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Effects of racecadotril and loperamide on bacterial proliferation and on the central nervous system of the newborn gnotobiotic piglet

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SUMMARY

Methods The effects of 4 days of oral administration of different doses of two drugs, an enkephalinase inhibitor (the antisecretory agent, racecadotril) and a μ-receptor agonist (loperamide), on intestinal growth of a bacterial nonpathogenic strain (*Escherichia coli* E 404) and on the central nervous system (CNS) were compared in newborn gnotobiotic piglets.

Results The *E. coli* content of the proximal jejunum (segment S_1) and the *E. coli* ratio of stomach:segment S_1 were similar in the racecadotril (20 mg/kg b.d., $n = 5$) and control groups. In contrast, in the loperamide group (1 mg/kg b.d., $n = 4$), the *E. coli* content of segment S_1 and the *E. coli* ratio stomach:S_1 were both significantly higher than with racecadotril or control ($P = 0.04$ and 0.005, respectively, for *E. coli* content; $P = 0.05$ and 0.03, respectively, for stomach:S_1). There were no clinical signs of neurotoxicity and no deaths with racecadotril given orally at a high dose of 130 mg/kg b.d. ($n = 5$) – nearly 60 times the paediatric dosage. In contrast, an equivalent high dose of loperamide (5 mg/kg b.d.) resulted in death in three out of four piglets.

Conclusions In contrast to loperamide, racecadotril did not induce bacterial overgrowth and did not produce central neurotoxicity.

INTRODUCTION

Racecadotril [racecadotril is the official international nonproprietary name (INN); the drug was known as acetorphan in early studies] and loperamide have both proved effective in the symptomatic treatment of diarrhoea.[1,2] However, they have very different mechanisms of action: racecadotril is an orally active, potent inhibitor of the membrane metalloendopeptidase enzyme, enkephalinase, and therefore its antidiarrhoeal activity is attributable to protection of endogenous enkephalins (δ-receptor opioid agonists). Racecadotril reduces intestinal secretion without increasing intestinal transit time.[3–5] In contrast, loperamide, a μ-receptor opioid agonist, is known to increase intestinal transit time,[6–9] in relation to its disruption of intestinal motor activity.[10,11]

The relationship between a reduction in intestinal motor activity and small bowel bacterial overgrowth has been demonstrated previously. Vantrappen *et al.*[12] reported that a reduction in number of phase III of the migrating myoelectric complex is associated with bacterial overgrowth, while a surgical technique that prevents propagation of the peristaltic waves has also been shown to promote bacterial overgrowth.[13] Hence, treatments are needed for infectious diarrhoea that do not promote intestinal bacterial overgrowth as a consequence of the reduction in intestinal motility.[14]

The aims of the present study were twofold: first, to compare the effects of the antisecretory agent, racecadotril, with those of the antimotility agent, loperamide, on the intestinal bacterial proliferation induced by oral

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administration of a nonpathogenic strain of *Escherichia coli* in newborn gnotobiotic piglets.

Second, as these newborn piglets share the blood–brain barrier immaturity of human infants, it was of interest to compare the central neurological tolerability of high doses of racecadotril and loperamide. Although both drugs are generally well tolerated, some neurotoxicity has been reported with loperamide in children aged less than 2 years.[15]

MATERIALS AND METHODS

Animal preparation

Twenty-seven Large White piglets were born at the Institut National de la Recherche Agronomique (INRA) via Caesarean section ($n = 8$) or natural delivery ($n = 19$). All the Caesarean piglets were obtained from one mother while the natural-born piglets came from three different mothers.[16] Immediately after birth, the piglets were placed in a sterile isolator for the duration of the study. Within 1 h of birth, each piglet received a single oral dose of an antibiotic combination (200 mg neomycin, 100 mg bacitracin, 100 mg streptomycin, and 100 mg ampicillin). They were fed with unskimmed milk prepared with powder (Gloria), irradiated (40 kiloGray), and containing autoclaved sterilized water. Food was available *ad libitum*. The mean weight of the animals was 1.5 kg. All piglets were confirmed as free of bacteria before treatment (on day 2 after their birth).

The study was carried out according to the laboratory's authorization from the French Ministry to perform such experiments with living animals.

Measurement of bacterial proliferation

A nonpathogenic strain of *E. coli* (E 404) was used. This strain is nontoxigenic, nonadhesive, and nonpathogenic, and was isolated and identified by the INRA microbioecology unit from the faeces of healthy piglets bred by the INRA. Each piglet was orally inoculated with a physiological serum suspension containing 5×10^7 colony forming units (cfu) on day 3 after birth, 1 h before drug treatment.

E. coli cultures were taken on a selective enterobacteria nutrient (deoxycholate agar). The main evaluation criterion was the *E. coli* content in segment S_1.

The *E. coli* content in the stools was measured before *E. coli* administration to confirm the axenic nature of the piglets; it was also measured 7 h after administration of *E. coli* to establish the transit time of the *E. coli* inoculum. Stool cultures were performed daily during the study period.

Treatments

Racecadotril was given orally in doses of 20 mg/kg b.d. ($n = 5$) or 130 mg/kg b.d. ($n = 5$). Loperamide was given orally in doses of 1 mg/kg b.d. ($n = 4$) or 5 mg/kg b.d. ($n = 4$). Treatment started on day 3 after birth and was given twice daily for 4 days, at 10:00 AM and 6:00 PM. The drugs were diluted in 9 mL of milk and administered via syringe. No other treatment was allowed during the study. Nine piglets raised under similar conditions acted as the control group.

After 4 days of treatment, animals were sacrificed using chloroform. The stomach and small bowel were removed and the small bowel divided into seven segments of identical length. These were identified as S_1 to S_7 from the proximal (Treitz ligament) to the distal part of the small bowel (the ileo-caecal end). The *E. coli* cfu per gramme of intestinal content was determined in the stomach and in segments S_1, S_3, and S_7.

Statistical analysis

The *E. coli* content was expressed as cfu/g of fresh intestinal content after logarithmic transformation. Results from the three groups – racecadotril, loperamide, and control – were compared using a nonparametric test (Kruskal–Wallis). If this ANOVA was significant, a Newman–Keuls' test was performed to establish where the difference lay, after the normality of the distribution and the homogeneity of variance had been checked by a Kolmogorov–Smirnov's test and Log–ANOVA test, respectively.

RESULTS

Bacterial proliferation

The *E. coli* content of the stomach did not differ significantly among the three groups of animals.

Analysis of the *E. coli* content of segment S_1 (proximal jejunum) showed a significant difference among the three groups ($P = 0.03$, Kruskal–Wallis test), and a significantly greater value with loperamide (120×10^6 cfu/g content) compared with both the control (4×10^6 cfu/g content; $P = 0.005$) and racecadotril (1×10^6 cfu/g

Table 1. *E. coli* content of the proximal jejunum (S_1) in gnotobiotic piglets: comparison of bacterial proliferation after administration of racecadotril or loperamide.

Number of *E. coli*/g content (median)	Racecadotril (20 mg/kg b.d.)	Loperamide (1 mg/kg b.d.)	Control	*P**
$\log_{10}$	6.0	8.1	6.6	0.03
10^6	1	120	4	
P†	0.04		0.005	
	NS (*P*† = 0.86)			

*Kruskal-Wallis test; †Newman-Keuls test.

content; $P = 0.04$) groups. No significant differences were seen between the control and racecadotril groups ($P = 0.86$) (Table 1).

The *E. coli* content from the stomach to segment S_1 decreased in both the racecadotril and control groups but increased in the loperamide group. The ratio stomach:S_1 of the *E. coli* content expressed as the median of 10^6 cfu/g was 10.7 with racecadotril and 2.8 in the control group. In contrast, this ratio was 0.14 in the loperamide group; thus the *E. coli* content in segment S_1 was 7.1-times higher than that of the stomach with loperamide.

The ratio stomach:S_1 of *E. coli* content expressed as $\log_{10}$cfu/g was similar for both the racecadotril and control groups ($P = 0.73$). In contrast, this ratio was significantly lower with loperamide than with racecadotril or control ($P = 0.05$ and 0.03, respectively). This difference corresponded to a bacterial proliferation of four generations with loperamide in comparison to the racecadotril and control groups.

The *E. coli* contents of segments S_3 and S_7 did not differ among the three groups. The S_3 values (mean $\pm$ s.d.; $\log_{10}$cfu/g) were 6.9 ± 1.7 for racecadotril, 7.9 ± 2.0 for loperamide, and 8.8 ± 0.6 for control animals. The S_7 values (mean $\pm$ s.d.; $\log_{10}$cfu/g) were 9.2 ± 0.6 for racecadotril, 10.1 ± 0.5 for loperamide, and 9.8 ± 0.7 for the control group.

The *E. coli* content of stools collected 7 h after *E. coli* administration was significantly different among the three groups ($P = 0.027$, Kruskal–Wallis test). Although no significant difference was seen between the racecadotril and control groups (10.1 ± 0.3 and 10.6 ± 0.2 $\log_{10}$cfu/g, respectively), the *E. coli* content of the stools was decreased with loperamide compared with control (9.9 ± 0.5 $\log_{10}$cfu/g; $P = 0.05$).

Tolerability

No mortalities or abnormal clinical signs occurred in the control and racecadotril groups at any dose. For example, the five piglets who received high-dose racecadotril (130 mg/kg b.d.) had the same clinical evolution as the control piglets from the commune litter.

In the loperamide group (5 mg/kg b.d., $n = 4$), three animals were found dead during the course of treatment. Two female piglets died on days 2 and 3 of treatment and one male on day 4. All deaths were preceded by weakness and paralysis immediately after each drug administration. Some major clinical signs, such as subdued behaviour, were noted during the 4 days of treatment in all loperamide-treated animals. Forelimb paralysis was also noted in the female who died on day 3 and in the one surviving male piglet, and fever was also reported in the male piglet found dead on day 4. Severe constipation and transitory green, hard faeces were noted in two animals found dead on days 3 and 4, respectively. In contrast, piglets born from the same litter and treated with racecadotril showed no signs of toxicity. Due to lack of tolerability, the dose of loperamide was decreased to 1 mg/kg b.d., a dose that did not appear to induce toxicity.

DISCUSSION

Bacterial proliferation

Usually, young axenic animals are obtained by surgical procedures involving hysterectomy or hysteromyotomy of the mother. In this study, piglets were rendered axenic via three steps: cleaning the perineal region of the mother with iodinated bactericidal solution (in piglets born spontaneously), washing the newborn piglets with the same solution in an airlock before placing them in the isolators, and treating them with a single dose of an antibiotic combination. All piglets were found to be axenic throughout their stay in the sterile isolators.[16] In addition, a nonenterotoxigenic, nonadhesive E 404 strain of *E. coli* was used to avoid any acute neonatal diarrhoea during the bacterial proliferation study.[17]

Using this validated experimental model of bacterial proliferation, the gnotobiotic piglet,[16–21] we have shown that 4 days of treatment with equivalent human therapeutic doses of the intestinal antisecretory drug, racecadotril,[1,3–5] did not modify the proliferation of a nonpathogenic *E. coli* strain in the jejunum. In contrast, the antimotility agent loperamide, at a dose that induced the same experimental antidiarrhoeal activity

as racecadotril,[4] promoted proliferation of *E. coli* in segment S_1 (proximal jejunum).

The present results should be compared with those of Runkel *et al.*[22] who showed that morphine, a μ-opioid receptor agonist, increases intestinal transit time, leading to an overgrowth of enteric bacteria in the intestinal lumen secondary to intestinal stasis. Loperamide also increases the intestinal transit time of *E. coli*, as demonstrated by the decrease in *E. coli* content of stools 7 h after administration of the strain compared with control values.

The decrease in *E. coli* content from the stomach to the jejunum in the control and racecadotril groups reflects normal intestinal peristaltic activity. In contrast, the increase in jejunal *E. coli* content with loperamide is related to impairment of normal peristaltic intestinal activity.[11] Such impairment has been associated with a slowing of intestinal transit,[7,8] an increase in gut capacitance,[23] and constipation.[24] The lack of modification of peristaltic intestinal activity with racecadotril is related to the lack of effect of the drug on intestinal transit[3,4] and the resultant lack of constipation.[1,25]

The disruption of interdigestive small bowel motility induced by morphine infusion in rats can induce bacterial overgrowth in the upper small bowel and translocation of enteric bacteria to abdominal organs (mesenteric lymph nodes, liver, and spleen).[26]

The consequences of bacterial overgrowth are many in human clinical practice. Adverse events with antimotility agents such as loperamide can be extremely severe: paralytic ileus, sometimes complicated with necrotizing enterocolitis,[27,28] and toxic megacolon.[29–35] More frequently, they can exacerbate infectious diarrhoea whatever the causative organism: *E. coli*,[36] *Shigella*,[37] *Salmonella*,[38] *Clostridium difficile*,[34] or amoebiasis.[39] The clinical consequences of chronic intestinal bacterial overgrowth are observed in the 'blind-loop syndrome'.[40] This syndrome associates diarrhoea with malabsorption and weight loss, and can be observed in every chronic slowing of intestinal transit whether the cause is visceral neuropathy[41] or ageing.[42] Bacterial translocation is also related to overgrowth of upper gastrointestinal tract microflora and is associated with increased septic complications in surgical patients.[43]

Tolerability

The second aim of this study was to investigate the central neurological tolerability of high doses of racecadotril and loperamide. Three of the four piglets treated with 10 mg/kg/day of loperamide exhibited neurotoxicity followed by death. This result can be related to the central nervous system (CNS) toxicity of loperamide in children: convulsions, lethargy, coma, and respiratory depression. Because of this toxicity, the use of loperamide is contra-indicated in children aged less than 2 years.[15] The FDA maximal recommended dosage for loperamide in children is 3 mg/day for those aged 2–5 years (13–20 kg).[44]

In contrast, the present study demonstrated that racecadotril, even at very high doses, is well tolerated in animals with an immature blood–brain barrier. Other studies have shown that racecadotril does not cross the mature blood–brain barrier.[45,46] In studies carried out in children, racecadotril has usually been given at a dose of 1.5 mg/kg three times daily.[47,48]

The effects of high doses of racecadotril and loperamide were therefore very different: racecadotril did not exhibit neurological toxicity at high doses while neurotoxicological effects were seen with loperamide. However, when the ratios that equated to the daily dosage in children (experimental dosage in piglets/therapeutic dosage in children) were calculated for each study, they were found to be of the same order for both drugs at a ratio of about 9 for the bacterial overgrowth study and a ratio of about 60 for the neurotoxicity study. This demonstrated that racecadotril and loperamide had been given at comparable high doses, although their neurological effects differed.

CONCLUSIONS

In conclusion, this study shows the potential benefits of using an intestinal antisecretory agent such as racecadotril in the symptomatic treatment of diarrhoea, due to the resultant lack of promotion of bacterial overgrowth, in contrast to antimotility agents such as loperamide. Furthermore, this study shows the better CNS tolerability of racecadotril compared with loperamide in animals that have an immature blood–brain barrier and, consequently, the potential of racecadotril for treating diarrhoea in the paediatric setting.

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