

EFFECT OF ANTISECRETORY DRUGS ON EXPERIMENTALLY INDUCED WEANLING DIARRHOEA IN PIGLETS

E. COX¹, V. COOLS AND A. HOUVENAGHEL

Laboratory of Veterinary Physiology, University of Antwerp, State University Centre, Slachthuislaan 68, B-2008 Antwerp, Belgium

¹Present address: Laboratory of Virology, Veterinary Faculty, State University of Ghent, Casinoplein 24, B-9000 Ghent, Belgium

ABSTRACT

Cox, E., Cools, V. and Houvenaghel, A., 1989. Effect of antisecretory drugs on experimentally induced weanling diarrhoea in piglets. *Veterinary Research Communications*, 13 (2), 159–170

In 45 newly-weaned 3 to 4-week-old piglets, diarrhoea was induced by a combined infection with transmissible gastroenteritis (TGE) virus and enterotoxigenic *E. coli* (ETEC) strains. In untreated control animals this dual inoculation resulted in profuse diarrhoea, vomiting, hypovolaemic shock and death of 77% of the animals within five days of TGE virus inoculation. Antisecretory drugs were administered intramuscularly for three consecutive days after experimental infection. The neurolepticum chlorpromazine, at 2 mg/kg/24 h, resulted in a significant inhibition of diarrhoea and vomiting, and in an increase in weight gain and survival. Sedation and hypothermia, however, were serious side-effects. The α_2 agonist clonidine, at 80 μ g/kg/12 h, induced a significant antidiarrhoeal effect and a reduction in mortality. The drug, however, provoked decreased activity of α_2 -adrenergic excitation and incoordination. The β -adrenergic antagonist propranolol, at 0.33 mg/kg/8 h, and the calcium channel blocker verapamil, at 2 mg/kg/8 h, had no beneficial effect on the experimentally induced diarrhoea.

Keywords: bacteriology, drugs, *Escherichia coli*, pigs, transmissible gastroenteritis virus, virology

INTRODUCTION

With improved knowledge of the mechanisms of action of specific diarrhoea-producing organisms, many therapeutic agents have been examined which might exert inhibitory effects at one or another level of the diarrhoeic pathways.

Enterotoxigenic *E. coli* (ETEC) strains adhere with fimbriae to villous epithelial cells, colonize the small intestinal surface and elaborate heat-labile (LT) and/or heat-stable (ST, type a and/or b) enterotoxins, which stimulate cyclic adenosine monophosphate (cAMP)-and/or cyclic guanosine monophosphate (cGMP)-mediated enteric fluid loss by inducing small intestinal hypersecretion. Treatment of secretory diarrhoea could be performed using drugs which inhibit this hypersecretion and/or stimulate intestinal absorption (Donowitz, Wicks and Sharp, 1986; Powell, 1986). Neuroleptics, e.g. chlorpromazine, are reported to induce inhibition of cAMP-mediated fluid secretion (Donowitz, Wicks and Sharp, 1986; Greenough and Rabbani, 1986). Adrenergic receptor agonists, especially the α_2 -agonists, are another promising group of potentially antidiarrhoeal drugs, acting primarily by increasing sodium and chloride absorption and by inhibiting bicarbonate secretion (Donowitz, Wicks and Sharp, 1986; Powell, 1986). The β -adrenergic antagonist, propranolol, has been shown

to be antisecretory for cholera toxin and dibutyl cAMP in rabbit and rat intestine (Donowitz, Wicks and Sharp, 1986). In animal studies verapamil, a well-known calcium channel blocker, increases basal ileal absorption of water, sodium and chloride. Some calcium channel blockers have also been shown to prevent secretion induced by cholera toxin and by ST-enterotoxin of *E. coli* (Donowitz, Wicks and Sharp, 1986).

In pigs post-weaning diarrhoea often results from a combined infection with *E. coli* and a viral enteropathogen (mostly rotaviruses, sometimes coronaviruses) (Tzipori, Chandler, Makin and Smith, 1980; Lecce, 1983; Wilson and Francis, 1986). The enteropathogenic viruses damage the small intestinal villi, resulting in villous atrophy and subsequently in small intestinal malabsorption (Moon, 1978). The dual infection induces a more severe diarrhoea than the infection with ETEC alone (Tzipori, Chandler, Makin and Smith, 1980).

We therefore established a combined hypersecretion-malabsorption diarrhoea model in newly-weaned piglets by inoculating them with transmissible gastroenteritis (TGE) virus, an enteropathogenic coronavirus, and ETEC. In preliminary experiments it has been shown that this combined viral-bacterial infection induces profuse diarrhoea and hypovolaemic shock, which provokes 100% mortality within five days (Cox, Cools, Charlier, Schrauwen and Houvenaghel, 1986).

In the present study the effects of chlorpromazine, clonidine, propranolol and verapamil were evaluated in this model.

MATERIALS AND METHODS

The experiments were performed on 45 newly-weaned, 3 to 4-week-old, female piglets, weighing 4.2 to 7.2 kg, from primiparous sows purchased from the same commercial farm. The piglets were of mixed breed (Pietrain × Belgian Landrace). They were individually housed at $27 \pm 2^\circ\text{C}$ and allowed to drink UHT sterilized whole cow's milk *ad libitum*. To enhance ETEC colonization of the small intestine, the piglets were orally pre-treated on days 1, 2 and 3 with chloramphenicol (Chloromycetin, Parke-Davis; 1875 mg/L milk). This pre-treatment with chloramphenicol renders the dual infection more severe (Cox, Schrauwen and Houvenaghel, 1986), probably by making the gut more susceptible to ETEC (Kaufman, 1984). After starvation for the first three hours of day 4, the piglets were orally inoculated with TGE virus (1.66×10^6 pig infective dose/animal). Twenty-four hours later (day 5) the piglets were starved again for three hours, after which 62 ml of a 1.4% NaHCO_3 solution was given intragastrically to neutralize the gastric acidity and so prevent ETEC destruction. Fifteen minutes later they were intragastrically inoculated with 10 ml of a bacterial suspension of two ETEC strains. Both strains, $\text{O}_{149}:\text{K}_{91}:\text{K}_{88\text{ac}}:\text{LT}$, STa and STb enterotoxin positive respectively, were grown on brain heart infusion agar (Oxoid) at 37°C for 24 h. Bacteria were suspended and diluted in sterile physiological saline to an A_{620} of 0.4, approximately 1.2×10^9 bacteria/ml as determined by viable count.

Antisecretory drug treatment was started 5 h after the ETEC inoculation and was continued for three consecutive days (Table I). Six piglets were injected with chlorpromazine hydrochloride (Rhone-Poulenc) at a dose rate of 1 mg/kg/24 h and six others were injected at a dose rate of 2 mg/kg/24 h. Another group of six piglets

TABLE I
 Experimental groups, dose and frequency of drugs studied, total number of piglets in each group (N1), number of piglets susceptible to K_{88ac} -adhesion (N2), percentage of piglets vomiting, percentage survival and mean survival time in shocked piglets

Group	Dosage mg/kg	Doses/day	N1	N2	Vomiting (%)	Survival (%)	Mean survival time of shocked pigs (days after TGE virus)
Control	-	-	9	6	50	33	3.3 ± 0.5
Chlorpromazine	1	1	6	5	80	0	4.1 ± 0.7
Chlorpromazine	2 ^a	1	6	5	0	80	3.0
Clonidine	0.04 ^b	2	6	5	40	40	3.8 ± 0.5
Clonidine	0.08 ^c	2	6	6	50	83*	6.0*
Propranolol	0.33	3	6	5	100	60	3.5 ± 0.5
Verapamil	2 ^c	3	6	6	50	33	2.9 ± 0.5

^a Third injection, 1.5 mg/kg; ^b first injection, 0.03 mg/kg; ^c first injection, 0.04 mg/kg

* Significant difference from the control group, $p < 0.05$

was injected with clonidine hydrochloride (Boehringer Ingelheim) at 0.04 mg/kg/12 h, whereas six animals received 0.08 mg/kg/12 h. A further six piglets were injected with propranolol hydrochloride (ICI) at a dose rate of 0.33 mg/kg/8 h. Finally, six piglets were treated with verapamil hydrochloride (Knoll AG) at a dose rate of 2 mg/kg/8 h. To reduce side-effects the first or last dosage was lower for several of the drugs (Table I). All the drugs were dissolved in distilled water and injected intramuscularly. The other nine piglets were kept as untreated controls.

During the experiment clinical signs, severity of diarrhoea, milk intake, weight change, body temperature and survival were recorded daily. The severity of the diarrhoea was evaluated by arbitrarily scoring the consistency of the faeces (0 = normal; 1 = pasty; 2 = semi-liquid; 3 = watery). Scoring was always performed by the same person.

Shocked piglets died spontaneously or were killed with an overdose of methomidate (Hypnodil, Janssen). The other piglets were killed with methomidate 10 days after the start of the experiment. All piglets were examined *post mortem*.

The genetic susceptibility of the piglets to K_{88ac} -ETEC adhesion to their small intestinal villi was determined by the *in vitro* technique described by Girardeau (1980).

The daily diarrhoea score was expressed as median and ranges or individual values and statistically analysed using the Mann-Whitney U or Wilcoxon tests. The one-way analysis of variance and simple contrasts were used to assess the statistical significance between the values of the different parameters after experimental infection and those before the infection on day 3. The Mann-Whitney U test was used to demonstrate significant differences for time-matched values between control and treatment groups. The differences in survival time between the treatment groups and the control group were evaluated using the Mantel modification of the Gehan generalized Wilcoxon test (Knapp and Wise, 1985).

RESULTS

The *in vitro* adhesion assay performed immediately *post mortem* revealed that the villi of seven piglets were genetically resistant to adhesion of K_{88ac} -ETEC to their enterocytes (Table I). Data from these piglets are not included in the results.

As previously described (Cox, Charlier and Houvenaghel, 1988), piglets developed diarrhoeic faeces during the chloramphenicol pre-treatment period (Figure 1, day 3).

In the control group diarrhoea seriously worsened after inoculation with TGE virus and ETEC ($p < 0.05$). Daily milk intake decreased (day 3, 1117 ± 132 ml; day 6, 630 ± 184 ml; $p > 0.05$), vomiting occurred in 50% of the animals (Table I) and there was a significant weight loss (Figure 2) and decrease in body temperature (Table II). Piglets became dehydrated as shown by bilateral retraction of the eyeballs and a decrease in skin turgor. Cyanosis of the extremities and depression occurred, and 77% of the animals developed hypovolaemic shock (Table I). In the terminal stage some animals lay in lateral decubitus, with sunken flanks and a prominent pelvis. Mean survival time of shocked piglets was 3.3 ± 0.5 days after TGE virus inoculation. The gross changes at necropsy were cyanosis of the extremities, a filled stomach, severe congestion of the gastric mucosa, watery contents in and congestion of the small intestine and sometimes of the colon, and petechial haemorrhages in the kidneys.

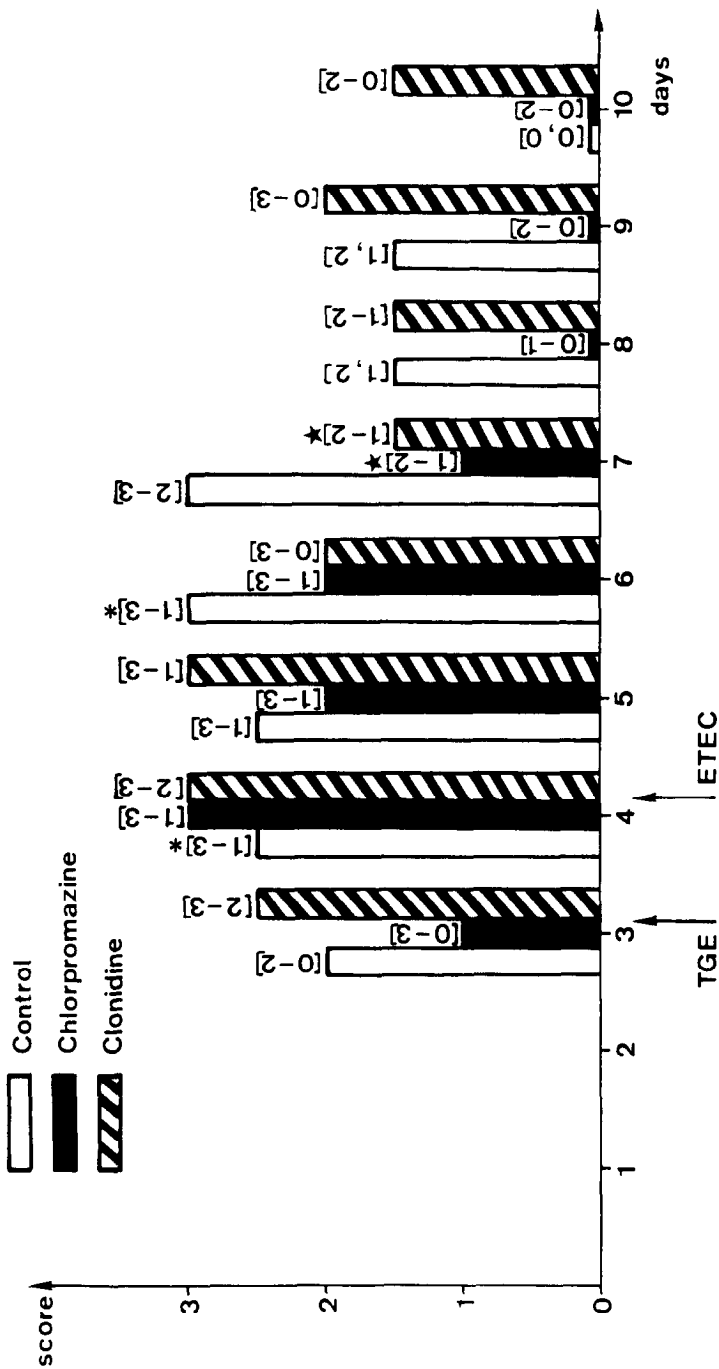


Figure 1. Diarrhoea scores (0 = normal; 1 = pasty; 2 = semi-liquid; 3 = watery) after a combined infection of newly-weaned piglets with TGE virus and ETEC (infected control group; $n = 6$) and the effect of treatment with chlorpromazine at 2 mg/kg/24 h (chlorpromazine group; $n = 5$) or with clonidine at 80 $\mu\text{g}/\text{kg}/12$ h (clonidine group; $n = 6$). Bars represent medians; ranges or individual values are given in brackets. Significant differences from the values prior to infection (day 3) are indicated by * ($p < 0.05$). Significant differences from the values of the control group are indicated by \star ($p < 0.05$).

TABLE II
Daily body temperature in °C (mean ± SEM or individual values) in the control and treatment groups

Groups (n)	Time (days)									
	3	4	5	6	7	8	9	10		
Control (6)	39.3 ± 0.1	39.1 ± 0.1	38.6 ± 0.4**	38.8 ± 0.3*	38.9 ± 0.2	39. & 39.2	38.9 & 39	38.6 & 39.3		
Chlorpromazine 1 mg (5)	39.7 ± 0.1 ^{†††}	39.6 ± 0.1 ^{††}	38.6 ± 0.2***	36.9 ± 0.7	38.4 & 41.1	36.1 & 39.8	41.7	-		
Chlorpromazine 2 mg (5)	39.7 ± 0.1 ^{††}	39.4 ± 0.2	38.9 ± 0.2	38.0 ± 0.6 [†]	38.8 ± 0.3	39.0 ± 0.2	-	38.9 ± 0.4		
Clonidine 0.04 mg (5)	39.4 ± 0.1	39.9 ± 0.2 ^{††}	39.9 ± 0.2 ^{††}	38.1 ± 0.5	38.6 ± 0.5	39.4 & 39.7	38.9 & 39.2	38.7 & 39.4		
Clonidine 0.08 mg (6)	39.1 ± 0.1	38.7 ± 0.1 [†]	38.9 ± 0.1	39.1 ± 0.2	39.8 ± 0.2 [†]	40.0 ± 0.3	39.7 ± 0.2	39.5 ± 0.3		
Propranolol (5)	39.2 ± 0.2	39.3 ± 0.2	39.5 ± 0.2 ^{††}	40.0 ± 0.3 [†]	39.4 ± 0.2	39.8 ± 0.1	39.4 ± 0.3	39.1 ± 0.4		
Verapamil 2 mg (6)	39.5 ± 0.1	39.1 ± 0.1	39.3 ± 0.3	38.8 ± 0.5	38.3 ± 1.0	39.5 & 39.8	38.9 & 39.2	38.7 & 39.1		

n = Number of piglets susceptible to K_{88ac} -adhesion in each group

Significant differences from the values prior to infection (day 3 values) are indicated by * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$)

Significant differences from the control group are indicated by [†] ($p < 0.05$); ^{††} ($p < 0.01$); ^{†††} ($p < 0.001$)

- = Not determined

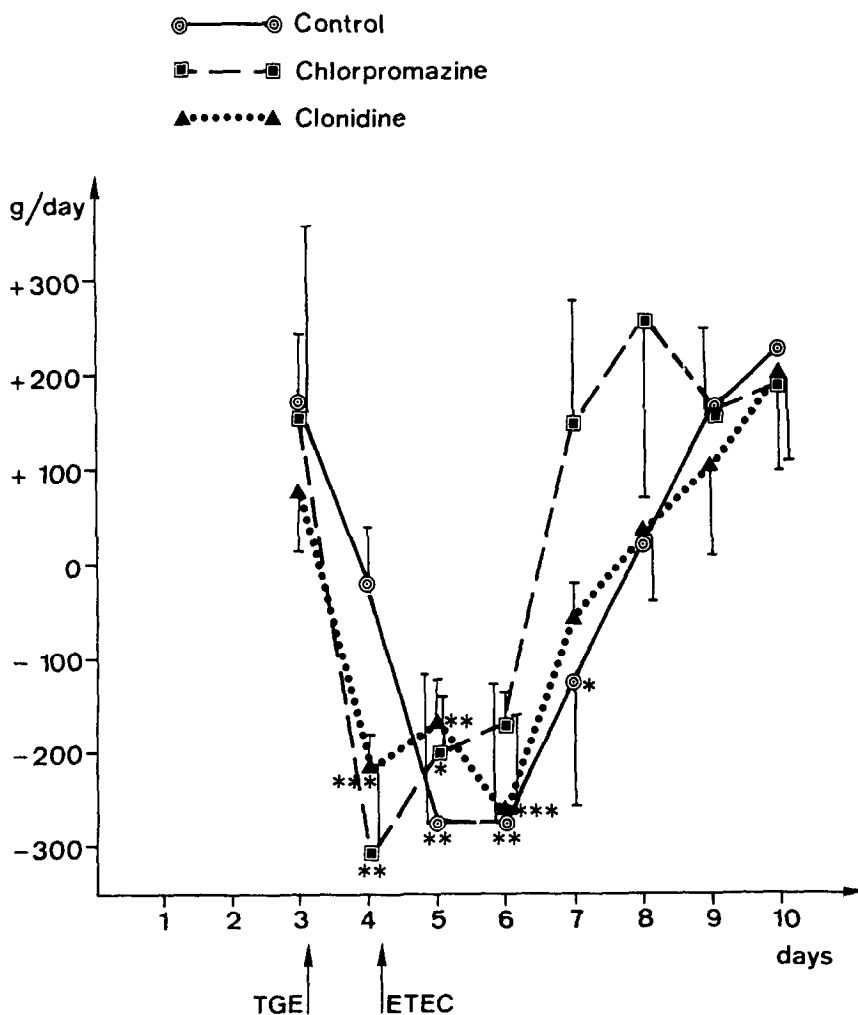


Figure 2. Weight change after a combined infection of newly-weaned piglets with TGE virus and ETEC (infected control group; $n = 6$) and effect of treatment with chlorpromazine at 2 mg/kg/24 h (chlorpromazine group; $n = 5$) or with clonidine at 80 $\mu\text{g}/\text{kg}/12$ h (clonidine group; $n = 6$). The values represent means \pm SEM. Significant differences from the values prior to infection (day 3) are indicated by * ($p < 0.05$); ** ($p < 0.01$) and *** ($p < 0.001$)

Treatment with the low dose of chlorpromazine at 1 mg/kg/24 h was without beneficial effect on diarrhoea score, vomiting or survival (Table I). However, the larger dose of this neurolepticum, 2 mg/kg/24 h, resulted in a significant decrease in the diarrhoea score on day 7 (Figure 1), a complete inhibition of vomiting (Table I), a less pronounced ($p > 0.05$) weight loss (Figure 2) and an increase, although not a significant one, in percentage survival (Table I). Chlorpromazine administration,

especially in the larger doses, also provoked marked sedation and a resulting further reduction in daily milk intake (340 ± 118 ml on day 6, as against 630 ± 184 ml in the control group; $p > 0.05$), and a significantly more pronounced decrease in body temperature on day 6 (Table II) as compared to the control piglets. The decrease in body temperature was observed within one hour of the first administration of the neurolepticum and became more pronounced after the second. On the third day we therefore injected 1.5 mg/kg instead of 2 mg/kg.

Administration of clonidine at the low dosage, 0.04 mg/kg/12 h, was without influence on the parameters studied, whereas at a dose rate of 0.08 mg/kg/12 h there was a decrease in diarrhoea score (Figure 1; day 7 values) and a significant reduction in mortality (Table I). Vomiting (Table I), daily milk intake and weight change (Figure 2) were not significantly influenced by this α_2 -adrenoceptor agonist, whereas body temperature did not undergo the significant decrease after experimental infection observed in the control pigs (Table II). In both clonidine groups serious side-effects were observed. Administration of the low dose induced decreased activity, whereas with the higher dose, although activity was usually decreased, there was sometimes excitation or even incoordination. No analgesic effects were noted 15 min or 1 h after clonidine injection as evaluated by gently pinching the coronets and the skin of the feet and thorax.

Propranolol at 0.33 mg/kg/8 h and verapamil at 2 mg/kg/8 h were without significant beneficial effect on diarrhoea score, vomiting, weight change or survival rate (Table I). The decrease in body temperature observed in control piglets after TGE virus and ETEC inoculation was not influenced by the calcium antagonist, whereas administration of the β -adrenergic antagonist resulted in an increase in temperature (Table II).

DISCUSSION

ETEC strains are involved in the multifactorial post-weaning diarrhoea syndrome in piglets, stimulating electrolyte and fluid secretion by the crypt cells (LT and STa enterotoxins) or diminishing absorption by the villous cells (LT enterotoxin). The mechanism of action of STb is still unclear. Recently rotavirus has also been shown to exert a role in the decrease of villous absorption (Tzipori, Chandler, Makin and Smith, 1980; Lecce, 1983). Since rotavirus is enzootic in Belgian piggeries (Debouck, 1984) and the experiments were performed on conventionally bred piglets, TGE virus, another enteropathogenic virus, was selected to induce a combined viral-bacterial enteritis. Whereas rotavirus only infects the upper part of the villi, TGE virus multiplies in and destroys the major part of the villous epithelium (Moon, 1978), resulting in more profuse diarrhoea and vomiting. In the very young piglets TGE is almost invariably fatal, whereas animals over three weeks old usually survive the infection (Aitken, 1983). In the present study most dually infected piglets in the control group and also in some of the treatment groups developed hypovolaemic shock as a result of vomiting, induced by the TGE virus, and diarrhoea, probably induced by a combination of malabsorption (TGE virus) and hypersecretion (ETEC) (Cox, Charlier and Houvenaghel, 1988). The diarrhoea occurring during the chloramphenicol pre-treatment can be explained by the change in diet from sow's to cow's milk, by the loss of maternal immunity, by environmental changes (Tzipori, Chandler,

Makin and Smith, 1980; Lecce, 1983) and perhaps also by a direct effect of the antibiotic on the intestine. It has been shown that chloramphenicol suppresses the anaerobic gut flora thus probably increasing the intestinal susceptibility to potentially pathogenic micro-organisms (Kaufman, 1984), whereas in calves a malabsorption diarrhoea has been reported following oral treatment with chloramphenicol (Mero, Rollin and Phillips, 1985). We recently evaluated the effect of the chloramphenicol pre-treatment on our diarrhoea model. Four piglets were treated with chloramphenicol for three days and were subsequently not inoculated with TGE virus and ETEC strains. Faeces became pasty to semi-liquid in two animals during treatment. After treatment, however, diarrhoea resolved within one day (Cox, Charlier and Houvenaghel, 1988).

The clinical signs in the markedly shocked piglets in our study agree with earlier observations on *E. coli* infections in gnotobiotic piglets (Kenworthy and de G. Mitchell, 1976), whereas our necropsy findings correspond to those observed in piglets which have died from post-weanling diarrhoea.

In the last decade there has been steady progress in research on potentially useful drugs which can reduce fluid loss in secretory diarrhoea (Greenough and Rabbani, 1986; Powell, 1986). These drugs, however, have never been tested in a combined secretion-malabsorption diarrhoea.

In animals chlorpromazine inhibits cAMP-induced hypersecretion provoked by the LT enterotoxin of *E. coli* and *Vibrio cholerae*, prostaglandins and dibutyryl cAMP, as well as cGMP-mediated hypersecretion due to the ST enterotoxin of *E. coli* (Lönnroth and Jennische, 1982; Donowitz, Wicks and Sharp, 1986). This neuroleptic probably also stimulates intestinal absorption of sodium and chloride, as demonstrated for promethazine in the rabbit ileum (Cohen, Sharp and Donowitz, 1985). In human medicine the beneficial effect of chlorpromazine as a therapeutic antisecretory drug was first reported in patients with severe diarrhoea due to cholera enterotoxin (Greenough and Rabbani, 1986). However, sedation was a serious disadvantage in its clinical use. In newborn piglets with experimentally ETEC-induced diarrhoea, a single intramuscular administration of chlorpromazine at 5 mg/kg one hour after the onset of diarrhoea completely normalized the intestinal fluid content within four hours but seriously sedated the animals, whereas at a dosage of 1-2 mg/kg there were no side-effects but an obvious antisecretory effect still occurred (Lönnroth, André, Lange, Martinsson and Holmgren, 1979). These authors confirmed their findings in a field trial in which the duration of diarrhoea in a spontaneous outbreak due to ETEC was significantly reduced by a single intramuscular injection of chlorpromazine at 1 mg/kg in addition to oral electrolyte solution and standard antimicrobial agents. In our present study, in which no additional therapy was performed, we only observed significant anti-diarrhoeic and antiemetic effects resulting in increased survival following the administration of 2 mg/kg/24 h of the neurolepticum for three consecutive days. However, it must be mentioned that the piglets in the present study were newly weaned and had been inoculated with an enteropathogenic virus prior to ETEC infection, resulting in a more pronounced small intestinal fluid loss. Sedation and suppression of vomiting are well-known pharmacological properties of neuroleptic agents (Booth, 1977; Baldessarini, 1980). These drugs also induce hypothermia (Booth, 1977; Baldessarini, 1980), which explains the significantly more pronounced decrease in body temperature in the chlorpromazine (2 mg/kg) group as compared to the control

group. Despite important side-effects such as sedation, decreased daily milk intake and hypothermia, chlorpromazine at 2 mg/kg/24 h would probably be useful in the treatment of severe diarrhoea of mixed viral-ETEC origin in weanling piglets.

Clonidine has been reported to inhibit castor oil-induced secretion diarrhoea in rats (Lal, Shearman and Ursillo, 1981; Spraggs and Bunce, 1983). In ligated jejunal loops in 2-week-old piglets this drug reduces the net secretion of water and electrolytes induced by the ST enterotoxin of *E. coli* (Ahrens and Zhu, 1982; Zhu and Ahrens, 1983). The α_2 -adrenergic agonist, however, was without effect on the ST-induced increase in chloride efflux from isolated porcine enterocytes (Ahrens and Panichkriangkrai, 1985). In a study on its antidiarrhoeal action in man, Schiller, Santa Ana, Morawski and Fordtran (1985) postulated that clonidine has two actions which might contribute to this effect. Firstly, the drug directly stimulates the rate of intestinal water and electrolyte absorption both *in vitro* and *in vivo* by means of an α_2 -adrenergic receptor. Secondly, clonidine has been found to inhibit intestinal motility. In the present porcine study, administration of clonidine at 80 $\mu\text{g}/\text{kg}/12$ h for three consecutive days resulted in beneficial effects on diarrhoea and survival rate. However, the drug also induced important side-effects such as decreased activity or excitation and incoordination, thus compromising its use as an antidiarrhoeal drug in practice. The sedative effects are similar to those of chlorpromazine (Blaschke and Melmon, 1980). Our observations do not confirm the analgesic effects of clonidine previously reported in pigs, horses and man especially after epidural administration (Wintzer, Krause, Siedentopf and Frey, 1985; Gordh, Feuk and Norlén, 1986).

The β -adrenergic antagonist, propranolol, has a species-specific action. It has been reported to be antisecretory for cholera toxin and dibutyryl cAMP in rat and rabbit but not in mice small intestine (Larsen, 1982; Donowitz, Wicks and Sharp, 1986). In human medicine, an increase in body temperature in response to propranolol is an infrequent side-effect, reflecting an allergic reaction (Weiner, 1980). The calcium antagonist, verapamil, has been observed to increase basal ileal absorption of sodium, chloride and water and to block jejunal secretory response to the ST enterotoxin of *E. coli* (Forsyth, Wong and Maenz, 1985; Donowitz, Wicks and Sharp, 1986). The results from our porcine study, however, failed to reveal any beneficial effects of verapamil or propranolol, at least in the dosage studied, on the experimentally induced diarrhoea.

Our results suggest that, of the drugs studied, only chlorpromazine and clonidine are likely to be of value in the treatment of severe diarrhoea of mixed viral-ETEC nature in newly-weaned piglets. Their observed side-effects are, however, a serious disadvantage for their practical use.

ACKNOWLEDGEMENTS

This work was supported by grant 4640A from the Belgian Institute for the Encouragement of Research in Industry and Agriculture (IWONL). The authors thank Professor Dr M. Pensacrt (University of Ghent, Belgium) for supplying the TGE virus, and Dr P. Pohl and Dr P. Lintermans (National Institute for Veterinary Research, Brussels) and Smith Kline-RIT (Genval) for the supply of the ETEC strains. Chlorpromazine, clonidine, propranolol and verapamil were generous gifts from Rhone-Poulenc, Boehringer Ingelheim, ICI-Pharma, and Knoll AG respectively. The technical assistance of Mr J. Bollé, Mrs C. De Schepper and Mrs R. Hens is gratefully acknowledged.

REFERENCES

- Ahrens, F. A. and Panichkriangkrai, W., 1985. Effects of *Escherichia coli* heat-stable enterotoxin, atropine, clonidine and morphine on chloride efflux from isolated enterocytes. *American Journal of Veterinary Research*, **46**, 2067-2071
- Ahrens, F.A. and Zhu, B., 1982. Effects of epinephrine, clonidine, 1-phenylephrine and morphine on intestinal secretion mediated by *Escherichia coli* heat-stable enterotoxin in pig jejunum. *Canadian Journal of Physiology and Pharmacology*, **60**, 1680-1685
- Aitken, I.D., 1983. Structural and functional damage caused by viral infection of the small intestine. In: R.M. Batt and T.L.J. Lawrence (eds.), *Function and Dysfunction of the Small Intestine*, (Liverpool University Press, Liverpool), 219-245
- Baldessarini, R.J., 1980. Drugs and the treatment of psychiatric disorders. In: A. Goodman Gilman, L.S. Goodman and A. Gilman (eds.), *The Pharmacological Basis of Therapeutics*, 6th edn., (MacMillan, New York), 391-447
- Biaschke, T.F. and Melmon, K.L., 1980. Antihypertensive agents and the drug therapy of hypertension. In: A. Goodman Gilman, L.S. Goodman and A. Gilman (eds.), *The Pharmacological Basis of Therapeutics*, 6th edn., (MacMillan, New York), 793-818
- Booth, N.H., 1977. Psychotropic agents. In: L.M. Jones, N.H. Booth and L.E. McDonald (eds.), *Veterinary Pharmacology and Therapeutics*, 4th edn., (Iowa State University Press, Ames, Iowa), 373-404
- Cohen, M.E., Sharp, G.W.G. and Donowitz, M., 1985. Promethazine stimulates NaCl absorption and inhibits Ca²⁺-calmodulin-dependent phosphorylation (P) in rabbit ileum at the same low concentrations suggesting a role for P in NaCl transport. *Federation Proceedings*, **44**, 1744
- Cox, E., Charlier, G. and Houvenaghel, A., 1988. Electron microscopic study of an experimental combined infection of just-weaned piglets with transmissible gastroenteritis virus and K88ac positive enterotoxigenic *Escherichia coli*. *Journal of Veterinary Medicine*, **35**, 317-330
- Cox, E., Cools, V., Charlier, G., Schrauwen, E. and Houvenaghel, A., 1986. Experimental combined infection of just-weaned piglets with transmissible gastroenteritis virus and enterotoxigenic *E. coli*. *IRCS Medical Science*, **14**, 934-935
- Cox, E., Schrauwen, E. and Houvenaghel, A., 1986. Experimental infection of piglets with enterotoxigenic *E. coli*: effect of oral chloramphenicol pretreatment. *IRCS Medical Science*, **14**, 374-375
- Debouck, P., 1984. Rotavirus infekties bij biggen: een epizootologische studie. *Vlaams Diergeneeskundig Tijdschrift*, **53**, 449-453
- Donowitz, M., Wicks, J. and Sharp, G.W.G., 1986. Drug therapy for diarrheal diseases: a look ahead. *Reviews of Infectious Diseases*, **8**, suppl. 2, S188-S201
- Forsyth, G.W., Wong, P.H. and Maenz, D.D., 1985. Calcium mediation of the pig jejunal secretory response. *Canadian Journal of Comparative Medicine*, **49**, 179-185
- Girardeau, J.P., 1980. A new *in vitro* technique for attachment to intestinal villi using enteropathogenic *Escherichia coli*. *Annales de Microbiologie*, **131B**, 31-37
- Gordh, T., Feuk, U. and Norlén, K., 1986. Effect of epidural clonidine on spinal cord blood flow and regional and central hemodynamics in pigs. *Anesthesia and Analgesia*, **65**, 1312-1318
- Greenough III, W.B. and Rabbani, G.H., 1986. Antisecretory and antimicrobial drugs for treating diarrhoea. In: J. Holmgren, A. Lindberg and R. Molby (eds.), *Development of Vaccines and Drugs against Diarrhea, 11th Nobel conference, Stockholm, 1985*, (Studentlitteratur, Lund, Sweden), 270-277
- Kaufman, J., 1984. Nosocomial infections: Klebsiella. *Compendium on Continuing Education for the Practicing Veterinarian*, **6**, 303-307
- Kenworthy, R. and de G. Mitchell, I., 1976. *Escherichia coli* infection of gnotobiotic pigs: significance of enterotoxin and endotoxin in the clinical state. *Journal of Comparative Pathology*, **86**, 275-284
- Knapp, R. and Wise, W., 1985. A more appropriate statistical method for analyzing mortality data in shock research. *Circulatory Shock*, **16**, 375-381
- Lal, H., Shearman, G.T. and Ursillo, R.C., 1981. Non-narcotic antidiarrheal action of clonidine and lofedidine in the rat. *Journal of Clinical Pharmacology*, **21**, 16-19
- Larsen, J.J., 1982. A study on inhibition of cholera toxin-induced intestinal hypersecretion by neuroleptics. *Acta Pharmacologica et Toxicologica*, **50**, 294-299
- Lece, J.G., 1983. Dietary regimen, rotavirus, and hemolytic enteropathogenic *Escherichia coli* in weanling diarrhea of pigs. *Annales de Recherches Vétérinaires*, **14**, 463-468
- Lönnroth, I., André, B., Lange, S., Martinssen, K. and Holmgren, J., 1979. Chlorpromazine reverses diarrhea in piglets caused by enterotoxigenic *Escherichia coli*. *Infection and Immunity*, **24**, 900-905
- Lönnroth, I. and Jennische, E., 1982. Reversal of enterotoxic diarrhoea by anaesthetic and membrane-stabilizing agents. *Acta Pharmacologica et Toxicologica*, **51**, 330-335
- Mero, K.N., Rollin, R.E. and Phillips, W., 1985. Malabsorption due to selected oral antibiotics. In: E. Hunt (ed.), *The Veterinary Clinics of North America: Food Animal Practice, Vol. 1*, (W.B. Saunders, Philadelphia), 581-588
- Moon, H., 1978. Mechanisms in the pathogenesis of diarrhea: a review. *Journal of the American Veterinary Medical Association*, **172**, 443-447

- Powell, D.W., 1986. Mechanisms of antisecretory agents and prospects for novel drugs. In: J. Holmgren, A. Lindberg and R. Molby (eds.), *Development of Vaccines and Drugs against Diarrhea, 11th Nobel conference, Stockholm, 1985*, (Studentlitteratur, Lund, Sweden), 257-269
- Schiller, L.R., Santa Ana, C.A., Morawski, S.G. and Fordtran, J.S., 1985. Studies of the antidiarrheal action of clonidine. Effects on motility and intestinal absorption. *Gastroenterology*, **89**, 982-988
- Spraggs, C.F. and Bunce, K.T., 1983. α_2 -Adrenoceptors and the delay of castor oil-induced diarrhoea by clonidine in rats. *Journal of Pharmacy and Pharmacology*, **35**, 321-322
- Tzipori, S., Chandler, D., Makin, T. and Smith, M., 1980. *Escherichia coli* and rotavirus infections in four-week-old gnotobiotic piglets fed milk or dry food. *Australian Veterinary Journal*, **56**, 279-284
- Weiner, N., 1980. Drugs that inhibit adrenergic nerves and block adrenergic receptors. In: A. Goodman Gilman, L.S. Goodman and A. Gilman (eds.), *The Pharmacological Basis of Therapeutics*, 6th edn., (MacMillan, New York), 176-210
- Wilson, R.A. and Francis, D.H., 1986. Fimbriae and enterotoxins associated with *Escherichia coli* serogroups isolated from pigs with colibacillosis. *American Journal of Veterinary Research*, **47**, 213-217
- Wintzer, H.J., Krause, D., Siedentopf, C.H. and Frey, H.H., 1985. Clonidin als Sedativum beim Pferd. *Berliner und Münchner Tierärztliche Wochenschrift*, **98**, 190-193
- Zhu, B. and Ahrens, F., 1983. Antisecretory effects of berberine with morphine, clonidine, l-phenylephrine, yohimbine or neostigmine in pig jejunum. *European Journal of Pharmacology*, **96**, 11-19

(Accepted: 9 January 1989)