J. Pharm. Pharmacol. 1993, 45: 919–921
Communicated November 6, 1992

Comparison of the effects of loperamide and loperamide oxide on absorptive processes in rat small intestine

JACQUELINE HARDCASTLE, P. T. HARDCASTLE, J. GOLDHILL*, *Department of Biomedical Science, The University, Western Bank, Sheffield S10 2TN, UK, and *Digestive Diseases Division, Department of Medicine, USUMS, 4301 Jones Bridge Road, Bethesda, MD 20814, USA*

Abstract—Mucosal loperamide inhibited the absorption of glycine by everted sacs of rat small intestine over 10-, 30- or 60-min incubation periods, but loperamide oxide was without effect. In stripped intestinal sheets, loperamide inhibited the rise in short-circuit current associated with Na^+-linked glucose absorption, but this effect was not observed with loperamide oxide. It is concluded that although there is evidence that loperamide oxide is converted to loperamide in the intestinal lumen, the rate at which free loperamide appears is not sufficient to inhibit absorptive processes.

Loperamide is a popular anti-diarrhoeal agent, providing long-lasting and effective protection against diarrhoea (Stockbroeckx et al 1973). This is due, at least in part, to its ability to inhibit intestinal secretion (Hardcastle et al 1981). In addition, however, loperamide also reduces the Na^+-linked absorption of nutrients by the intestine (Hardcastle et al 1986). Since net fluid movement results from the balance between absorptive and secretory solute fluxes, this action will reduce its effectiveness as an anti-diarrhoeal agent. The pro-drug of loperamide, loperamide oxide, has recently been developed and since this is converted to loperamide by the luminal contents of the intestine (Lavrijsen et al 1984; Hardcastle et al 1990) it was the aim of this study to determine whether the oxide exhibited the same anti-absorptive actions as the parent compound.

Materials and methods

Male Wistar rats, 230–250 g, from the Sheffield Field Laboratories, were allowed free access to food and water, and were anaesthetized with sodium pentobarbitone (Sagatal, 60 mg kg^{-1}, i.p.).

Measurement of fluid, glycine and Na^+ absorption. Small intestinal fluid, Na^+ and nutrient absorption was measured using the everted sac preparation (Wilson & Wiseman 1954). Two 10-cm sacs were made from the mid-intestine. Each was filled with 0·5 mL serosal fluid and incubated for varying times at 37°C in 25 mL mucosal fluid. Mucosal and serosal solutions were Krebs bicarbonate saline (Krebs & Henseleit 1932) equilibrated with 95% O_2–5% CO_2 and containing additions as described.

Fluid transport was measured gravimetrically and the volume of fluid taken up by the sac, mucosal fluid transport, was expressed as mL (g initial wet wt)$^{-1}$ time^{-1}.

Glycine absorption was determined using [^{14}C]glycine (10 nCi mL^{-1}) added to the mucosal fluid which contained unlabelled glycine at a concentration of 7·5 mM. At the end of the incubation, the serosal fluid was collected and the empty sac was deproteinized with 0·33 M H_2SO_4 and 10% sodium tungstate and homogenized. Samples were added to scintillation fluid (Optiphase Safe) and counted in a liquid scintillation counter (LKB, 1215 Rackbeta). Glycine absorption was expressed as the amount taken up by the sac in μmol (g initial wet wt)$^{-1}$ time^{-1}, and as the T/M ratio; the glycine concentration in tissue water compared with that in the mucosal solution at the end of the incubation. Tissue concentration was calculated from the amount of glycine in the homogenate and the volume of tissue water (80% initial wet weight plus the volume of fluid taken up during the incubation). The T/M ratio provides an index of active nutrient uptake.

Na^+ transport was determined from the difference in the Na^+ content of the sac (gut + serosal fluid) at the beginning and the end of the incubation. Following incubation, each sac was deproteinized with 5% trichloroacetic acid and the Na^+ concentrations of the homogenate and the serosal fluid samples were analysed by a Corning flame photometer (model 430), allowing the final Na^+ content of the sac to be determined. The initial Na^+ content of the gut wall was measured in separate experiments and that of the initial serosal solution calculated from the initial serosal volume and the Na^+ concentration of the Krebs bicarbonate saline. Uptake was expressed as μmol (g initial wet wt)$^{-1}$ time^{-1}.

Measurement of transintestinal electrical activity. Transintestinal electrical activity was measured in sheets of mid-intestine from which the muscle layers had been removed. These were mounted in Ussing chambers (exposed tissue area = 1·925 cm^2) and incubated at 37°C in Krebs bicarbonate saline gassed with 95% O_2–5% CO_2 as described previously (Hardcastle et al 1981; Hardcastle & Hardcastle 1986, 1987). The serosal fluid contained 10 mM glucose and the mucosal solution 10 mM mannitol and each had a volume of 5 mL. The potential difference (PD) was measured using salt bridge electrodes connected via calomel half-cells to a differential input electrometer. Current was applied across the tissue by Ag/AgCl electrodes which made contact with mucosal and serosal solutions via wide-bore salt bridges and the short-circuit current (SCC) was determined as described by Field et al (1971). Three tissues were taken from each animal. One acted as the control, one received loperamide and one loperamide oxide. Tissues were left to stabilize for 10 min following mounting and readings were then taken at 1 min intervals. After 5-min basal readings, loperamide, loperamide oxide (10^{-4} M in both cases) or ethanol (1·4% v/v) was added to the mucosal solution and after a further 10 min, glucose (10 mM) was added.

Expression of results. Results were expressed as mean values ± s.e.m. for the number of observations indicated. The significance of drug action was assessed using Student's *t*-test, paired or unpaired as appropriate.

Chemicals. Glucose and glycine were obtained from BDH Chemicals, Poole, UK; [U-^{14}C]glycine from Amersham International, Amersham, UK. Loperamide and loperamide oxide were generously provided by Janssen Pharmaceutica, Beerse, Belgium.

Results

Effects on basal fluid and Na^+ absorption by everted sacs. In the absence of nutrient the intestine exhibited a net absorption of fluid and Na^+ (Table 1). Neither loperamide nor the oxide (both

Correspondence: J. Hardcastle, Department of Biomedical Science, The University, Western Bank, Sheffield S10 2TN, UK.

Table 1. Effects of loperamide and loperamide oxide on mucosal fluid transport and Na^+ uptake by everted sacs of rat mid-intestine. Loperamide or loperamide oxide was present in the mucosal fluid at a concentration of 10^{-4} M, with control sacs receiving an equivalent volume of the ethanol vehicle (0·2% v/v). The incubation period was 30 or 60 min as indicated.

	30 min		60 min	
	Mucosal fluid transport (mL g^{-1})	Na^+ uptake (μmol g^{-1})	Mucosal fluid transport (mL g^{-1})	Na^+ uptake (μmol g^{-1})
Ethanol	0·46±0·03 (11)	63·9±3·5 (11)	0·69±0·02 (7)	105·7±2·8 (7)
Loperamide	0·44±0·01 (12)	57·1±2·4 (12)	0·55±0·03 (8)**	91·3±4·8 (8)*
Loperamide oxide	0·47±0·02 (12)	61·6±2·5 (12)	0·68±0·03 (8)	108·4±3·8 (8)

Each value represents the mean±s.e.m. of the number of observations in parentheses and an unpaired *t*-test was used to assess the significance of loperamide or loperamide oxide action. *$P<0·05$, **$P<0·01$.

at 10^{-4} M) affected fluid and Na^+ uptake over 30 min incubation, but after 60 min loperamide caused a significant inhibition although loperamide oxide was still without effect (Table 1).

Effects on fluid and glycine absorption by everted sacs. Loperamide (10^{-4} M) reduced the amount of glycine taken up by the sacs during 10, 30 and 60 min incubation (Table 2). During the two longer periods this was associated with a significant inhibition of the T/M ratio and net fluid uptake (Table 2). In contrast, loperamide oxide at the same concentration failed to influence either glycine or fluid uptake (Table 2). Increasing the concentration to 2×10^{-4} M did not increase the inhibitory actions of loperamide nor did it reveal any effect of loperamide oxide (Table 2).

Effects on basal and glucose-stimulated transintestinal electrical activity. The addition of 10^{-4} M loperamide to the mucosal solution caused a small but significant ($P<0·05$) reduction in the basal SCC of 4±1 (6) μA cm^{-2} (Fig. 1). The equivalent volume of the ethanol vehicle (1·4%, v/v) had no effect (change in SCC = −1±1 (6) μA cm^{-2}, $P>0·05$). Loperamide oxide (10^{-4} M) had no effect on basal SCC (change in SCC = 0±1 (6) μA cm^{-2}, $P>0·05$). The addition of 10 mM glucose to the mucosal

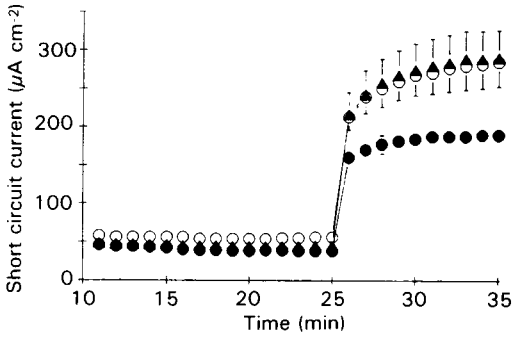

FIG. 1. Effects of loperamide and loperamide oxide (both at 10^{-4} M) on the short-circuit current generated by stripped sheets of rat mid-intestine under basal conditions and in the presence of glucose (10 mM). Loperamide (●) or loperamide oxide (○) was added to the mucosal solution 15 min after the sheets were mounted, while control sheets (▲) received an equivalent volume of the ethanol vehicle (1·4%, v/v). After a further 10 min glucose was added to the mucosal solution. Each point represents the mean±s.e.m. of six observations. The absence of an error bar indicates that the s.e. is less than the size of the symbol.

Table 2. Effects of loperamide and loperamide oxide on mucosal fluid transport and glycine transport by everted sacs of rat mid-intestine. Loperamide or loperamide oxide was present in the mucosal fluid at the concentration indicated, with control sacs receiving an equivalent volume of the ethanol vehicle (0·2%, v/v). The incubation period was 10, 30 or 60 min as indicated.

	Mucosal fluid transport (mL g^{-1})	Total amount transferred (μmol g^{-1})	T/M ratio
10-min incubation			
Ethanol (6)	0·23±0·01	8·90±0·59	1·06±0·06
10^{-4} M Loperamide (6)	0·22±0·02	7·40±0·32*	0·92±0·06
10^{-4} M Loperamide oxide (6)	0·20±0·02	7·82±0·76	0·93±0·08
30-min incubation			
Ethanol (10)	0·58±0·03	26·53±1·51	2·29±0·12
10^{-4} M Loperamide (10)	0·43±0·02*	16·35±1·13*	1·50±0·07*
10^{-4} M Loperamide oxide (10)	0·51±0·03	25·35±1·50	2·28±0·13
Ethanol (8)	0·56±0·04	28·16±1·79	2·51±0·14
2×10^{-4} M Loperamide (8)	0·45±0·05	16·02±1·89*	1·41±0·14*
2×10^{-4} M Loperamide oxide (8)	0·61±0·04	28·22±1·84	2·49±0·14
60-min incubation			
Ethanol (8)	0·82±0·07	40·14±4·29	3·25±0·29
10^{-4} M Loperamide (8)	0·64±0·02*	22·79±1·67**	1·75±0·14***
10^{-4} M Loperamide oxide (8)	0·76±0·05	39·87±1·69	3·47±0·10

Each value represents the mean±s.e.m. of the number of observations in parentheses and an unpaired *t*-test was used to assess the significance of loperamide or loperamide oxide action. *$P<0·05$, **$P<0·01$, ***$P<0·001$.

solution increased the SCC by 246 ± 32 (6) μA cm^{-2} in control tissues. This response was unaffected by the presence of loperamide oxide (change in SCC $= 225 \pm 29$ (6) μA cm^{-2}, $P > 0.05$), but it was significantly reduced by loperamide (change in SCC $= 152 \pm 7$ (6) μA cm^{-2}, $P < 0.05$).

Discussion

It has previously been demonstrated that as well as inhibiting the secretory response of the small intestine (Hardcastle et al 1981), loperamide also reduces its ability to absorb fluid, nutrients and Na^+ (Hardcastle et al 1986). There are several mechanisms for the active transport of Na^+ across the intestine: electrogenic Na^+ absorption where its movement is not coupled to that of any other solute, Na^+ absorption coupled to the absorption of nutrients and the neutral cotransport of NaCl (Fondacaro 1986). The inhibition of nutrient-linked Na^+ absorption by loperamide is more marked than that of nutrient-independent Na^+ movement. This could account for the greater effect of loperamide on glycine transport than on net fluid transport, as the latter has a component not linked to nutrient uptake. This anti-absorptive action will reduce its effectiveness in combating the fluid loss resulting from excessive intestinal secretion. A prodrug of loperamide, loperamide oxide, has been developed to overcome the occasional reports of loperamide overdosage (Friedli & Haenggeli 1980; Minton & Smith 1987). Like other *N*-oxides (Bickel 1969) loperamide oxide itself is inactive, but on contact with the luminal contents it is converted to the parent compound which produces its normal therapeutic actions (Lavrijsen et al 1984; Hardcastle et al 1990). It might therefore be expected that the oxide would exhibit the same effects as loperamide and in its ability to inhibit prostaglandin-induced intestinal secretion this is certainly the case (Hardcastle et al 1990). However, as this study shows, loperamide oxide does not share the anti-absorptive actions of loperamide (Tables 1, 2; Fig. 1). It is not clear why this is so. The finding that 2×10^{-4} M loperamide produces the same inhibition of fluid and glycine transport as 10^{-4} M indicates that a maximal effect is attained. Extending the incubation period to 60 min did not reveal any inhibitory actions of the oxide and previous studies have shown that 10-min incubation of loperamide oxide with the contents of the intestinal lumen is adequate to activate loperamide oxide so that it exerts an antisecretory effect (Hardcastle et al 1990).

Both compounds, administered orally, lead to the appearance of loperamide in the portal vein (Monbaliu et al 1984) so it is evident that loperamide can cross the intestinal mucosa. This is confirmed by the recent observation that loperamide in the lumen of one intestinal loop can influence fluid movement in an adjacent, yet separate, loop where the only means of access is via the circulation (Hardcastle et al 1993). It is therefore possible that in the present experiments, loperamide that is produced from the oxide is taken up by the intestinal mucosa so that its concentration in the mucosal fluid never reaches the minimal level necessary to inhibit absorptive processes. In addition, in the in-vitro preparations employed, the area of intestine in contact with the oxide-containing medium may not provide sufficient converting factor to produce an effective concentration of loperamide. Whatever the reasons, the oxide possesses a potential therapeutic advantage over loperamide in that it is as effective in terms of its ability to inhibit secretion, but it lacks loperamide's anti-absorptive actions. This will result in a more pronounced reduction in net fluid secretion and hence greater anti-diarrhoeal efficacy.

We gratefully acknowledge financial support from Janssen Pharmaceutica, Beerse, Belgium.

References

Bickel, M. H. (1969) The pharmacology of *N*-oxides. Pharmacol. Rev. 21: 325–355

Field, M., Fromm, D., McColl, I. (1971) Ion transport in rabbit ileal mucosa. I. Na and Cl fluxes and short-circuit current. Am. J. Physiol. 220: 1388–1396

Fondacaro, J. D. (1986) Intestinal ion transport and diarrheal disease. Am. J. Physiol. 250: G1–G8

Friedli, G., Haenggeli, C. A. (1980) Loperamide overdose managed by naloxone. Lancet i: 1413

Hardcastle, J., Hardcastle, P. T. (1986) The involvement of basolateral potassium channels in the intestinal response to secretagogues in the rat. J. Physiol. 379: 331–335

Hardcastle, J., Hardcastle, P. T. (1987) The secretory actions of histamine in rat small intestine. J. Physiol. 388: 521–532

Hardcastle, J., Hardcastle, P. T., Read, N. W., Redfern, J. S. (1981) The action of loperamide in inhibiting prostaglandin-induced intestinal secretion in the rat. Br. J. Pharmacol. 74: 563–569

Hardcastle, J., Hardcastle, P. T., Cookson, J. (1986) Inhibitory actions of loperamide on absorptive processes in rat small intestine. Gut 27: 686–694

Hardcastle, J., Hardcastle P.T., Goldhill, J. (1990) The effect of loperamide oxide on prostaglandin-stimulated fluid transport in rat small intestine. J. Pharm. Pharmacol. 42: 364–366

Hardcastle, J., Hardcastle, P. T., Goldhill, J. (1993) Local and systemic actions of loperamide on fluid transport and transmural potential difference across rat small intestine. J. Pharm. Pharmacol. 45: 210–214

Krebs, H.A., Henseleit, K. (1932) Untersuchungen über die Harnstoff bildung im Tierkörper. Hoppe-Seyler's Z. Physiol. Chem. 210: 33–66

Lavrijsen, K., Meuldermans, W., Hendrickx, J., Swysen, E., Heykants, J. (1984) The reductive in vitro metabolism of loperamide *N*-oxide by rat liver homogenates, red blood cells and gut contents. Arch. Int. Pharmacodyn. 270: 174–175

Minton, N. A., Smith, P. G. D. (1987) Loperamide toxicity in a child after a single dose. Br. Med. J. 294: 1383

Monbaliu, J., Michiels, M., Geuens, I., Wiestenborghs, R., Heykants, J. (1984) Plasma concentration of loperamide in male and female rats during subchronic administration of loperamide (R18 553) and of loperamide oxide (R58 425) admixed with food at 25, 100 or 400 ppm. Janssen Pharmaceutica Preclinical Research Report N59672.

Stockbroeckx, R. A., Vandenberk, J., Van Heertum, A. H. M. T., Van Laar, G. M. L. W., Van der Aa, M. J. M. C., Van Bever, W. F. M., Janssen, P. A. J. (1973) Synthetic antidiarrheal agents: 2,2-diphenyl-4-(4′aryl-4′-hydroxypiperidino) butyramides. J. Med. Chem. 16: 782–786

Wilson, T. H., Wiseman, G. (1954) Use of sacs of everted small intestine for the study of the transference of substances from the mucosal to the serosal surface. J. Physiol. 123: 116–125