal water flux and tissue morphology; and (b) the rectal uptake of [^{3}H]prednisolone from aqueous solution. The 1% sodium salicylate and 2.5% mannitol solutions induced water movement into the lumen but caused no apparent change in the tissue morphology. The more hypertonic 5% salicylate solution and the equiosmotic mannitol solution induced water flux into the lumen and resulted in extensive cell loss. The uptake of [^{3}H]prednisolone from the loop was confirmed by a simultaneous increase in plasma tritium levels.

Introduction

A variety of in situ procedures have been used in the study of gastrointestinal drug absorption (Houston and Wood, 1980) but few have been applied to the rectum. Drug absorption in this region of the bowel has frequently been assessed by determining the plasma drug level following the administration of microenemas

Correspondence: N.W. Thomas, Dept. of Human Morphology, Queen's Medical Centre, University of Nottingham, Nottingham, U.K.

0378-5173/84/$03.00 © 1984 Elsevier Science Publishers B.V.

(Nishihata et al., 1982) but this does not allow the luminal disappearance to be quantified. Where the latter has been measured the constant perfusion technique has been the preferred method (Shanker, 1959; Crommelin et al., 1979). Closed loops such as described by Dolusio (1969) in experiments on the small intestine have not been used for rectal studies. This may be due to inherent difficulties in setting up a loop in a section of the bowel that: (i) is not suspended by an extensive mesentery; (ii) for much of its course is retroperitoneal; and (iii) is inaccessible in the pelvis. In addition there are technical problems associated with sampling from a small initial luminal volume.

A method based on the closed loop principle has been developed to study rectal drug absorption and tissue interactions. The method is simple requiring minimal surgical intervention yet allowing serial sampling of luminal fluid.

Materials and Methods

Test solutions

Krebs buffer modified to give a solution isotonic with body tissue (NaCl 107.00 mM; KCl 4.96 mM; $NaHCO_3$ 5.00 mM; $CaCl_2$ 1.00 mM; $MgCl_2$ 0.98 mM; KH_2PO_4 1.03 mM; glucose 20.00 mM; all reagents were Analar grade) was used as the control solution. Test solutions of 1% w/v and 5% w/v sodium salicylate (BDH Chemicals, Poole Dorset) were prepared in the control buffer and the osmotic pressure determined to be 443 and 974 mOsm/kg, respectively, using an Osmet osmometer. Equiosmotic solutions of mannitol (2.5% w/v and 11.8% w/v, respectively) were prepared. A solution containing prednisolone (0.1 mg/ml; MSD, Hoddesdon, Herts.) and [^{3}H]prednisolone (30 μCi/ml; Amersham, Slough, Berks.) was prepared in the control buffer. All test and control solutions contained [^{14}C]polyethylene glycol 4000 ([^{14}C]PEG 4000, 2.5 μCi/100 ml; Amersham, Slough, Berks.) as a non-absorbable marker.

In situ procedure

Male Wistar rats weighing between 190 and 210 g were fasted overnight with water ad libitum, and then anaesthetized with pentobarbitone (i.p. Sagatal 90 mg/kg). The tip of a three-way tap coated with cyanoacrylate adhesive was held at the anus and the scrotal skin and the base of the tail drawn against the tap to form a watertight seal. The lower abdomen was incised in the mid-line and a ligature tied loosely around the large intestine approximately 5 cm from the anus. 1 ml of test solution was injected into the rectum through the three-way tap and the syringe left in place (Fig. 1). The ligature was tightened and the wound closed. At 10-min intervals the luminal fluid was withdrawn slowly into the syringe then returned to the lumen in order to facilitate mixing. Immediately a second smaller volume was withdrawn and diverted into the third port of the tap from which two 10-μl samples were taken. The solution was then returned to the rectum. Following injection of the prednisolone solution blood samples were taken from the tail tip at each time interval and the plasma collected and weighed. Each sample of rectal fluid and

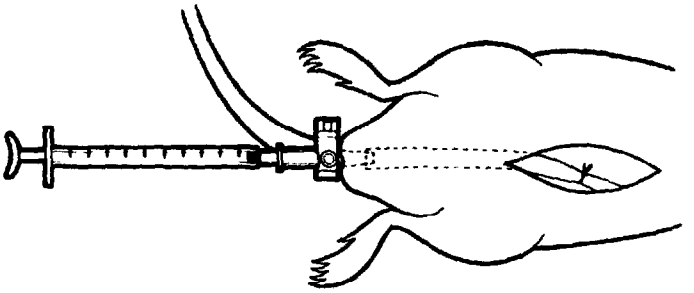

Fig. 1. Schematic diagram of the in situ rectal loop preparation.

plasma was dispersed in 10 ml 'Fisofluor 1' (Fisons, Loughborough, Leics.) and the concentration of [^{14}C]PEG 4000 and [^{3}H]prednisolone determined by scintillation counting.

Histology

Segments of the rectal wall from the loop and from adjacent untreated rectum were fixed in either 10% neutral formalin and processed for light microscopy, or a mixture of 2% glutaraldehyde and 1% paraformaldehyde in 0.1 M cacodylate buffer

Fig. 2.

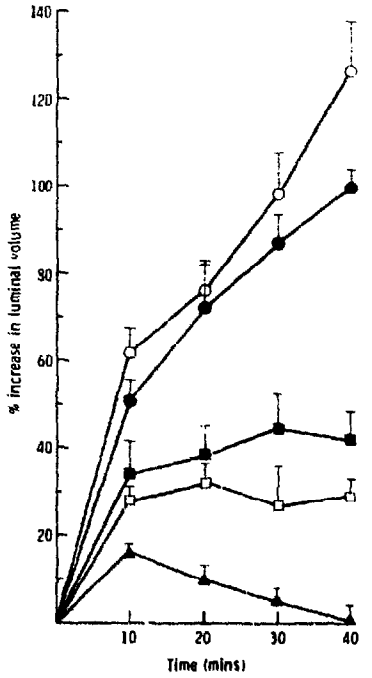

Fig. 3.

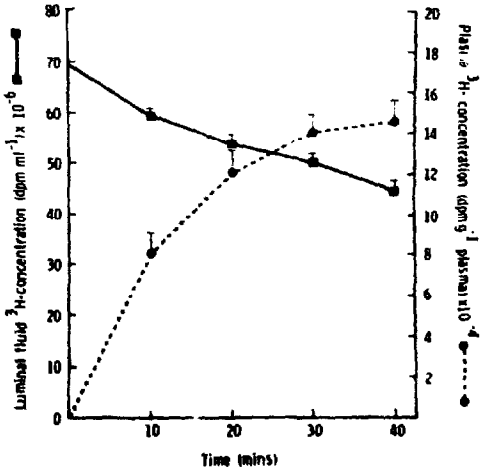

Fig. 2. Water flux in the rat rectum in the presence of different aqueous solutions: ○, 11.8% mannitol; ●, 5% sodium salicylate: □, 2.5% mannitol; ■, 1% sodium salicylate; ▲, Krebs control buffer. Each value is the mean ± S.E.M. of more than 6 animals.

Fig. 3. The changes in the luminal and plasma concentrations of tritium against time: ■, luminal fluid tritium concentration; ●, plasma tritium concentration.

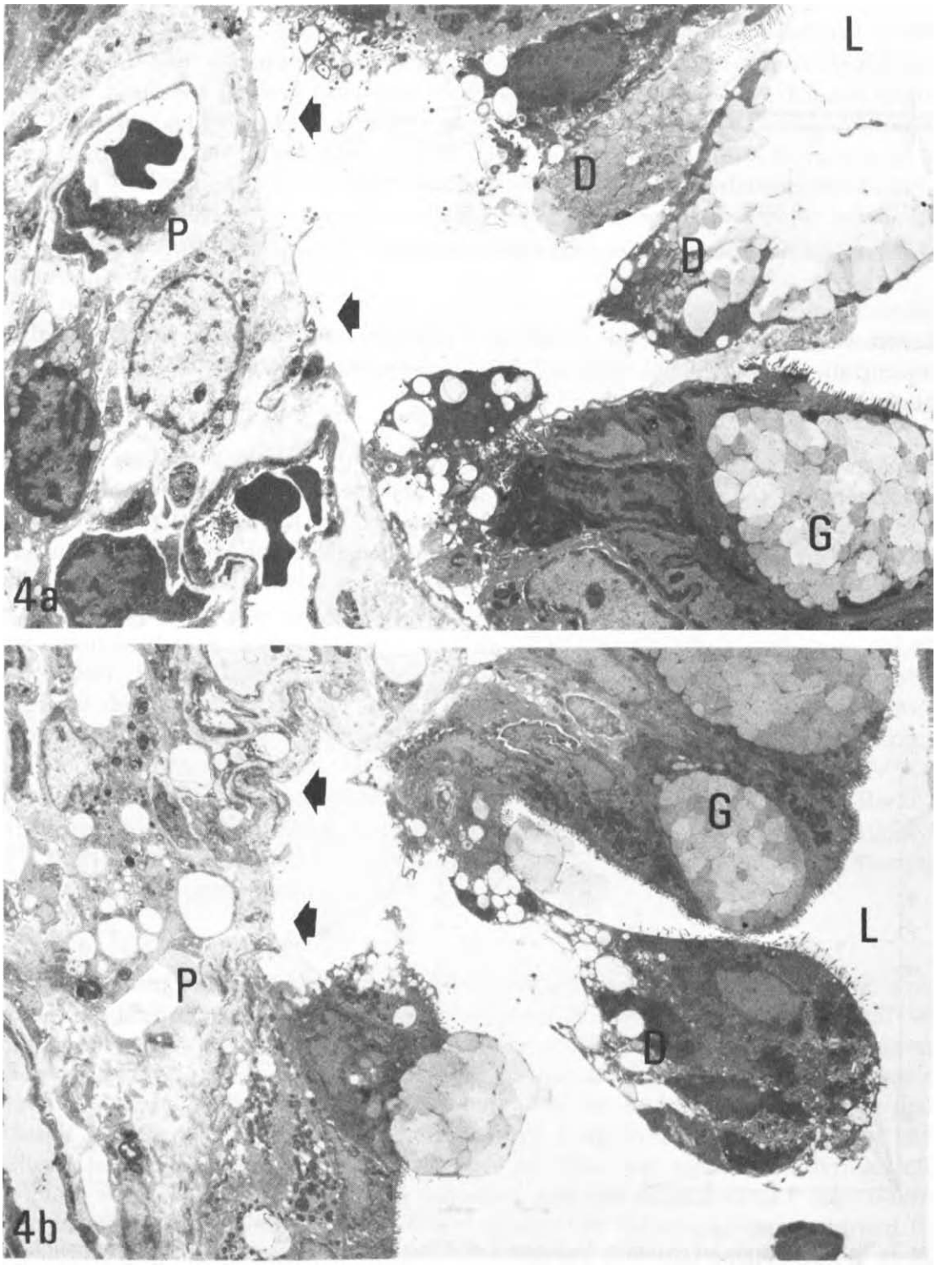

Fig. 4. Electron micrographs showing damage to the rectal epithelium after (a) exposure to a 5% solution of sodium salicylate, and (b) after exposure to a 11.8% solution of mannitol. P = lamina propria; D = detached cell; G = goblet cell; L = lumen; arrows = sites of epithelial desquamation. a and b; ×4000

at pH 7.2 and processed for electron microscopy. Wax sections cut at 6 μm were stained with haematoxylin and eosin. Sections for electron microscopy were stained with lead citrate and examined in a Phillips 410 electron microscope.

Results

Rectal water flux was calculated from the change in [^{14}C]PEG 4000 activity and expressed as the percentage change in the luminal volume from time zero (Fig. 2). Statistical comparison of the data using the Mann-Whitney U-test, showed that after 40 min there was a significant ($P < 0.05$) increase in rectal luminal fluid volume with increasing osmotic pressure of the test solution. No difference in fluid flux was found between the equiosmotic solutions containing 1% sodium salicylate and 2.5% mannitol. However, the 11.8% mannitol solution induced a greater ($P < 0.05$) increase in luminal fluid than the equiosmotic solution containing 5% sodium salicylate. The control solution induced an initial increase in luminal volume followed by a gradual return to the starting volume.

At each time interval the [^{3}H]prednisolone concentration was determined. These values were corrected for changes in activity due to fluid flux and expressed as dpm · ml^{-1} of luminal fluid (Fig. 3). Regression analysis of the semilogarithmic plot of these values against time showed that prednisolone disappearance followed

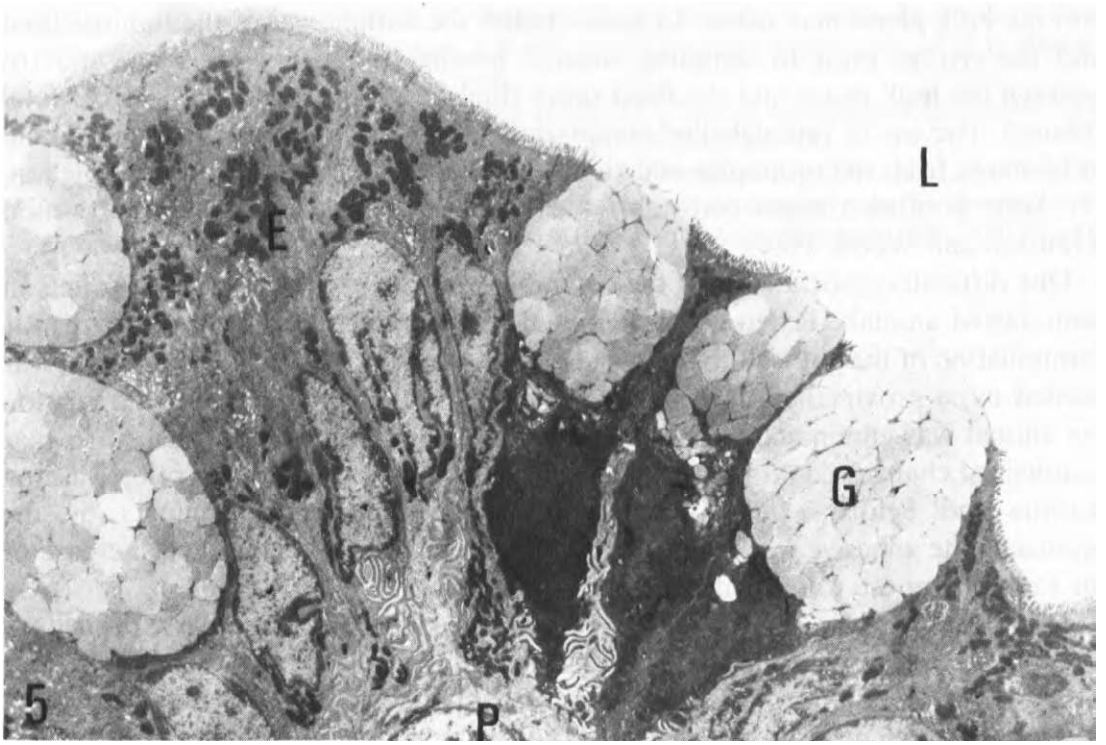

Fig. 5. Electron micrograph showing the normal appearance of the rectal epithelium after exposure to the control solution for 40 min. L = lumen; G = goblet cell; E = enterocyte; P = lamina propria. ×3060.

first-order kinetics with a disappearance rate constant (K) of -0.011 ± 0.003 min^{-1} (mean ± S.D.). Plasma [^{3}H]prednisolone levels were determined as dpm · g^{-1} plasma (Fig. 3).

The rectal tissue exposed to the concentrated solutions of mannitol and salicylate showed evidence of extensive cell loss from the surface epithelium (Fig. 4a and b). In some instances epithelial desquamation extended into the intestinal glands. In areas of cell loss the basement membrane remained as an intact layer and there was no obvious change in the structure of the underlying lamina propria. The rectal tissues exposed to the lower concentrations of salicylate and mannitol, and to prednisolone, resembled control tissue (Fig. 5) and showed no evidence of epithelial cell damage or cell loss.

Discussion

The anatomical limitations imposed by the rectum on investigations of drug absorption using in situ loops have been overcome. This was achieved by sealing a three-way tap at the anal canal thereby providing a route for periodic sampling.

The rate of perfusion in a constant perfusion system is an important factor in determining the rate of intestinal absorption for it reduces or removes the unstirred water layer immediately adjacent to the epithelial surface (Winne, 1979; Savina et al., 1981). Although this effect is eliminated in a static loop, the problem of laminated static layers forming a concentration gradient from the unstirred layer into the bulk phase may occur. In these studies the withdrawal of the luminal fluid into the syringe prior to sampling ensured mixing: (a) within the loop; and (b) between the bulk phase and the dead space fluid in the three-way tap (< 10% total volume). The use of radiolabelled compounds allowed the analysis of luminal fluid to be made from microsamples and therefore minimized luminal volume depletion. The latter is often a major constraint when serial sampling of closed loops is used (Houston and Wood, 1980).

One difficulty associated with the technique was the presence of faecal pellets in some fasted animals. Pellets in the terminal rectum were easily removed by gentle manipulation of the gut wall through the scrotal skin. Occasionally when a pellet was located more proximally in the bowel and could not be removed by manipulation the animal was eliminated from the study. The loops were not prewashed so that histological changes could be attributed to the treatment and not to prior washing (Levine and Pelikan, 1961). Possible irritation of the rectal mucosa by the cyanoacrylate adhesive was avoided by using the loose scrotal skin and the base of the tail to form the watertight seal.

In pilot studies it was noted that by 60 min some loops showed areas of transitory tissue blanching which indicated sites of vascular shut down. In order to minimize disturbances to normal physiology which would follow vascular stress all experiments were restricted to 40 min. Histological examination of the control and experimental tissues showed no evidence of changes which could be related to a compromised vascular perfusion and thus those changes present in the experimental

tissue could be related to the composition of the luminal solutions.

In conclusion investigations of fluid flux, drug absorption and gut histology were used to assess a simple in situ loop technique for use in the rectum. The data confirm the suitability of the method for acute studies and provide an alternative to the constant perfusion system.

Acknowledgements

We are grateful to Dr. C.G. Wilson for his advice in preparing this paper and the staff in the EM unit of the Department of Human Morphology for their technical support.

References

Crommelin, D.J.A., Modderkolk, J. and de Blaey, C.J., The pH dependence of rectal absorption of theophylline from solutions of aminophylline in situ in rats. Int. J. Pharm., 3 (1979) 299–309.

Dolusio, J.T., Billups, N.F., Dittert, L.W., Sugita, E.T. and Swintosky, J.V., Drug absorption I: An in situ rat gut technique yielding realistic absorption rates. J. Pharm. Sci., 58 (1969) 1196–1200.

Houston, J.B. and Wood, S.G., Gastrointestinal absorption of drugs and other xenobiotics. In Bridges, J.W. and Chasseaud, L.F. (Eds.), Progress in Drug Metabolism, Vol. 4, John Wiley, London, 1980, pp. 57–129.

Levine, R.R. and Pelikan, E.W., The influence of experimental procedures and dose on the intestinal absorption of an onium compound benzomethamine. J. Pharmacol. Exp. Ther., 131 (1961) 319–327.

Nishihata, T., Rytting, J.H., Caldwell, L., Yoshioka, S. and Higuchi, T., Adjuvant effects on rectal absorption. In Bundgaard, H., Hansen, A.B. and Kofod, H. (Eds.), Optimization of Drug Delivery, Munksgaard, Copenhagen, 1982, pp. 17–34.

Savina, P.M., Staubus, A.E., Gaginella, T.S. and Smith, D.F., Optimal perfusion rate determined for in situ intestinal absorption studies in rats. J. Pharm. Sci., 70 (1981) 239–243.

Schanker, L.S., Absorption of drugs from the rat colon. J. Pharmacol. Exp. Ther. 126 (1959) 283–290.

Winne, D., Rat jejunum perfused in situ: effect of perfusion rate intraluminal radius on absorption rate and effective unstirred layer thickness. Naumyn-Schmiedeberg's Arch. Pharmacol., 307 (1979) 265–274.