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Effects of diflunisal on fever in the rabbit and the rat

M. J. DASCOMBE

Department of Pharmacology, Materia Medica and Therapeutics, Medical School, University of Manchester, Oxford Road, Manchester M13 9PT, UK

The antipyretic efficacy of diflunisal was assessed in rats made febrile by yeast and in rabbits made febrile by bacterial endotoxin. Diflunisal was a more potent antipyretic than aspirin in rats, reducing a maximum fever in doses not producing overt toxic effects. In contrast, submaximal fever in rabbits was not reduced by diflunisal. Fatal hyperthermia of rapid onset was observed in rats and rabbits receiving high doses of diflunisal after administration of microbial pyrogen but not in control animals. These data indicate the toxicity of diflunisal may be potentiated by the presence of pyrogens. It is concluded that the apparent antipyretic efficacy of a drug can depend on the species-pyrogen combination used to screen for antipyresis.

Diflunisal is a salicylic acid derivative (5-(2,4-difluorophenyl salicylic acid) introduced as 'a promising anti-inflammatory-analgesic agent with an enhanced potency, and with longer duration of action and better tolerance than aspirin' (Hannah et al 1977). Despite some pharmacological similarities, diflunisal differs from aspirin by not having a significant antipyretic effect in man (Thomas Morson Pharmaceuticals, personal communication). Antipyretic drugs, like aspirin, inhibit fatty acid cyclo-oxygenase and reduce the synthesis and release of prostanoids, which are thought to cause pyrexia by altering the activity of thermoregulatory neurons in the cns (Milton 1982). In contrast with observations in man, preliminary screening for antipyretic activity in yeast-treated rats indicated diflunisal was antipyretic (Stone et al 1977). The aim of the present study was to determine the effect of diflunisal on fever induced in rabbits by bacterial endotoxin to determine whether this animal model of fever resembles the clinical situation more closely than the yeast-treated rat.

MATERIALS AND METHODS

Female albino half lop rabbits (3–4·5 kg) and male Sprague-Dawley rats (200–400 g) from the Manchester Medical School Animal Unit were used. Colonic temperature was measured by a Yellow Springs Instrument model 401 thermistor held in position continuously in rabbits restrained in stocks. Colonic temperature in rats was measured at 30 min intervals while the animals were held by hand; rats were not restrained between observation times. Temperatures were displayed on either a Yellow Springs Instrument 47-TA Scanning Telethermometer or a digital thermometer (Brown & Dascombe 1982). Experiments were conducted between 10.00–17.00 h at an ambient temperature of 20–23 °C.

The O-somatic antigen of *Shigella dysenteriae* (Humphrey & Bangham 1959) was dissolved in 0·9% w/v sodium chloride solution for injection intravenously (i.v.) into rabbits. Rabbits were used at intervals of not less than 7 days to minimize the development of tolerance to the pyrogen. Brewer's bottom yeast (Sigma Chemical Company) was suspended in 0·5% w/v methylcellulose (Methocel A15, Dow Chemical Company) in water and heated at 37 °C for 30 min before injection subcutaneously (s.c.) in rats which were used once only. Doses of yeast are expressed as dry weight of yeast before activation. Diflunisal (Merck Sharp & Dohme) and aspirin (Sigma Chemical Company) were suspended in 0·5% w/v methylcellulose in 0·9% saline for injection intraperitoneally (i.p.). Control animals received vehicle acidified by the addition of hydrochloric acid to pH 2·9, intermediate between the pHs of the drug solutions employed. All glassware, saline and water for injection (Travenol) were sterile and pyrogen-free. Disposible sterile, pyrogen-free syringes and hypodermic needles were used to administer pyrogens and drug solutions.

Temperature responses were assessed as changes from pre-injection values (Δ °C) and as a temperature response index (TRI) integrating Δ °C against time (h), a single TRI unit (TRI 1 °C × h) being equivalent to a 1 °C rise in temperature lasting 1 h. The value of x in TRI_x is the time for which the response has been assessed. Results are expressed as the mean ± s.e. mean for n experiments. Differences between the means of experimental data have been evaluated by a 2-tailed Student's *t*-test for related or unrelated data as appropriate.

RESULTS

Shigella dysenteriae lipopolysaccharide (LPS) injected i.v. in rabbits produced a dose-dependent rise in body temperature (Table 1) associated with ear skin vasoconstriction and sedation. The submaximal pyrexia induced by LPS 5 μg kg^{-1} i.v. was attenuated by aspirin (200 mg kg^{-1}, $P < 0.05$) injected i.p. 1 h after administration of pyrogen (Fig. 1a). Aspirin (25–200 mg kg^{-1} i.p.) had no significant effect on temperature in afebrile rabbits although the mean values for temperature in aspirin-treated animals were less than control values (Fig. 1a).

Diflunisal (8·75–69·4 mg kg^{-1}, doses equimolar with aspirin 6·25–50 mg kg^{-1}) was not antipyretic in febrile rabbits (Fig. 1b). The low doses of diflunisal tested (8·75 and 34·7 mg kg^{-1} i.p.) produced small but insignificant reductions in fever ($P > 0.05$) comparable with those produced by low doses of aspirin (25 and 100 mg kg^{-1} i.p.). Unlike aspirin, high doses of diflunisal (69·4 mg kg^{-1} i.p.) had no significant antipyretic effect (Fig. 1b). One LPS-treated rabbit developed a fatal hyperthermia after receiving diflunisal (69·4 mg kg^{-1}). Associated with cutaneous vasodilatation, tachypnoea, salivation and restlessness, body temperature began to rise about 30 min after injection of the drug and reached a maximum of 43·4 °C (+4·2 °C) concommitant with convulsions and death 2·5 h after drug injection. A single LPS-treated rabbit died 35 min after receiving diflunisal 138·8 mg kg^{-1} i.p. Body temperature began to rise in this animal after 20 min and was greater than 42·2 °C (>+2·7 °C, the maximum temperature displayed on a Yellow Springs Instrument), at death 35 min after injection of diflunisal. Fatal hyperthermia did not occur in one afebrile rabbit receiving diflunisal 138·8 mg kg^{-1} i.p. or in afebrile rabbits receiving lower doses of diflunisal although one animal receiving diflunisal 69·4 mg kg^{-1} developed a sustained rise in temperature, maximum increase +1·6 °C 4 h after drug administration (Fig. 1b).

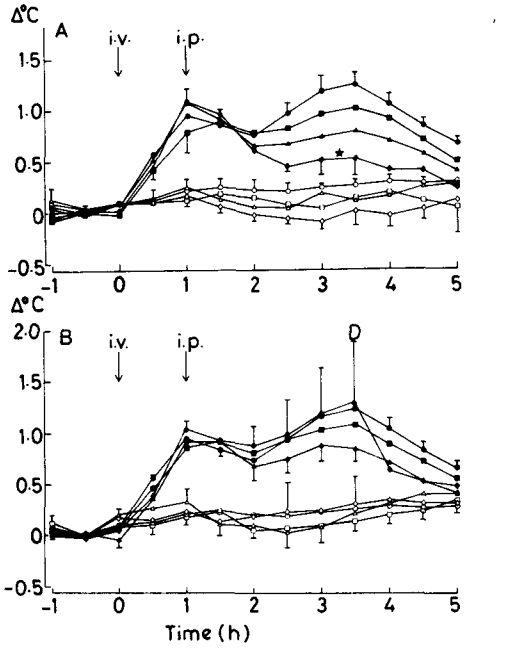

FIG. 1. Effect of aspirin (A) and diflunisal (B) on lipopolysaccharide (LPS)-induced fever in rabbits at an ambient temperature of 20–23 °C. At the first arrow (i.v.) LPS 5 μg kg^{-1} (solid symbols) or 0·9% sodium chloride 0·2 ml kg^{-1} (open symbols) was injected i.v. At the second arrow (i.p.) A: aspirin 25 mg kg^{-1} (□ ■), 100 mg kg^{-1} (△ ▲), 200 mg kg^{-1} (◇ ◆) or vehicle 1 ml kg^{-1} (○ ●), B: diflunisal 8·75 mg kg^{-1} (□ ■), 34·7 mg kg^{-1} (△ ▲), 69·4 mg kg^{-1} (◇ ◆) or vehicle 1 ml kg^{-1} (○ ●) was injected i.p. Values are the mean changes in colonic temperature from pre-injection values (Δ °C), with or without standard errors of mean, for 6 experiments except for ○, ● and ▲ where n = 10. At the time indicated by D one rabbit died in the group receiving LPS + diflunisal 69·4 mg kg^{-1}, thereafter n = 5. ★ indicates significant difference ($P < 0.05$) from control fever (●).

Table 1. Effect of *Shigella dysenteriae* lipopolysaccharide (LPS) on deep body temperature after i.v. injection in rabbits at an ambient temperature of 20–23 °C. Values are means ± s.e.m. for n observations.

Dose of LPS (μg kg^{-1})	n	Latency of febrile response (min)	Maximum rise in temperature (°C)	TRI$_5$ (°C × h)
0 (saline)	6	—	0·16 ± 0·10	0·31 ± 0·21
1	6	23·2 ± 4·4	1·18 ± 0·60*	3·57 ± 0·28*
5	6	19·0 ± 0·7	1·32 ± 0·20*	4·11 ± 0·50*
10	5	10·6 ± 1·7	2·31 ± 0·13*	7·50 ± 0·51*
20	6	11·8 ± 1·7	2·16 ± 0·26*	7·48 ± 0·86*

* $P < 0.001$ compared with response to saline, 2-tailed Student's *t*-test.

Yeast injected s.c. in rats produced pyrexia after a latency of 3–3·5 h (Fig. 2). The maximal febrile response to yeast 750 mg kg^{-1} (Fig. 2) was reduced by aspirin 100 mg kg^{-1} i.p. ($P < 0.001$) but not by aspirin 25 mg kg^{-1} (Fig. 3a). Diflunisal (8·75 and 34·7 mg kg^{-1} i.p.) was antipyretic in yeast-treated rats (Fig. 3b). Fatal hyperthermia was not seen in these animals. A single yeast-treated rat injected i.p. with diflunisal 138·8 mg kg^{-1} developed a rise of 2·3 °C in body temperature after 15 min. Convulsions and death occurred about 30 min after injection. Hyperthermia and death did not occur in one control rat (0·5% methylcellulose 10 ml kg^{-1} s.c.) receiving diflunisal 138·8 mg kg^{-1}.

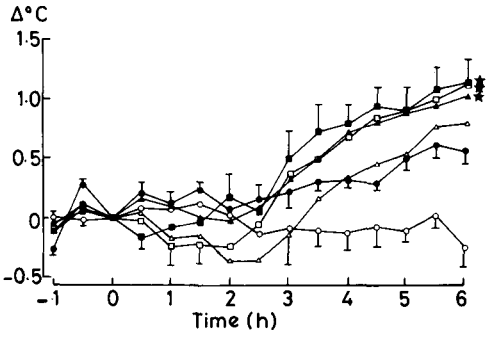

FIG. 2. Effect of brewer's bottom yeast on colonic temperature in rats at an ambient temperature of 20–23 °C. A subcutaneous dose of 30 (●), 125 (△), 250 (▲), 500 (□) or 750 mg kg^{-1} (■) was given to 8 rats at 0 h. Vehicle, 0·5% methylcellulose 10 ml kg^{-1} (○) was given to 8 rats. Values shown are mean changes in temperature from pre-injection values (Δ °C) with or without standard errors of mean. ★ indicates significant difference ($P < 0·05$) from the TRI_6 for vehicle.

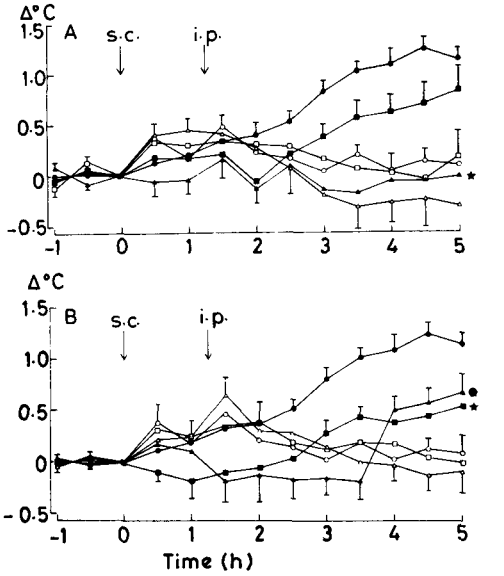

FIG. 3. Effect of aspirin (A) and diflunisal (B) on yeast-induced fever in rats at an ambient temperature of 20–23 °C. At the first arrow (s.c.) brewer's bottom yeast 750 mg kg^{-1} (solid symbols) or 0·5% methylcellulose 10 ml kg^{-1} (open symbols) was injected s.c. At the second arrow (i.p.) A: aspirin 25 mg kg^{-1} (■ □), aspirin 100 mg kg^{-1} (△ ▲) or vehicle 10 ml kg^{-1} (○ ●), B: diflunisal 8·75 mg kg^{-1} (□ ■), diflunisal 34·7 mg kg^{-1} (△ ▲) or vehicle 10 ml kg^{-1} (○ ●) was injected i.p. Values are the mean changes in colonic temperature from pre-injection values (Δ °C) with or without standard errors of mean (n = 5). ★ indicates significant difference ($P < 0·005$) from control fever (●).

DISCUSSION

The results presented here confirm the observations of Stone et al (1977) that diflunisal is a more potent antipyretic than aspirin in yeast-treated rats. Unlike aspirin, however, diflunisal did not attenuate the febrile response to bacterial pyrogen in rabbits. A similar lack of antipyretic effect by diflunisal is reported for man by the marketers of the drug, Thomas Morson Pharmaceuticals (personal communication). The reasons for the difference in sensitivities of yeast-induced fever in the rat and LPS-induced fever in the rabbit to the antipyretic effect of diflunisal are not known. The absence of a significant antipyretic effect by diflunisal in rabbits cannot be attributed, however, to either the magnitude of the fever, which was submaximal and reduced by aspirin, or the dose of diflunisal tested in this study. Diflunisal 138·8 mg kg^{-1} i.p. (equimolar with 100 mg aspirin) was originally intended to be the highest dose used in these experiments but toxicity, manifest as hyperthermia and death in both rabbits and rats, militated a reduction in the maximum dose tested to 69·4 mg kg^{-1} i.p. This dose of diflunisal produced fatal hyperthermia of rapid onset in 1 of 12 rabbits but did not reduce the febrile response to LPS. Hyperthermia can be produced by high doses of salicylates and is attributed to uncoupling of oxidative phosphorylation (Bowman & Rand 1980). This effect appears from the data presented here to be produced more readily by diflunisal than by aspirin, and to be facilitated by the presence of a pyrogen, because fatal hyperthermia of rapid onset was observed only in rabbits or rats receiving diflunisal after being treated with a microbial pyrogen.

Although diflunisal is not marketed as an antipyretic drug, antipyretic activity is commonly associated with salicylates and there is a report that diflunisal reduces fever in children aged 3 months to 10 years, average age 2·14 years (Similä & Keinänen-Kiukaanniemi 1982). No clear dose-dependency was observed in children and increasing the dose did not increase the antipyretic effect. Similä and Keinänen-Kiukaanniemi concluded diflunisal can be used as an antipyretic in selected cases because in single dose usage, as in the treatment of fever, side effects would, they presumed, be very rare. The toxicity of diflunisal in rabbits and rats, however, appeared in this study to be potentiated by the presence of microbial pyrogens. In case a similar interaction could occur in man, perhaps after increased dosage with diflunisal in an attempt to compensate for the low antipyretic potency of the drug, it seems prudent at this time to emphasize the qualifications attached by Similä & Keinänen-Kiukaanniemi (1982) to their recommended use of diflunisal as an antipyretic.

Diflunisal 'is a new drug' and 'it must be kept in mind that perhaps rare and serious side-effects have not yet been revealed'.

In summary, this study has shown that diflunisal has no significant antipyretic activity in the rabbit in contrast with the rat. Reliable extrapolation, from animals to man, of data relating to antipyretic activity may depend, therefore, on the species-pyrogen combination used to screen for antipyresis. In this instance, data for the rabbit, not the rat as used by Stone et al (1977), appears to agree more closely with clinical experience to date with diflunisal.

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REFERENCES

Bowman, W. C., Rand, M. J. (1980) Textbook of Pharmacology, 2nd edn, Blackwell Scientific, Oxford, pp 16.1–16.42

Brown, T., Dascombe, M. J. (1982) Br. J. Pharmacol. 77: 581P

Hannah, J., Ruyle, W. V., Jones, H., Matzuk, A. R., Kelly, K. W., Witzel, B. E., Holtz, W. J., Houser, R. W., Shen, T. Y., Sarett, L. H. (1977) Br. J. Clin. Pharmacol. 4: 7S–13S

Humphrey, J. H., Bangham, D. R. (1959) Bull. Wld. Hlth. Org. 20: 1241–1244

Milton, A. S. (1982) Handb. Exp. Pharmacol. 60: 257–303

Similä, S., Keinänen-Kiukaanniemi, S. (1982) Clin. Trials J. 19: 31–37

Stone, C. A., Van Arman, C. G., Lotti, V. J., Minsker, D. H., Risley, E. A., Bagdon, W. J., Bokelman, D. L., Jensen, R. D., Mendlowski, B., Tate, C. L., Peck, H. M., Zwickey, R. E., McKinney, S. E. (1977) Br. J. Clin. Pharmacol. 4: 19S–29S