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Delay of castor oil diarrhoea in rats: a new way to evaluate inhibitors of prostaglandin biosynthesis

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Forty-four non-steroidal anti-inflammatory compounds were tested for possible effects on castor oil-induced diarrhoea in rats. A small but significant delay of intestinal evacuations was found with all compounds. Quantitatively, the oral doses required to delay diarrhoea beyond the first hour after castor oil challenge reflected the acute anti-inflammatory potency of the tested compounds. Qualitatively, the evolution of the effective doses with increasing delay was linear for potent inhibitors of prostaglandin biosynthesis. The evolution for less potent compounds was markedly different and suggested the earlier occurrence of non-specific drug effects. Suprofen, the most potent of the series of compounds, produced the 1 h delay at an oral dose of 1·11 mg kg^{-1}; the ED50 increased linearly to 115 mg kg^{-1} for a 4 h delay. Compared with other compounds the activity pattern of suprofen was consistent with that of a very potent, short-acting inhibitor of prostaglandin biosynthesis, which maintains its specific action over a wide dose range. It is concluded that delay of castor oil-induced diarrhoea in rats allows a detailed characterization of aspirin-like compounds, and that inhibition of prostaglandin biosynthesis is insufficient to suppress the intestinal effects of the oil.

The castor oil test in the rat has been used extensively in our laboratories as a basic pharmacological test to screen and evaluate antidiarrhoeal drugs. This application has resulted in the development of the potent and increasingly specific antidiarrhoeal drugs diphenoxylate, difenoxin and loperamide (Niemegeers, Lenaerts & Awouters, 1976). One of the assets of the castor oil model is the very reproducible evacuation of watery stools within 1 h of oral administration of the oil; treatment with effective doses of antidiarrhoeal compounds changes the timing, and also reduces the weight of the intestinal evacuations (Awouters, Niemegeers & others, 1975). Ricinoleic acid, the active component of castor oil, produces effects, which are traditionally described as those of an intestinal stimulant. Recent investigations, in which contact with ricinoleic acid induced depression of intestinal contractile activity rather than stimulation, have challenged this view (Gaginella, Stewart & others, 1975; Stewart, Gaginella & Bass, 1975; Stewart & Bass, 1976). However, irrespective of changes in contractile activity, fluid accumulation in the lumen of the intestinal tract is a generally recognized effect of ricinoleic acid; this accumulation, together with the histological evidence of damage to mucosal cell layers would define ricinoleic acid as an irritant which induces inflammation of the intestine upon oral administration.

In the light of this inflammatory activity many parallels can be drawn between the intestinal effects of ricinoleic acid and those of prostaglandins (Wilson, 1974), which are capable of inducing diarrhoea by themselves. Furthermore, inflammatory reactions have now for some time been associated with stimulation of prostaglandin biosynthesis (Piper & Vane, 1971). This prompted us to investigate the effects of inhibitors of prostaglandin biosynthesis on castor oil-induced diarrhoea.

We have studied a large number of compounds of this class. Without exception they delayed the appearance of diarrhoea at oral doses known to produce an acute anti-inflammatory effect. The delay did not last unless high doses were used, but the evolution of the effective doses with increasing delay revealed interesting differences between the compounds.

MATERIALS AND METHODS

Most of the pure compounds were obtained through the courtesy of the companies that reported their original synthesis. They were all suspended in water containing 1% polysorbate 80 and the suspensions were treated with ultrasound shortly before oral administration. The castor oil test was performed as previously described (Niemegeers, Lenaerts & Janssen, 1974). Male Wistar rats, 220–250 g, were starved overnight and treated orally by gavage (1 ml/100 g) the next morning with a selected dose of the compounds under investigation (0·16, 0·31, 0·63 . . . 40·0, 80·0, 160, 320 mg kg^{-1}). One hour

* Correspondence.

after treatment 1 ml castor oil was administered orally, also by gavage. One, 2, 3 and 4 h after the castor oil challenge the transparent plastic dishes beneath the individual rat cages were inspected for the presence of characteristic diarrhoeal droppings; their absence was recorded as a positive result, indicating protection from diarrhoea at that time. The experimental data on at least 5 animals per dose were used for computation of ED50 values according to Finney (1962).

RESULTS

The detailed results obtained with the recently described prostaglandin biosynthesis inhibitor, suprofen (Janssen, 1975), are reported in Table 1. After administration of low doses a considerable number of animals was protected at the earliest inspection time, i.e. 1 h after castor oil administration. The delay of diarrhoea was, however, short-lived and markedly higher doses were required to prolong the protection time beyond the first hour. The dose protecting 50% of the animals at 1 h was 1·11 mg kg^{-1}; the same protection at the 4 h interval was obtained with a dose of 115 mg kg^{-1}.

Many non-steroidal anti-inflammatory compounds were tested this way and the ED50 values for protection 1 h after castor oil challenge ranged between 1·11 mg kg^{-1} (suprofen) and 131 mg kg^{-1} (paracetamol). They are listed in the order of decreasing potency in Table 2. This Table further lists the relative potency of the compounds with respect to indomethacin, which was found to be active at 2·41 mg kg^{-1}. For comparison with their acute anti-inflammatory activity, literature data on the carrageenan foot inflammation test have been used; the activity has been uniformly expressed as a potency ratio with respect to indomethacin. The relative potencies of 31 compounds in both tests

Table 1. *The number of protected rats out of ten to which suprofen was administered orally, at the inspection times of 1, 2, 3 and 4 h after castor oil challenge.*

Dose mg kg^{-1}	Inspection time 1 h	2 h	3 h	4 h
0·31	0			
0·63	2	0		
1·25	7	0		
2·50	8	2	0	
5·00	9	1	0	
10·0	10	2	1	1
20·0	10	8	2	1
40·0	10	8	6	5
80·0	10	9	4	1
160	10	10	10	5
320	10	10	9	9
ED50, mg kg^{-1} (limits)	1·11 (0·74–1·67)	14·1 (7·64–26·1)	47·2 (29·2–76·4)	115 (65·6–202)

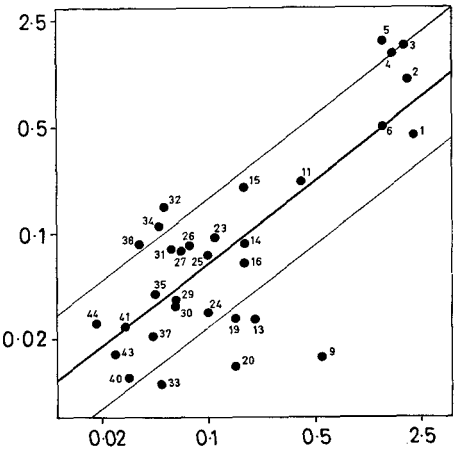

FIG. 1. Correlation between the relative potencies of non-steroidal anti-inflammatory drugs in the castor oil and the carrageenan inflammation test. Ordinate: Potency ratio *vs* indomethacin carrageenan oedema test. Abscissa: Potency ratio *vs* indomethacin castor oil test. Numbers relate to drugs in Table 2.

(Fig. 1) correlated significantly (r = 0·77). The regression line, log y = 0·42 + 0·78 log x, was not statistically different from the line of equal activity in both tests.

When tested up to 320 mg kg^{-1} several compounds afforded significant protection from diarrhoea for at least 4 h. Fig. 2 shows the evolution of the ED50 values with time after castor oil challenge for five potent compounds which were active at the 1 h interval at doses between 1 and 2·5 mg kg^{-1}. A regular linear increase in ED50 was observed with suprofen and indomethacin. Ketoprofen and indoprofen showed a very similar rapid evolution, whereas the evolution of ED50 values for sudoxicam levelled off after 3 h.

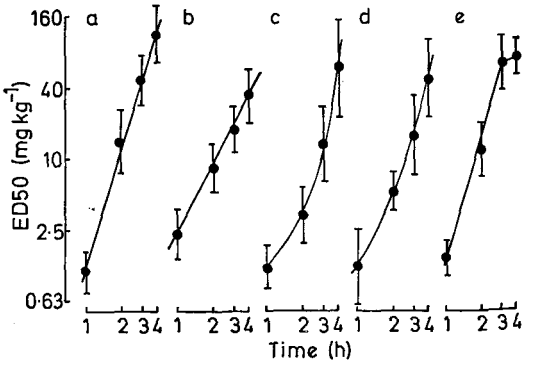

FIG. 2. Orally administered doses of a—suprofen, b—indomethacin, c—ketoprofen, d—indoprofen and e—sudoxicam, which produce delay of diarrhoea at 1, 2, 3 and 4 h after castor oil administration.

Table 2. *Activity of aspirin-like drugs in the castor oil test and in the carrageenan paw oedema test.*

Compound	Castor oil test		Carrageenan paw oedema test				
	ED50 (95% confidence limits) mg kg^{-1}; oral	PR *vs* indomethacin	Median PR *vs* indomethacin	Extreme PR values		Reference Nos	Abbreviated references
1 Suprofen	1·11 (0·74–1·67)	2·17	0·44	0·41	0·48	11, 19	1 Adams (1969). *Archs int. Pharmacodyn. Thér.*, **178**, 115.
2 Ketoprofen	1·19 (0·80–1·78)	2·03	1·0	0·27	1·43	14, 18, 19	2 Berger (1973). *Pharmacology.* **9**, 164.
3 Indoprofen	1·25 (0·59–2·65)	1·93	1·7			3	3 Buttinoni (1973). *Arzneim.-Forsch.*, **23**, 1100.
4 Sudoxicam	1·49 (1·04–2·13)	1·62	1·50	0·38	2·91	6, 18, 31	4 Carrano (1972). *J. pharm. Sci.*, **61**, 1450.
5 TAI–284	1·76 (1·12–2·77)	1·37	1·81			15	5 Colot (1973). *Thérapie*, **28**, 775.
6 Naproxen	1·77 (1·11–2·83)	1·36	0·50	0·25	1·10	6, 10, 18, 19	6 Dipasquale (1975). *Agents and Actions*, **5**, 256.
7 Indomethacin	2·41 (1·47–3·95)	1					7 Fleming (1969). *Archs int. Pharmacodyn. Thér.*, **178**, 423.
8 Cliprofen	2·50 (1·23–5·08)	0·96					8 Flower (1972). *Nature*, **238**, 104. Millonig (1973). *J. med. Chem.*, **16**, 780.
9 Bufexamac	4·40 (2·35–8·25)	0·55	0·015	0·015	0·016	5, 18	9 Glenn (1967). *J. Pharmac. exp. Ther.*, **155**, 157.
10 Protizinic acid	5·78 (2·97–11·2)	0·42					10 Ham (1972). In: *Prostaglandins in cellular biology* (Ramwell and Phariss), Plenum, p. 345. Winter (1964). In: *Proceedings of an international symposium on non-steroidal anti-inflammatory drugs.* Excerpta Medica, p. 190.
11 Meclofenamic acid	6·00 (2·48–14·5)	0·40	0·22	0·10	0·47	8, 21	11 Hirayama (1976). Unpublished results.
12 Flutiazin	7·02 (4·93–9·99)	0·34					12 Horodniak (1975). *Res. Commun. Chem. Path. Pharmac.*, **11**, 533.
13 Clonixin	11·8 (8·61–16·3)	0·204	0·027			30	13 Jahn (1969). *Arzneim.-Forsch.*, **19**, 36.
14 Tolmetin	14·0 (10·1–19·5)	0·172	0·085	0·035	0·58	6, 19, 32	14 Julou (1971). *J. Pharmac. (Paris)*, **2**, 259.
15 Ibuprofen	14·2 (8·31–24·1)	0·170	0·20	0·083	0·40	1, 10, 11, 18, 21	15 Kawai (1971). *Jap. J. Pharmac.*, **21**, 621.
16 Alclofenac	14·2 (8·31–24·1)	0·170	0·063	0·056	0·07	5, 18, 21	16 Kimura (1973). *Archs int. Pharmacodyn. Thér.*, **202**, 119.
17 Clonixeril	14·2 (9·70–20·7)	0·170					17 Levy (1976). *J. Pharmac. exp. Ther.*, **198**, 473.
18 Namoxyrate	14·2 (8·31–24·1)	0·170					18 Lombardino (1975). *Arzneim.-Forsch.*, **25**, 1629.
19 Aspirin	16·2 (10·9–24·0)	0·149	0·027	0·0025	0·10	1, 2, 4–12, 18–20, 22–25, 27–30, 33	19 Lwoff (1976). Personal communication.
20 Fenoprofen	16·3 (9·02–29·5)	0·148	0·013			18	20 Marazzi-Uberti (1972). *Arzneim.-Forsch.*, **22**, 213.
21 Fluindarol	16·2 (9·20–28·9)	0·148					21 Mörsdarf (1972). *Ibid.*, **22**, 2105.
22 Fenbufen	20·0 (11·4–35·2)	0·121					22 Niemegeers (1964). *J. Pharm. Pharmac.*, **16**, 810.
23 Flufenamic acid	22·3 (15·6–32·0)	0·108	0·095	0·054	0·22	4, 7, 10, 12, 13, 18, 21, 22, 29, 30	23 Nuss (1976). *Agents and Actions*, **6**, 735.
24 Benzydamine	23·6 (16·1–34·8)	0·102	0·030			20	24 Peterfalvi (1975). *Archs int. Pharmacodyn. Thér.*, **216**, 97.
25 Intrazole	24·3 (14·2–41·6)	0·099	0·072			7	25 Randall (1976). *Ibid.*, **220**, 94.
26 Phenylbutazone	32·5 (18·8–56·3)	0·074	0·082	0·016	0·23	1–10, 13, 15, 16, 18–23, 25–30, 32, 33	26 Riedel (1973). *Ibid*, **23**, 1215.
27 Prenazone	36·4 (20·5–64·7)	0·066	0·077	0·045	0·13	18, 20	27 Sciatti (1974). *Ibid.*, **24**, 2003.
28 Tesicam	37·1 (18·6–73·9)	0·065					28 Sofia (1974). *Eur. J. Pharmac.*, **26**, 51.
29 Ibufenac	40·0 (23·4–68·5)	0·060	0·036	0·017	0·072	1, 21	29 Vigdahl (1977). *Biochem. Pharmac.*, **26**, 307. Swingle (1971). *Archs int. Pharmacodyn. Thér.*, **189**, 129.
30 Indoxole	40·0 (15·1–106)	0·060	0·033	0·024	0·098	9, 10, 21	30 Watnick (1971). *Ibid.*, **190**, 78.
31 Fenclozic acid	42·4 (30·0–59·9)	0·057	0·078	0·050	0·12	10, 21	31 Wiseman (1972). *Biochem. Pharmac.*, **21**, 2323.
32 Metiazinic acid	47·3 (34·6–64·6)	0·051	0·15	0·096	0·23	10, 18	32 Wong (1973). *J. Pharmac. exp. Ther.*, **185**, 127.
33 Oxaprozine	48·8 (28·4–83·8)	0·049	0·010			33	33 Rosenthale (1974). *Agents and Actions*, **4**, 131.
34 Mefenamic acid	51·5 (25·9–103)	0·047	0·11	0·017	0·24	4, 8, 10, 13, 15, 20, 22	
35 Phenacetin	55·0 (32·0–94·7)	0·044	0·039			22	
36 Dipyrone	56·3 (39·9–79·4)	0·043					
37 Flazalone	56·3 (34·1–92·9)	0·043	0·021			17	
38 Azapropazone	68·8 (41·9–113)	0·035	0·083	0·045	0·11	10, 13, 18, 26	
39 Furobufen	80·0 (47·8–134)	0·030					
40 Chloroquine	80·0 (47·8–134)	0·030	0·011			4	
41 Cinchophen	85·0 (59·9–121)	0·028	0·024			22	
42 Tribenoside	95·0 (70·5–128)	0·025					
43 Sodium salicylate	100 (60·1–167)	0·024	0·016	0·010	0·023	21, 22	
44 Paracetamol	131 (80·2–213)	0·018	0·025			22	

For less potent compounds, as those shown in Fig. 3, the evolution of ED50 values with time always followed a particular pattern. The ED50 for protection at 2 h was markedly higher than at 1 h, but beyond the 2 h interval the increase of the effective doses of tolmetin, ibuprofen, acetylsalicylic acid, flufenamic acid and phenylbutazone slowed down.

DISCUSSION

Absence of diarrhoea 1 h after the administration of castor oil to starved rats is a significant delay, as only 4·6% of 1000 control rats failed to show diarrhoea at that time (Niemegeers & others, 1976). The 44 non-steroidal anti-inflammatory drugs used were all capable of delaying diarrhoea at doses, which are similar to those commonly found to produce an acute anti-inflammatory effect. Indomethacin was active in the castor oil test at 2·41 mg kg^{-1}. This dose corresponds to the ED50 of indomethacin in a relatively sensitive carrageenan test (the median ED50 in 27 of the consulted studies was 3 mg kg^{-1}, with extreme values of 1·5 and 18·4 mg kg^{-1}). The activity of the other compounds has been expressed relative to indomethacin, rather than in absolute ED50, to obviate the pronounced differences in sensitivity of the carrageenan test in different laboratories; when several results were available, the median potency ratio was used. The relative potencies of the compounds in the castor oil test and in the compiled carrageenan test correlate well (Fig. 1); in fact, when considering the occurrence of very divergent extreme potency ratios in the carrageenan test (Table 2) only a few compounds deviate significantly from equal potency in both tests. Bufexamac shows the largest deviation and is much more active in the intestinal than in the foot pad model; this hydroxamic acid may have gastrointestinal effects which differ from the remaining anti-inflammatory drugs. Acetylsalicylic acid is also more active in the castor oil test than is predicted by the large number of evaluations in the carrageenan test. It is possible that the peculiar property of this drug of acetylating proteins (Pinckard, Hawkins & Farr, 1968), including prostaglandin synthetase (Roth, Stanford & Majerus, 1975), plays a more decisive role in the rapidly developing intestinal inflammation.

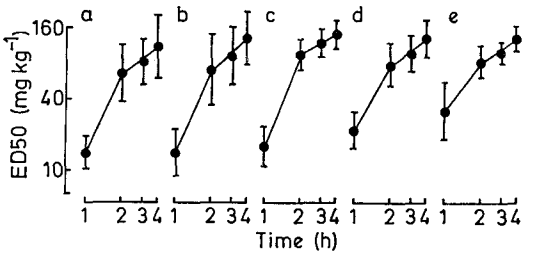

FIG. 3. Orally administered doses of a—tolmetin, b—ibuprofen, c—acetylsalicylic acid, d—flufenamic acid and e—phenylbutazone, which produce delay of diarrhoea 1, 2, 3 and 4 h after castor oil administration.

When an anti-inflammatory dose of the compounds is used the protection from diarrhoea does not last and markedly higher doses are required to delay diarrhoea for more than 1 h. The relation between effective doses and protection time reveals two clearly distinct patterns. Of some compounds, including the potent inhibitors of prostaglandin biosynthesis, regularly increasing doses are required to afford protection up to 4 h. As a rule the less potent aspirin-like drugs show a different pattern: a delay of more than 2 h is obtained with a smaller increase in dose than expected from the activity at 1 and 2 h. In fact, this ceiling of effective doses occurs for all compounds when more than about 60 mg kg^{-1} is administered.

It is unlikely that this response to doses in excess of a certain absolute level is an expression of gastrointestinal toxicity. The ulcerogenic doses of the tested compounds vary widely and in our own study (Niemegeers, Lenaerts & others, 1975) they were as low as 9·30 mg kg^{-1} for indomethacin, or close to the ED50 at the 2 h interval, and as high as 1190 mg kg^{-1} for acetylsalicylic acid, which is far higher than the dose range studied in the castor oil test. Furthermore, that the different patterns of the time-activity curves, linear or not linear, are related to the duration of activity of the compounds can be excluded. A relatively long acting compound like indomethacin has a linear evolution, as does suprofen, a short acting drug. The differences in slope, however, may allow a good estimate of the activity of these compounds in chronic compared to acute inflammation.

Studies on the mechanism of action of aspirin-like compounds may provide the answer to the difference in pattern between the compounds of Fig. 2 and those of Fig. 3. The activity these drugs develop in the lower dose range is inhibition of endoperoxide formation from arachidonic acid (Vane, 1971; Flower, 1974). On the other hand a variety of biochemical and cellular effects have been observed, usually with higher concentrations of these drugs (Hichens, 1974). The ceiling of effective doses in excess of a defined level may be the conse-

quence of the superposition of non-specific drug effects on their basic mode of action. It is suggested that the first linear portion of the curves for delay of diarrhoea offers a good estimate of the dose range of a compound, within which inhibition of prostaglandin biosynthesis is operating exclusively.

Non-steroidal anti-inflammatory drugs inhibit various prostaglandin-dependent functions of the gut. In relation to the propulsion of the intestinal contents, it has been reported that continuous prostaglandin biosynthesis, which is required to sustain peristaltic activity of the ileum and colon *in vitro*, is inhibited by relatively low concentrations of aspirin-like drugs (Bennett, Eley & Stockley, 1976; Fontaine, van Nueten & Reuse, 1977). *In vivo* the propulsive activity of the intestine is activated by castor oil. This may well be associated with increased prostaglandin biosynthesis, in the non-specific way many agents produce membrane perturbation (Piper & Vane, 1971). Alternatively, the accumulating ricinoleic acid may catalyse peroxidations of various polyunsaturated lipids (Schauenstein, 1967), which would, at least in part, be inhibited by aspirin-like drugs. Ultimately, however, ricinoleic acid may increase neuronal sensitivity and induce spasmogenic activity by itself, in the typical manner of hydroxy-fatty acids (Ambache, 1966).

Irrespective of the exact impact of aspirin-like drugs, the type of activity they display in the castor oil test is a characteristic delay of diarrhoeal evacuations; this delay can be increased with doses, which rapidly fall within the toxic range. This agrees with the fact that therapeutic use of aspirin-like drugs for diarrhoea is not established. Inhibition of prostaglandin biosynthesis is clearly an insufficient basis to counteract the intestinal effects of castor oil. The analysis of the transient effects of these drugs is, however, of great pharmacological and pathological interest.

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