Drug Development Research 8:279–286 (1986)

Antidiarrheal Specificity and Safety of the N-Oxide of Loperamide (R 58 425) in Rats

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ABSTRACT

Neimegeers, C.J.E., F. Awouters, F.M. Lenaerts, K.S.K. Artois, and J. Vermeire: Antidiarrheal specificity and safety of the N-oxide of loperamide (R 58 425) in rats. Drug Dev. Res. **8**:279–286, 1986.

The pharmacological and toxicological effects of loperamide and the N-oxide of loperamide were studied in adult and 19-day-old rats and in 1-day-old pups. In adult rats both orally administered compounds were equipotent and specific antidiarrheals, but the N-oxide was slightly less toxic. In these rats, i.v. injected N-oxide, in contrast to loperamide, did not induce opiate-like central effects and its intestinal activity developed more gradually. In 19-day-old rats high oral doses of both compounds induced opiate-like behavioral effects, but the i.v. injected N-oxide was again virtually devoid of these effects and proved 5 times less toxic than i.v. loperamide. The orally administered compounds in 1-day-old pups induced lethality at doses slightly higher than the i.v. lethal doses of loperamide in 19-day-old and adult rats. The results are in agreement with rapid and efficient conversion of the N-oxide to loperamide in intestine and in liver, and a much slower and pharmacologically hardly detectable conversion of circulating N-oxide. The visceral barriers, which limit access of the orally administered compounds to the systemic circulation, appear fully developed in 19-day-old rats; the blood-brain barrier at this age is still more permeable than in adult rats. The more gradual development of antidiarrheal activity and the reduced risk of acute systemic intoxication are the major pharmacological differences between orally administered N-oxide and loperamide.

Key words: castor oil diarrhea, opiate effects, acute toxicity

Received, final version March 3, 1986; accepted March 3, 1986.

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INTRODUCTION

The specific antidiarrheal, loperamide [Niemegeers et al., 1974], has recently been shown to be both effective and safe in very young patients, even at high doses [Aubry et al., 1984; Chapoy and Lonchet, 1983; Evans et al., 1984; Excler et al., 1985; Hamdi and Dodge, 1985; Kassem et al., 1983; Sandhu et al., 1983; Soeparto et al., 1981; Vesikari and Isolauri, 1985]. However, these data were often obtained in well-nourished, hospitalized children who were provided with other therapeutic measures such as rehydration. In the absence of optimal conditions, loperamide treatment for critical diarrhea in malnourished infants is not generally accepted. Improvement of safety, rather than potency, therefore appears to be the primary objective for the more generally useful antidiarrheal of the future.

The present study describes some pharmacological and toxicological data obtained in rats of different ages with a prodrug of loperamide, the trans N-oxide of loperamide. Based on the results obtained, a further increase in safety may be expected.

MATERIALS AND METHODS

Wistar rats of both sexes and different ages (adults, 19-day-old and 1-day-old) were used. The compounds studied were loperamide and the trans N-oxide of loperamide (Fig. 1). The different doses were given either as solutions in PPG 20% (i.v. injections in one of the tail veins) or as suspensions homogenized by ultrasonic waves (oral administration by gavage).

The castor oil test was used to determine antidiarrheal activity [Niemegeers et al., 1972, 1974, 1976a, 1979]. The test compounds were administered orally or intravenously at various doses to food-deprived (overnight) rats. One hour later 1 ml of castor oil was given orally. Presence of diarrhea was recorded 1, 2, 3, 4, 6, and 8 hr after castor oil and absence of diarrhea was the criterion of drug effectiveness.

The tail withdrawal reaction [Janssen et al., 1963; Niemegeers, et al., 1976b] was used to determine opiate-like central activity. The test compounds were given intravenously or orally to adult rats, followed by exposure of the tail to water heated at 55°C. The reaction time to withdrawal of the tail was recorded electronically. Absence of tail withdrawal response within 10 sec (cut-off time) was considered to be a drug-induced effect. The reaction times were measured repeatedly at fixed time intervals up to 8 hr after compound administration. Both the castor oil test and the tail withdrawal reaction test were performed in adult rats and were selected because of their high reliability and clinical validity [Niemegeers et al., 1976a].

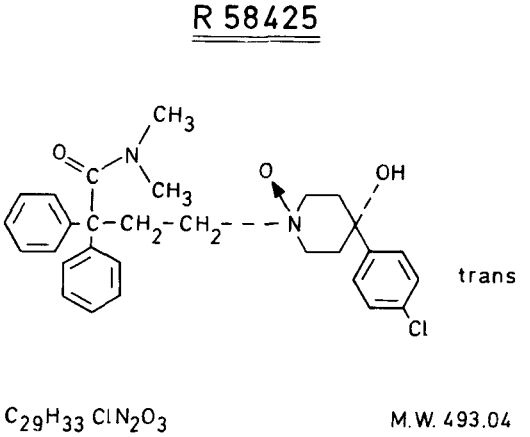

Fig. 1. Chemical structure of the N-oxide of loperamide. *Trans*-4-(4-chlorophenyl)-4-hydroxy-*N*,*N*,-dimethyl-α,α-diphenyl-1-piperidine-butanamide-*N*-oxide.

The acute toxicity test in adult rats was used to evaluate the relative safety margins of the compounds. Mortality was recorded up to 14 days after compound administration. Opiate-like behavioral effects such as muscle rigidity and blockade of pinna and cornea reflexes were also recorded at regular time intervals.

In 19-day-old rats (weaning age), behavioral effects as described above were studied in males and females in single dose toxicity studies (intravenously and orally).

In the studies with 1-day-old rats, groups of at least 50 1-day-old pups were sexed and given orally various doses of the test compounds. Five male and five female pups receiving the same treatment were assigned to a randomly chosen nursing female. The number of surviving pups was recorded daily up to 14 days later.

ED50s and LD50s with 95% confidence limits were calculated according to Finney's iterative method [Finney, 1962].

RESULTS

Adult Rats

The results obtained with orally administered loperamide and with the N-oxide of loperamide in the castor oil test are shown in Table 1. The antidiarrheal activity of both compounds started at the same dose level (lowest ED50s 0.15 and 0.16 mg/kg, respectively) and parallel time-effect curves [with statistically identical ($p > 0.05$) slopes of 0.130 and 0.127] were obtained (Fig. 2). At non-lethal doses, both loperamide and the N-oxide were devoid of opiate-like effects (tail withdrawal, pinna and cornea blockade, and induction of muscle rigidity). Both compounds were found to be more toxic in female than in male rats, but in both sexes the N-oxide was less toxic than loperamide (factor: 1.75).

The results obtained in the castor oil test after intravenous injections are shown in Table 2. The lowest ED50 of the N-oxide (1-hr interval) was about 3.5 times higher than that of loperamide, but 8 hr after injection the ED50s were virtually identical. Both compounds showed a non-linear time-effect curve, but the deviation from linearity was more marked with

TABLE 1. Adult Rats—Results Obtained with Orally Administered Loperamide and the N-Oxide of Loperamide*

Time (hr)	Castor oil ED50 and confidence limits in mg/kg		Tail withdrawal ED50 and confidence limits in mg/kg	
	Loperamide	N-oxide	Loperamide	N-oxide
1/4	—	—	>160	>160
1/2	—	—	>160	>160
1	0.15 (0.11–0.20)	0.16 (0.12–0.21)	>160	>160
2	0.29 (0.23–0.38)	0.34 (0.27–0.42)	>160	>160
3	0.43 (0.34–0.56)	0.42 (0.31–0.56)	>160	>160
4	0.61 (0.45–0.83)	0.59 (0.42–0.83)	>160	>160
6	1.07 (0.77–1.51)	1.02 (0.70–1.50)	>160	>160
8	1.81 (1.25–2.63)	1.91 (1.30–2.80)	>160	>160
Opiate-like effects				
Pinna blockade	—	—	>160	>160
Cornea blockade	—	—	>160	>160
Lead-pipe rigidity	—	—	>160	>160
LD50 mg/kg				
Males	—	—	185 (135–254)	320 (240–426)
Females	—	—	98.4 (72.6–133)	172 (122–241)

*Antidiarrheal doses (castor oil test), centrally acting doses (tail withdrawal reaction and observations), and LD50s.

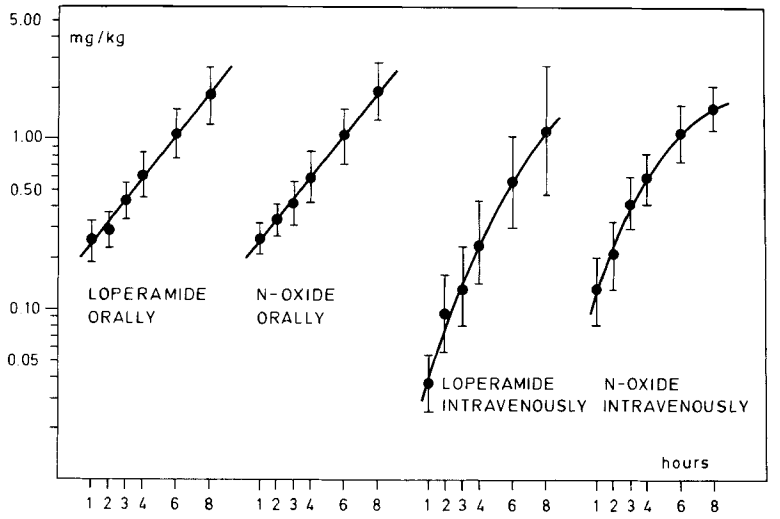

Fig. 2. Time-related protection from diarrhea (castor oil test) after oral and intravenous administration of loperamide and its N-oxide. ED50-values in mg/kg at different time intervals. The one-hour ED50 was corrected for 10.2% false positives (N = 2770 controls).

TABLE 2. Adult Rats—Results Obtained with Intravenously Injected Loperamide and the N-Oxide of Loperamide*

Time (hr)	Castor oil ED50 and confidence limits in mg/kg		Tail withdrawal ED50 and confidence limits in mg/kg	
	Loperamide	N-oxide	Loperamide	N-oxide
1/4			3.18 (2.60–3.89)	>20.0
1/2			2.83 (2.28–3.51)	>20.0
1	0.020 (0.010–0.038)	0.069 (0.044–0.11)	3.85 (2.99–4.95)	>20.0
2	0.095 (0.056–0.16)	0.21 (0.13–0.33)	4.50 (3.51–5.77)	>20.0
3	0.13 (0.080–0.23)	0.42 (0.30–0.58)	4.72 (3.61–6.17)	>20.0
4	0.24 (0.14–0.43)	0.59 (0.42–0.82)	5.02 (4.11–6.13)	>20.0
6	0.56 (0.30–1.05)	1.09 (0.74–1.60)	>8.00	>20.0
8	1.10 (0.46–2.64)	1.54 (1.16–2.05)	>8.00	>20.0
Opiate-like effects				
Pinna blockade	—	—	3.42 (2.55–4.60)	>20.0
Cornea blockade	—	—	2.67 (1.73–4.13)	>20.0
Lead-pipe rigidity	—	—	3.70 (3.25–4.21)	>20.0
LD50 mg/kg				
Males	—	—	5.92 (5.36–6.54)	28.3 (24.4–3.28)
Females	—	—	5.92 (5.36–6.54)	28.3 (24.4–32.8)

*Antidiarrheal doses (castor oil test), centrally acting doses (tail withdrawal reaction and observations), anc LD50s.

the N-oxide (Fig. 2). When injected intravenously loperamide induced typical opiate-like central effects (Table 2). The lowest ED50 in the tail withdrawal reaction test was 2.83 mg/kg; other opiate-like effects occurred in a dose range of 2.67 mg/kg (blockade of cornea) to 3.70 mg/kg (muscle rigidity). The N-oxide of loperamide, when given intravenously, was devoid of any opiate-like activity up to 20.0 mg/kg, the highest non-lethal dose. The acute intravenous LD50 of loperamide was found to be 5.92 mg/kg in both sexes; with the N-oxide the LD50 was 28.3 mg.

Nineteen-Day-Old-Rats

The results of the repeated observation and testing of young rats after oral administration of loperamide and the N-oxide are summarized in Table 3. Opiate-like central activities (blockade of the cornea and pinna reflexes and induction of rigidity) were found in 50% of the animals after oral loperamide within the dose range of 21.5 to 49.2 mg/kg and after the oral administration of the N-oxide within the dose range of 37.4 to 49.4 mg/kg. There were no significant differences between male and female rats. The oral LD50s were statistically identical in both sexes, but again the N-oxide was less toxic than loperamide (ratio: 1.32). The results of the comparative intravenous study are also summarized in Table 3. Loperamide was found active in a dose range of 0.71 to 1.20 mg/kg; the N-oxide was virtually inactive. No opiate-like central effects were observed at the dose of 10.0 mg/kg, whereas at 40.0 mg/kg all animals died within 5 min without showing any opiate-like central activity. At 20.0 mg/kg in some of the animals cornea and pinna reflexes were blocked and muscle rigidity was induced. These effects started between 2 and 3 hr after injection. In contrast, with loperamide the opiate-like effects were immediate. The LD50s were identical in both sexes with both compounds but the N-oxide was much less toxic (ratio: 5).

One-Day-Old-Rats

The results of the mortality studies in 1-day-old rats are summarized in Table 4. It is assumed that all deaths were due to the test compounds, although in solvent-treated pups (n=170) the mortality was 8.2%. The N-oxide was less toxic than loperamide (ratios of 1.24 in male and 1.43 in female pups), and there were no differences between male and female pups. The lethality in both the loperamide and the N-oxide treated pups occurred 1 to 2 days after administration.

DISCUSSION

In adult rats, both compounds when administered orally are equipotent and specific antidiarrheals (no opiate-like central effects). The safety margin, however, increases from 54.4

TABLE 3. Nineteen-Day-Old Rats—Central Behavioral Effects and Toxicity After Oral and Intravenous Administration of Loperamide and the N-Oxide of Loperamide

		ED50 and confidence limits in mg/kg			
		Oral administration		Intravenous administration	
		ED50 and confidence limits in mg/kg		ED50 and confidence limits in mg/kg	
Tests	Sex	Loperamide	N-oxide	Loperamide	N-oxide
Cornea block	M	21.5 (13.3–34.8)	37.4 (23.2–60.5)	0.71 (0.54–0.93)	≈20.0
Pinna block	M	37.4 (23.2–60.5)	37.4 (23.2–60.5)	0.85 (0.64–1.13)	≈20.0
Rigidity	M	43.0 (26.2–69.4)	49.4 (32.9–74.0)	1.11 (0.85–1.45)	≈20.0
LD50	M	98.6 (65.8–148)	130 (86.8–195)	5.61 (4.29–7.33)	28.3 (24.4–32.8)
Cornea block	F	21.5 (13.3–34.8)	37.4 (25.0–56.1)	1.12 (0.86–1.46)	>20.0
Pinna block	F	37.4 (25.0–56.1)	37.4 (25.0–56.1)	0.92 (0.65–1.29)	≈20.0
Rigidity	F	49.2 (36.4–66.9)	43.0 (31.7–58.2)	1.20 (0.92–1.57)	>20.0
LD50	F	113 (75.6–170)	149 (99.7–224)	5.78 (4.42–7.15)	28.3 (24.4–32.8)

TABLE 4. One-Day-Old Rats—Toxicity Study

	LD50 and confidence limits in mg/kg orally	
Compounds	Males	Females
Loperamide	6.17 (4.21–9.04)	7.08 (4.83–10.4)
N-oxide	7.63 (5.44–10.7)	10.1 (7.56–13.4)

(LD50: ED50 castor oil 8 hr) for loperamide to 90.1 for the N-oxide. Upon intravenous injection, the peripheral effects of loperamide are also observed (dose range: 0.02 to 1.10 mg/kg), but only slightly higher doses produce opiate-like central effects (2.83 mg/kg tail withdrawal test) and lethality (LD50: 5.92 mg/kg). The i.v.-injected N-oxide shows a more progressively developing peripheral effect (0.069 to 1.54 mg/kg) but remains devoid of central effects (> 20.0 mg/kg) and is 4.5 times less toxic than loperamide (LD50: 28.3 mg/kg).

In 19-day-old rats, both orally administered compounds induce opiate-like behavioral effects at doses that exceed 20.0 mg/kg for loperamide and 35.0 mg/kg for the N-oxide. A corresponding difference in safety (ration: 1.32) is also observed. Upon intravenous injection the opiate-like behavioral effects of loperamide are present around 1.00 mg/kg and for the N-oxide at doses around 20.0 mg/kg, which are close to lethal. The LD50 of the intravenously injected N-oxide is, as in adult rats, 5 times higher than that of the intravenously injected loperamide. With loperamide the opiate-like behavioral effects appear immediately after i.v. injection. In contrast, with the N-oxide these effects develop very slowly and can only be observed in some rats injected with 20.0 mg/kg if lethality does not occur within the first 3 hr. In one-day old pups, the LD50-values of oral loperamide approach the intravenous LD50-values in adult and 19-day-old rats. The LD50s of the oral N-oxide are only slightly higher (ratios: 1.24 and 1.43) than the loperamide LD50s.

The present data in rats are consistent with the concept that the N-oxide of loperamide is a prodrug. The antidiarrheal effect appears to require conversion of the N-oxide to loperamide. Efficient conversion is known to occur in the presence of intestinal contents or of liver microsomes [Lavrysen et al., 1984] and this is in agreement with the identical oral ED50-values in the castor oil test. The injected N-oxide also acts peripherally but with a pronounced delay with respect to injected loperamide. A less rapid conversion of the N-oxide has also been described in the presence of red blood cells, but not of plasma [Lavrysen et al., 1984]. The opiate-like behavioral effects of the injected N-oxide, if any, appear with a corresponding delay relative to injected loperamide. The lethality caused by the injected N-oxide, however, does not require metabolic conversion, since the rapid death of the injected animals is not accompanied by opiate-like behavior. In both adult and 19-day-old rats, circulating, unconverted N-oxide is thus 5 times less toxic than circulating loperamide. The difference between the N-oxide and loperamide in terms of binding to μ-opiate receptors is 2-fold: the affinity of the N-oxide is 80 times lower and the dissociation is much faster [Gommeren and Leysen, 1985].

From the present studies certain conclusions can also be drawn with respect to the different barriers that limit access of the antidiarrheals to various body compartments. Injected loperamide induces opiate-like behavioral effects in 19-day-old rats at doses about 3 times lower than in adult rats. The blood-brain barrier, therefore, does not appear to have reached at weaning age the degree of impermeability that is found in mature rats. The visceral barriers (intestinal wall, liver) on the other hand, appear to function equally well in both age groups, since oral administration of the N-oxide in 19-day-old rats leads to opiate-like behavioral effects. This points to rather efficient conversion of loperamide and confirms at the same time the relative immaturity of the blood-brain barrier. The most pronounced intestinal changes of structure in intestine, such as increased crypt depth, and of function, such as enzyme patterns, occur in rats in the third week of postnatal life [Henning and Kretchmer, 1973]. Liver function with respect to uptake, metabolism, and biliary excretion of drugs is markedly underdeveloped in newborn rats [Klinger, 1982] and may have reached at weaning age nearly the optimal level of young adults. None of the barriers appear to function adequately in the 1-day-old pups.

The N-oxide of loperamide may have distinct advantages over loperamide in the treatment of diarrhea. Orally administered N-oxide acts on the intestine after conversion to loperamide. Even though this conversion is rapid and efficient, the oral antidiarrheal activity of the N-oxide develops more slowly than that of loperamide (unpublished results in adult rats). The switch from an actively secreting and abnormally contracting intestinal wall to a more normal pattern will, therefore, always be more gradual with the N-oxide than with

loperamide itself, but the ultimate intensity of action may be similar. The virtual absence of visceral and central barriers in the 1-day-old pups is probably the main reason why loperamide is lethal at doses only slightly above the i.v. toxic doses in older rats. Lethality after the oral N-oxide is observed 1 to 2 days after administration, a time sufficient for complete conversion to loperamide. In conditions such as malnutrition [Brunser, 1977] and infectious gastro-enteritis [Schreiber et al., 1973] villous shortening and absorptive cell abnormalities may greatly enhance the passive transport of lipophilic drugs. There are probably many other factors, including accidental overdosage, which may produce high plasma levels. High plasma levels of the prodrug, however, are unlikely to induce acute intoxication.

In summary, the present studies suggest two potential clinical advantages of the N-oxide over loperamide. The first is related to the more gradual development of its intestinal activity and the second to accidental excessive uptake into the circulation, in which case the N-oxide is not likely to produce morphine-like actions and is expected to be much less toxic.

ACKNOWLEDGMENTS

This study was supported in part by a grant from the I.W.O.N.L., Brussels, Belgium.

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