J. Pharm. Pharmacol. 1993, 46: 1082–1084
Communicated March 25, 1993

COMMUNICATIONS

Do antidiarrhoeal opiates accumulate in the rat intestinal lumen?

ANTHONY DE LUCA, GERARD MURRAY, IAN M. COUPAR, *Unit of Addictive Drug Research, School of Pharmaceutical Pharmacology, Victorian College of Pharmacy, Monash University, Parkville, 3052 Australia*

Abstract—The opiate antidiarrhoeal drugs loperamide (0·6 mg kg^{-1}, i.p.) or difenoxin (0·77 mg kg^{-1}, s.c.), were administered in an anaesthetic mixture (pentobarbitone 60 mg kg^{-1}) to rats. A length of jejunum (approx. 30 cm) was cannulated, washed and then perfused with iso-osmotic saline for 20 min. The perfusion commenced 50 min after drug administration and continued for 20 min. The perfusates were collected for analysis of fluid transport rates and antidiarrhoeal drug content. These doses of the antidiarrhoeals caused marked inhibition of intestinal fluid secretion induced by intra-arterial infusion of vasoactive intestinal peptide. However, neither of the antidiarrhoeal drugs were detected in the intestinal perfusates (< 0·5 ng by HPLC). The results indicate that loperamide and difenoxin have a different pharmacokinetic profile compared with that previously found for morphine under the same conditions.

Opiates have a wide spectrum of pharmacological effects on the mammalian intestine. These include reductions in motility (propulsive peristalsis), secretions (pancreatic, biliary and electrolyte/fluid) and increases in intestinal fluid absorption and blood flow (see Brown & Miller 1991). These effects may contribute to a desirable antidiarrhoeal action or, under different conditions, to constipation.

Previous studies have established that morphine accumulates in intestinal tissue of rats after intravenous or intraperitoneal administration (Bianchi et al 1983) where it inhibits gastrointestinal transit (Brown et al 1988). This observation could help explain how morphine produces such potent constipating and antidiarrhoeal effects.

The opiates morphine and codeine, and the opiate antidiarrhoeals such as diphenoxylate and loperamide, also produce their antidiarrhoeal effect through blocking intestinal fluid secretion. The secretion is produced by the mucosal epithelium in response to endogenous secretagogues (e.g. vasoactive intestinal peptide, prostaglandins and acetylcholine) as well as exogenous secretagogues such as bacterial enterotoxins.

We have demonstrated that morphine appears in the intestinal lumen of laboratory rats, approximately 10–20 min after intravenous administration where it persists for a long period of time (Margaritis et al 1991). This could also help explain the potent intestinal actions of morphine, especially if the intestinal mucosa is a possible site of action. Indeed, there is evidence to show that morphine stimulates mucosal sensory receptors which in turn activate a reflex-arc to further increase intestinal fluid absorption (Mailman 1984; Brown & Miller 1991).

The aim of this study was to determine whether other widely used opiate antidiarrhoeals also accumulate in the lumen. Difenoxin was selected as it is the active metabolite of the widely used antidiarrhoeal drug diphenoxylate (Heykants et al 1972). The doses used in this study were selected from the ED50 values for protection of rats against castor oil-induced diarrhoea (Niemegeers et al 1974) and the doses producing a large inhibition of intestinal fluid secretion (De Luca & Coupar 1992).

Materials and methods

Surgical and analytical procedures in fluid transport studies. Hooded Wistar rats of either sex, 230–290 g, were anaesthetized with a subcutaneous injection of pentobarbitone sodium (60 mg kg^{-1}). Difenoxin was dissolved in the anaesthetic mixture so it could be administered subcutaneously to maintain consistency with the previous morphine study which also used this route (Margaritis et al 1991). However, subcutaneous administration of loperamide did not result in a dose-related effect in a previous study (De Luca & Coupar 1992) and therefore this compound was administered intraperitoneally.

A cannula was introduced into the left common carotid artery for constant intra-arterial infusion of vasoactive intestinal peptide (VIP; 0·8 $\mu g\ min^{-1}$) in saline at a rate of 40 $\mu L\ min^{-1}$ to induce intestinal fluid secretion.

A 20–30 cm loop of jejunum was cannulated so preventing the entry of bile into the intestinal lumen. The loop was washed 50 min after drug administration, then perfused with 9 mL iso-osmotic saline (NaCl 148, KCl 5 and dextrose 5·5 mM) for 20 min. The solution was contained in a reservoir maintained at 37 C and recirculated through the lumen of the jejunum by a gas-lift column of moistened 5% CO_2 in O_2.

The fluid from the loop and reservoir was recovered at the end of the 20 min perfusion period. Aliquots of the samples were diluted with buffer and peak absorbances were measured at 560 nm and at 520 and 600 nm to correct for non-specific interferences as described by Miller & Schedl (1972). Results are expressed as the net amount of water absorbed or secreted (−) in $\mu L\ (g\ wet\ wt)^{-1}$ of jejunum during the 20 min perfusion.

Sample preparations. The method of sample preparation and HPLC detection was that described by Pierce et al (1992). In the present study, a 0·9 mL sample was mixed with 0·1 mL internal standard solution (10 $\mu g\ mL^{-1}$) and loaded into a reverse-phase C18 Sep Pak cartridge (Waters). The cartridge was then washed with 4 mL water:acetonitrile (9:1) 90% containing 0·08% diethylamine (pH adjusted to 2·3 with orthophosphoric acid), at a flow rate of 1·5 mL min^{-1}, maintained with a B Braun-Melsungen AG syringe pump. Standards, samples and the internal standard in the samples were then eluted separately using water:acetonitrile (6:4) containing 0·08% diethylamine, pH 2·3 (4 mL for difenoxin; 6 mL for loperamide, retaining the last 4 mL for analysis) at a flow rate of 5 mL min^{-1}.

Apparatus. The separation and detection system consisted of a Rheodyne 7125 injection valve fitted with a 100 μL injection loop, a C18 guard column and an Ultracarb reverse-phase 30 μm analytical column (150 mm × 4·6 mm; Phenomenex). A programmable multiwavelength detector was used (Waters Model 490E Spectrophotometer) set at 210 nm. Samples (100 μL) were injected onto the column, and chromatograms were recorded on a BAS RYT chart recorder set at a chart speed of 2 mm min^{-1}.

Drug standards and internal standards. Standards were sonicated in ethanol (1 mg mL^{-1}) and diluted to the required concentration in mobile phase. Difenoxin served as the internal standard for loperamide and loperamide for difenoxin.

Mobile phase. The mobile phase consisted of a water:acetonitrile solution (65:35) containing 0·08% diethylamine. The pH of the solution was adjusted to 2·3 with orthophosphoric acid and it was then filtered through 0·45 μm Millipore cellulose filters

Correspondence: I. M. Coupar, Unit of Addictive Drug Research, School of Pharmaceutical Pharmacology, Victorian College of Pharmacy, 381 Royal Parade, Parkville, 3052 Australia.

before use. The mobile phase was freshly prepared each day and the system was allowed to equilibrate for 1 h at a flow rate of 1 mL min^{-1} before carrying out any separations.

Statistics. Pairs of means were analysed by Student's unpaired two-tailed *t*-test. Probability levels of (*P*) less than 0·05 were taken to indicate a significant difference.

Drugs and chemicals. Difenoxin and loperamide were obtained from Janssen (Beerse, Belgium) and Ethnor (Sydney, Australia), respectively. Pentobarbitone sodium (Nembutal) was from Boehringer Ingelheim (Artarman, Australia). Acetonitrile was from Mallinckrodt (Clayton, Australia), diethylamine and orthophosphoric acid from British Drug Houses (Poole, UK). Analytical grade ethanol was used.

Results

Antisecretory effects of difenoxin and loperamide. The rate of fluid absorption was 235 ± 22 μL (g tissue)$^{-1}$ in 20 min (saline intra-arterially; n = 7). This was reversed to net secretion of 339 ± 48 μL g^{-1} in 20 min by intra-arterial infusion of VIP at 0·8 μg min^{-1} (n = 5). This rate of infusion produces a maximal secretory response to VIP in pentobarbitone-anaesthetized rats (Coupar 1985).

Difenoxin (0·77 mg kg^{-1}) inhibited VIP-induced intestinal fluid secretion from 339 ± 48 to 92 ± 61 μL g^{-1} in 20 min (n = 7; $P < 0·05$). Similarly, loperamide (0·6 mg kg^{-1}) blocked the VIP-induced secretion to produce a fluid rate of transport of 0 ± 52 μL g^{-1} (n = 5; $P < 0·05$).

Determination of calibration lines. Calibration lines for the loperamide and difenoxin standards prepared in mobile phase (100 μL injected) were linear from 0·25–1 μg mL^{-1}. The correlation coefficients of the detector responses against the standards were 0·999 and 0·998 for difenoxin and loperamide, respectively. The lowest limit of detection corresponded to 0·5 ng for each antidiarrhoeal drug.

Chromatograms of difenoxin (1 μg mL^{-1}) and loperamide (2.5 μg mL^{-1}) standards in mobile phase are shown in Fig. 1. The retention times were approximately 7 and 13 min in all experiments for difenoxin and loperamide, respectively.

Determination of extraction efficiencies. Mobile phase and perfusate were each mixed with difenoxin (1 μg mL^{-1}) to determine the extraction efficiency. Separate samples were mixed with loperamide (2·5 μg mL^{-1}). The samples were then loaded onto reverse-phase C18 Sep Pak cartridges. Analysis of the eluents indicated that recovery from the mobile phase was complete, but the recovery was reduced from the samples of perfusate (Table 1).

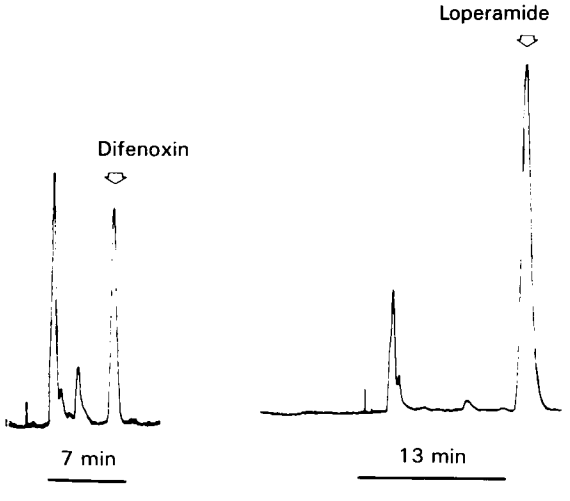

FIG. 1. Chromatogram of drug standards in mobile phase (0·005 aufs).

Table 1. Extraction efficiencies (%) of standards diluted in mobile phase (blank) and in perfusate circulated through the intestinal lumen, and subjected to the extraction procedure using reverse phase C18 Sep Pak cartridges.

Diluent	Difenoxin (1 μg mL^{-1})	Loperamide (2·5 μg mL^{-1})
Blank	$95 \pm 1·7$ (n = 3)	$99·7 \pm 0·3$ (n = 3)
Perfusate	55 ± 18 (n = 3)	79 ± 11 (n = 3)

Table 2. Morphine, loperamide and difenoxin entering the intestinal loop during a 20 min perfusion period. For comparison, data in the table includes that for morphine from a previous study (Margaritis et al 1991).

Opiate	Dose administered (mg kg^{-1})	Route	Time elapsed before perfusing lumen (min)	n	Amount (ng g^{-1} in 20 min)
Morphine	2·5	i.v.	10	5	3433 ± 573
Loperamide	0·6	i.p.	50	5	< 20*
Difenoxin	0·77	s.c.	50	4	< 20*

n = the number of experiments. *Lower limit of detection.

Examination of the chromatograms in this group of experiments indicated that there were no endogenous substances present in the perfusate samples that could interfere with peak detection.

Analysis of perfusates for difenoxin and loperamide. Neither of the antidiarrhoeal drugs were detected in the luminal perfusates of treated rats (Table 2).

Discussion

This study is an extension of previous studies in which we detected morphine in the intestinal lumen of rats (Margaritis et al 1991). The method of sample preparation and HPLC detection used in this study was devised by Pierce et al (1992) for detecting methadone using difenoxin as an internal standard. Analytical methods already available for measuring loperamide are radioimmunoassay (RIA) (Michiels et al 1977), UV spectrophotometry and colorimetry (Tu et al 1989). For difenoxin, radioimmunoassay (Jackson & Stafford 1987) and gas chromatography-mass spectrometry (Ford et al 1976) have been used. Although radioimmunoassay is the preferred analytical method in clinical (human plasma) determinations for these antidiarrhoeals, high performance liquid chromatographic (HPLC) methods are now utilized for determinations in laboratory based research (Tu et al 1989; Margaritis et al 1991; Pierce et al 1992). The HPLC method described in this paper compares favourably with RIA, the lower limits of detection being in the low ng and pg range, respectively.

Using HPLC we report here that the clinically used opiate antidiarrhoeals loperamide and difenoxin do not accumulate in the intestinal lumen. This result is in marked contrast to morphine, which we demonstrated previously to accumulate rapidly in the lumen of the rat jejunum soon after parenteral

administration (Margaritis et al 1991). Morphine and the antidiarrhoeal drugs all share the property of becoming localized within the gut. For example, it has been established that morphine accumulates in intestinal tissue of rats in relatively high concentrations following intravenous or intraperitoneal administration (Bianchi et al 1983). In addition to this we have demonstrated that morphine passes from the tissues of the intestine into the lumen. Relatively large amounts of both morphine and its primary metabolite appeared in the lumen of the small intestine of rats soon after they were injected with an antisecretory dose (Margaritis et al 1991). Like morphine, the antidiarrhoeal drugs have also been shown to concentrate within intestinal tissues after parenteral administration. For example, most of an intravenous dose of loperamide concentrates in the intestine (Wuster & Herz 1978). No information is available for difenoxin by the intravenous route, but it has been shown to accumulate following oral administration (Heykants et al 1972). However, our results show that these antidiarrhoeals do not then pass from tissue to lumen at doses that cause marked inhibition of intestinal secretion.

The present results are in agreement with those of Wuster & Herz (1978), who showed that opiate antidiarrhoeals such as loperamide, but not morphine, possess strong surface tension-lowering activity. They suggested that this physicochemical property results in fixation of the antidiarrhoeals to the intestinal tissue such that they are adsorbed onto membrane surfaces from which they are slowly released.

The results of the present study provide further information regarding the differences in pharmacokinetic profiles of the opiate antidiarrhoeals loperamide and difenoxin compared with morphine.

This work was supported by a Special Initiative Project Grant in Addictive Behaviour by the National Health and Medical Research Council of Australia. We thank Janssen and Ethnor for their generous gifts of respective drugs.

References

Bianchi, G., Feretti, P., Recchia, M., Rocchetti, M., Tavani, A., Manara, L. (1983) Morphine tissue levels and reduction of gastrointestinal transit in rats. Gastroenterology 85: 852–858

Brown, D. R., Miller, R. (1991) Neurohormonal control of fluid and electrolyte transport in intestinal mucosa. In: Field, M., Frizell, R. A. (eds) Handbook of Physiology, Section 6, The Gastrointestinal System. Vol. iv, Intestinal Absorption and Secretion. Oxford University Press, New York, pp 527–589

Brown, N. J., Coupar, I. M., Rumsey, R. D. E. (1988) The effect of acute and chronic administration of morphine and morphine withdrawal on intestinal transit time in the rat. J. Pharm. Pharmacol. 40: 844–848

Coupar, I. M. (1985) Opioid action on the intestine: the importance of the intestinal mucosa. Life Sci. 41: 917–925

De Luca, A., Coupar, I. M. (1992) Difenoxin and loperamide: studies on possible mechanisms of intestinal antisecretory action. Naunyn Schmiedebergs Arch. Pharmacol. 347: 231–237

Ford, G. C., Haskins, N. J., Palmer, R. F. (1976) The measurement of diphenoxylic acid in plasma following administration of diphenoxylate. Biomed. Mass Spectrom. 3: 45

Heykants, J. J. P., Lewi, P. J., Janssen, P. A. J. (1972) Difenoxine (R 15403), the active metabolite of diphenoxylate (R 1132). Arzneim. Forsch. 22: 520–526

Jackson, L. S., Stafford, J. E. H. (1987) The evaluation and application of a radioimmunoassay for the measurement of diphenoxylic acid, the major metabolite of diphenoxylate hydrochloride (Lomotil) in human plasma. J. Pharmacol. Methods 18: 189–197

Mailman, D. (1984) Morphine-neural interactions on canine intestinal absorption and blood flow. Br. J. Pharmacol. 81: 263–270

Margaritis, J., Coupar, I. M., Bentley, G. A. (1991) Studies to determine whether there is tolerance or cross-tolerance to the antisecretory effect of morphine and clonidine in the rat intestine. J. Pharm. Pharmacol. 43: 655–658

Michiels, M., Hendriks, R., Heykants, J. (1977) Radioimmunoassay of antidiarrhoeal loperamide. Life Sci. 21: 451–460

Miller, D. L., Schedl, H. P. (1972) Non-absorbable indicators: a comparison of phenol red and inulin ^{14}C and effects of perfusion techniques. Gastroenterology 62: 48–55

Niemegeers, C. J. E., Lenaerts, F. M., Janssen, P. A. J. (1974) Loperamide (R 18 553), a novel type of antidiarrheal agent. Part I: in vivo oral pharmacology and acute toxicity. Comparison with morphine, codeine, diphenoxylate and difenoxine. Arzneim. Forsch. 24: 1633–1636

Pierce, T. L., Murray, A. G. W., Hope, W. (1992) Determination of methadone and its metabolites by high performance liquid chromatography following solid-phase extraction in rat plasma. J. Chromatogr. Sci. 30: 443–447

Tu, Y. H., Allen, L. V., Wang, D. P. (1989) Stability of loperamide hydrochloride in aqueous solutions as determined by high performance liquid chromatography. Int. J. Pharm. 51: 157–160

Wuster, M., Herz, A. (1978) Opiate agonist action of antidiarrheal agents in vitro and in vivo—findings in support for selective action. Naunyn Schmiedebergs Arch. Pharmacol. 301: 187–194