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AD-1590, a potent antagonist of lipopolysaccharide-induced fever in rabbits

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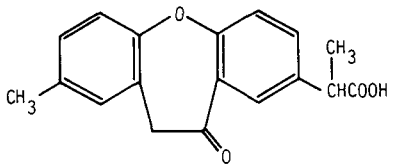
The antipyretic activity of AD-1590 (2-[8-methyl-10,11-oxodibenz[b,f]oxepin-2-yl]propionic acid), a non-steroidal anti-inflammatory drug with a novel chemical structure, was investigated in rabbits with lipopolysaccharide (LPS)-induced fever and monkeys with leucocytic pyrogen-induced fever. AD-1590 produced a dose-related inhibition of the LPS-fever at oral doses of 0·1 mg kg^{-1} or more (ED50 = 0·089 mg kg^{-1}). Its potency was 10–12, 20–35, 100–170, 400–540, >1500 and >2000 times that of ketoprofen, diclofenac sodium, indomethacin, ibuprofen, mefenamic acid and aspirin, respectively. The fever caused by leucocytic pyrogen was significantly inhibited by intravenous administration of 0·1–0·2 mg kg^{-1} of AD-1590. AD-1590 (10 mg kg^{-1} oral or i.v.) did not affect body temperature in afebrile rabbits or monkeys. These results suggest that AD-1590 shows a potent antipyretic activity in the rabbit and monkey and is a potent antagonist of LPS-fever.

AD-1590, a non-steroidal anti-inflammatory drug (NSAID) with a novel chemical structure (I), has been reported to have more potent antipyretic activity in comparison with, and anti-inflammatory and analgesic activity at least equivalent to, indomethacin (Nagai et al 1982; Nakamura et al 1983). AD-1590 produces antipyretic activity at oral doses as low as 0·02–0·1 mg kg^{-1} in rats with fever induced by yeast or adjuvant and its potency is about 10 times that of indomethacin. However, there is a species difference in the antipyretic activity of NSAIDs between the rat and the rabbit; the antipyretic activity of ibuprofen is 30–85 times and about 6 times that of aspirin against yeast-induced fever in rats and LPS (lipopolysaccharide)-induced fever in rabbits, respectively (Nakamura et al 1981). There appears to be a correlation between the antipyretic activities of NSAIDs in rabbits with LPS-induced fever and in man, but not so with yeast-induced fever in rats. Prostaglandins of the E-series seem to act as mediators of fever during infectious disease in man as well as in rabbits with the fever caused by exogenous pyrogen such as LPS (Philipp-Dormston & Siegert 1975). We wanted to know whether AD-1590 had antipyretic activity in species other than the rat, especially the rabbit. In this paper, the antipyretic activity of AD-1590 has been compared with other NSAIDs against LPS-induced fever in rabbits and its inhibitory activity against fever caused by human leucocytic pyrogen was also tested in monkeys.



I. AD-1590

MATERIALS AND METHODS

Antipyretic assay

Male albino rabbits, 2·3–3 kg, were used. Pyrexia was caused by an intravenous injection of 1 μg kg^{-1} of LPS (lipopolysaccharide B, *E. coli* 026 : B_6, Difco) dissolved in aseptic 0·9% NaCl (saline). One hour after LPS, drugs were administered orally to those rabbits showing an increase of 1 °C or more in rectal temperature (monitored by means of a thermistor probe Takara Thermistor K-700, Takara Ind., Japan, and recorded automatically at 5 min intervals for 2 h before and 5 h after LPS injection, Nakamura et al 1981). The dose (antipyretic ED50), at which the fever index is inhibited by 50% of the vehicle control, was calculated from the regression equation for each drug. The fever index refers to the area under the fever curve in 4 h after drug administration; one unit of the fever index is equivalent to a 1 °C change lasting for 1 h (Clark & Cumby 1975).

Leucocytic pyrogen preparation

Human leucocytic pyrogen (human LP) was prepared from human peripheral blood leucocytes

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(Clark & Moyer 1972; Clark & Cumby 1975). Heparinized blood (60–120 ml) was first incubated with shaking for 2 h at 37 °C with LPS (final concentration 100 ng ml^{-1}) dissolved in aseptic saline to activate the leucocytes. After the plasma had been centrifuged at 3000 rev min^{-1} for 10 min, the same volume of saline was added and incubation and shaking were continued for 2 h at 37 °C to release pyrogen. After centrifuging, the supernatant containing the pyrogen (crude human LP) was stored at 4 °C for later use. A blank of human LP (LP-blank) was prepared by the same procedure except that the LPS solution was replaced by saline.

Antipyretic assay against the pyrexia caused by human LP was in male cynomolgus (crab-eating macaque) monkeys (2·2–3·6 kg) adaptable to the monkey chair for restraint and with a stable body temperature. Three of 4 monkeys showed an increase of about 0·6 °C in rectal temperature after an intravenous injection of 1 ml kg^{-1} of human LP or rabbit LP (prepared from rabbit blood by the same procedure). Then, drugs were administered intravenously to the monkeys 5 min before human LP injection; each monkey was given 0·1 and 0·2 mg kg^{-1} of AD-1590 and saline in random order at an interval of 7 days. Rectal temperature was measured by the procedure described above for 2 h before and 3 h after human LP injection. Each animal was placed in a cage kept at 24 ± 1 °C throughout the test; drugs were given about 1200 h.

Drugs

Drugs used were: 2-[8-methyl-10,11-oxodibenz[b,f]-oxepin-2-yl]propionic acid (AD-1590, I), indomethacin, diclofenac sodium, ketoprofen, mefenamic acid, ibuprofen, aspirin and tolmetin sodium. Drugs were suspended in aqueous solution of 0·5% gum tragacanth for oral administration or dissolved in 0·1 M NaOH or 0·1 M phosphate buffer (pH 7·4) for intravenous administration.

Statistical analysis

Student's *t*-test was used for the statistical analyses.

RESULTS

LPS-induced fever

The time-course of LPS-induced fever in rabbits is shown in Fig. 1. LPS produced a dose-related rise in rectal temperature at intravenous doses of 0·1 to 10 μg kg^{-1} in rabbits; 1 μg kg^{-1} of LPS caused a biphasic rise. But in monkeys, LPS did not produce any clear rise in temperature at doses of up to 10 μg kg^{-1} (Fig. 1), while both human and rabbit LP (1 ml kg^{-1}) did produce a rise. Therefore, LPS (1 μg kg^{-1}) and LP (1 ml kg^{-1}) were used as pyrogen for the antipyretic test in rabbits and monkeys, respectively.

Antipyretic activity in LPS-induced febrile rabbits

Fig. 2 shows the time-course of antipyretic activity of AD-1590 after oral administration. When given 1 h

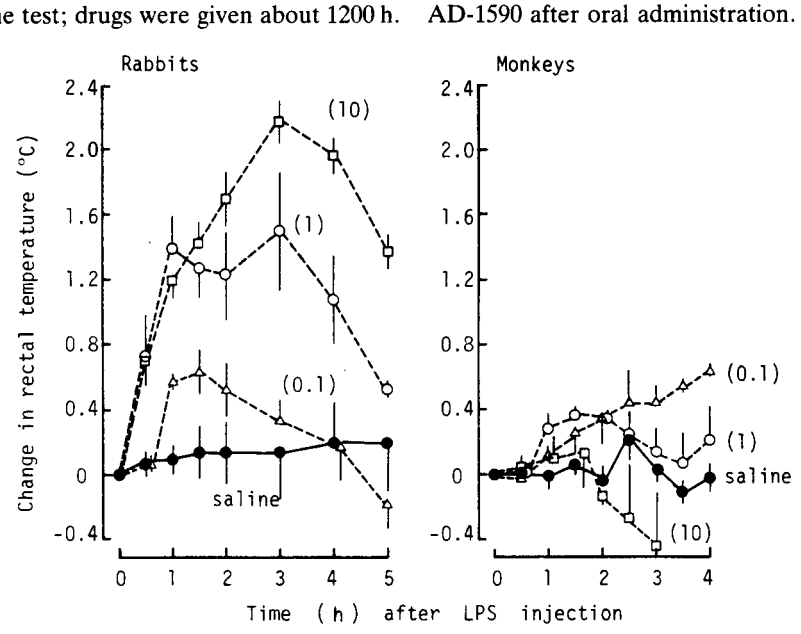

FIG. 1. Pyretic activity of LPS in rabbits and monkeys. Rectal temperature of rabbits and monkeys before LPS injection ranged from 38·0 to 39·5 °C and 37·4 to 38·3 °C, respectively. For each dose 3 rabbits and 2 monkeys were used. ( ) Dose in mg kg^{-1}, i.v.

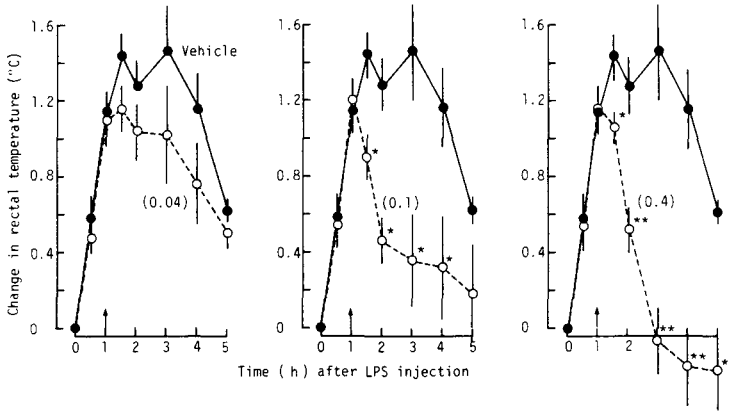

FIG. 2. Time-course of antipyretic activity of AD-1590 in LPS-induced fever in rabbits. AD-1590 was given 1 h (arrow marks) after i.v. injection of LPS (1 μg kg^{-1}). ( ) Dose of AD-1590 in mg kg^{-1} orally, * $0{\cdot}01 < P < 0{\cdot}05$, ** $P < 0{\cdot}01$ Significantly different from the vehicle control.

after LPS challenge, AD-1590 inhibited significantly the rise in rectal temperature at doses of 0·1 mg kg^{-1} or more; the temperature returned approximately to normal after 0·4 mg kg^{-1}.

AD-1590 showed a dose-related antipyretic activity, but there was not a dose-response relationship with indomethacin and diclofenac sodium, at least at high doses (Table 1). So the antipyretic activity of AD-1590 based on the fever index cannot be compared simply with that of other NSAIDs tested. The antipyretic ED50 of AD-1590 was 0·089 mg kg^{-1}, and its potency was about 20–35, 100–170, 400–540, 10–12 and >2000 times that of diclofenac sodium, indomethacin, ibuprofen, ketoprofen and aspirin, respectively, if the comparison is made on the basis of the ED50 and minimum effective dose (Table 1).

When AD-1590 was given intravenously to the febrile rabbits 1 h after LPS challenge, it produced a significant antipyretic activity at doses of 0·04 mg kg^{-1} or more and its activity was similar to that achieved by the oral route (Table 2). However, the antipyretic activity of indomethacin after intravenous administration was about 3 times more potent than that after oral administration. The pyrexia was significantly inhibited for 150 min beginning from 30 min after LPS challenge when 0·1 mg kg^{-1} of AD-1590 was given intravenously just before LPS challenge (Fig. 3). AD-1590 inhibited both the phases of the pyrexia caused by LPS.

Antipyretic activity in LP-induced febrile monkeys

Human LP produced a monophasic rise in rectal temperature after the intravenous injection of 1 ml kg^{-1}; the rise reached a maximum 50 min after

Table 1. Antipyretic activity of AD-1590 and other non-steroidal anti-inflammatory drugs in LPS-induced febrile rabbits.

Drug	Dose mg kg^{-1} orally	n	Fever index[a] Mean (°C h) ± s.e.	Inhibition (%)	ED50[b] mg kg^{-1} oral
Vehicle		5	4·90 ± 0·67		
AD-1590	0·04	5	3·67 ± 0·62	25·1	
	0·1	5	1·87 ± 0·87	61·9*	0·089
	0·4	5	0·84 ± 0·55	82·8**	
Vehicle		8	5·12 ± 0·36		
Indomethacin	4	4	4·04 ± 0·98	21·1	
	10	8	2·43 ± 0·90	52·5*	15·4
	20	4	2·51 ± 0·45	49·0**	
Vehicle		6	4·40 ± 0·39		
Diclofenac sodium	1	4	3·47 ± 0·62	21·1	
	2	4	1·88 ± 0·56	57·3**	3·15
	4	4	2·24 ± 0·33	49·1**	
	8	4	1·54 ± 0·18	65·0**	
Vehicle		4	6·00 ± 0·45		
Ketoprofen	0·4	4	5·17 ± 0·60	13·8	
	1	5	2·99 ± 0·62	50·2**	1·08
	2	3	1·95 ± 0·24	67·5**	
	4	3	−1·60 ± 0·83	100·0**	
Vehicle		3	5·84 ± 0·59		
Mefenamic acid	80	3	3·51 ± 0·93	39·9	
	160	3	4·34 ± 0·72	25·7	>160
Vehicle		5	4·60 ± 0·65		
Ibuprofen	10	4	3·79 ± 1·06	17·6	
	20	4	3·81 ± 0·56	17·2	48·3
	40	4	1·88 ± 0·28	51·7*	
Vehicle		5	4·60 ± 0·65		
Tolmetin sodium	20	4	3·79 ± 1·06	17·6	
	40	4	3·48 ± 0·62	24·3	70·9
	80	4	1·88 ± 0·28	59·1**	
Vehicle		6	5·01 ± 0·66		
Aspirin	80	3	3·88 ± 0·37	22·6	
	160	3	3·74 ± 0·59	25·3	237
	240	3	2·08 ± 1·20	58·5	

[a] The area under the fever curve during 4 h after drug administration; one unit of the fever index is equivalent to a 1 °C change lasting for 1 h.
[b] The median effective dose required to inhibit the fever index by 50% of the vehicle control.
* $0{\cdot}01 < P < 0{\cdot}05$, ** $P < 0{\cdot}01$ Significantly different from each vehicle control.

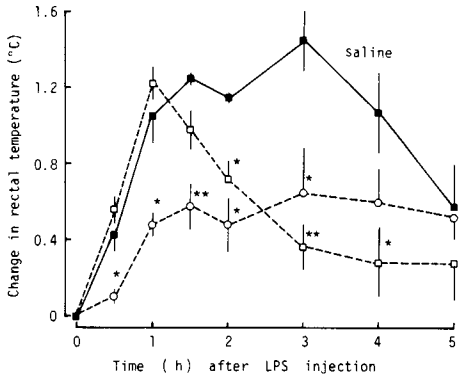

FIG. 3. Antipyretic activity of AD-1590 in LPS-induced fever in rabbits. AD-1590 (0·1 mg kg^{-1}) was given i.v. just (○) before or 1 h (□) after i.v. injection of LPS (1 μg kg^{-1}). Each point and vertical bar represent the mean and s.e.m. from 4 to 5 rabbits. * $0{\cdot}01 < P < 0{\cdot}05$, ** $P < 0{\cdot}01$ Significantly different from the saline group.

dosing and then subsided gradually (Fig. 4). LP-blank (1 ml kg^{-1} i.v.) did not produce a clear rise in rectal temperature. AD-1590 (0·1 and 0·2 mg kg^{-1}) showed a dose-related inhibition of the rise in rectal temperature, when given intravenously to monkeys just before human LP injection (Fig. 4).

Effect on body temperature in afebrile animals

AD-1590, 10 mg kg^{-1}, was administered orally to 4 male albino rabbits (2·3–3·0 kg) and intravenously to 3 male cynomolgus monkeys (2·4–3·7 kg), and rectal temperature was measured for 4 and 3 h, respectively, after dosing. No significant change in rectal temperature was observed after dosing compared with vehicle controls (data not shown).

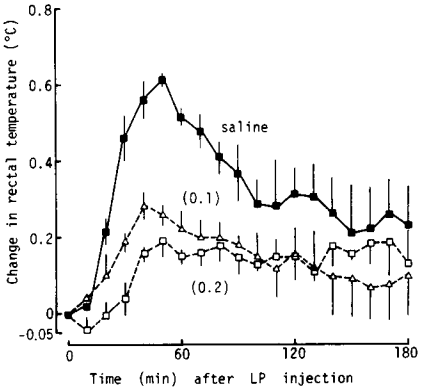

FIG. 4. Antipyretic activity of AD-1590 in human LP-induced fever in monkeys. AD-1590 was given i.v. 5 min before i.v. injection of human LP (1 ml kg^{-1}). Each point and vertical bar represent the mean and s.e.m. from 3 monkeys. ( ) Dose in mg kg^{-1}, i.v.

Table 2. Antipyretic activity of intravenously administered AD-1590 and indomethacin in LPS-induced febrile rabbits.

Drug	Dose mg kg^{-1} i.v.	n	Fever index: Mean (°C h) ± s.e.	Fever index: Inhibition (%)	ED50 mg kg^{-1} i.v.
Vehicle		4	4·54 ± 0·44		
AD-1950	0·04	5	2·70 ± 0·54	40·5*	
	0·1	5	2·12 ± 0·49	53·3**	0·080 Ca.
Vehicle		4	4·54 ± 0·44		
Indomethacin	1	4	4·35 ± 0·16	4·2	
	2	4	2·52 ± 0·37	44·5*	
	4	3	1·67 ± 0·40	63·2**	4·59
	10	3	2·14 ± 0·85	52·9*	

See explanations to Table 1. Drugs were given 1 h after LPS.

DISCUSSION

AD-1590 showed antipyretic activity in the LPS-febrile rabbits as well as in yeast-febrile rats at oral doses as low as 0·1 to 0·4 mg kg^{-1}. This result demonstrates that there was not a species difference in the antipyretic activity of AD-1590 between rats and rabbits. On the contrary, the antipyretic effective doses of other NSAIDs tested in the rabbit were higher than that in the rat (Nakamura et al 1982). Accordingly, the potency ratio of AD-1590 to indomethacin in the rabbit (more than 100) was at least 10 times larger than that (8·7–11) in the rat. The enteral absorption of AD-1590 after oral administration was so good that the antipyretic activity after oral administration was approximately comparable to that after intravenous administration, although the potency ratio of indomethacin after intravenous administration to oral administration was about 3 (Table 2). However, the antipyretic potency of AD-1590 was about 57 times that of indomethacin, even after intravenous administration.

The antipyretic effective doses of NSAIDs in the rabbit with fever caused by bacterial pyrogen have been reported as follows; 5–20 mg kg^{-1} oral for indomethacin (Kawai et al 1971; Fujimura et al 1974), 30–60 mg kg^{-1} i.v. for ibuprofen (Van Miert & Van Duin 1977), 140 mg kg^{-1} i.v. for sodium acetylsalicylate (Nishio & Kanoh 1981) and 5–10 mg kg^{-1} oral for ketoprofen (Fujimura et al 1974; Julou et al 1976). The present results agree generally with these results. Moreover, the antipyretic effective doses of flurbiprofen and clidanac, which are more potent than indomethacin in the rat (Kawai et al 1971; Adams et al 1975), have been reported to be 4–8 mg kg^{-1} i.v. (Van Miert & Van

Duin 1977) and 5–10 mg kg^{-1} oral (Kawai et al 1971), respectively. It has been reported that there is a correlation between the antipyretic activity of NSAIDs tested in LPS-febrile rabbits, and their clinical antipyretic activity but this does not exist with yeast-febrile rats (Nakamura et al 1981). From these results, AD-1590 appears a very potent antipyretic NSAID.

Monkeys, including cynomolgus monkeys are known to be weak responders to bacterial endotoxin with fever (Feldberg & Milton 1978). In the present experiment, an intravenous administration of up to 10 μg kg^{-1} of LPS to cynomolgus monkeys resulted in a failure to produce fever. But, it has been reported that monkeys respond to LP with fever and LP-induced fever is inhibited by sodium salicylate (100–250 mg kg^{-1} i.v.) (Chai et al 1971) and indomethacin (total 105–120 mg kg^{-1} i.v., monkeys of 5–6·5 kg) (Perlow et al 1975). So we tested the LP-febrile monkeys to see whether AD-1590 had antipyretic activity in the monkey as well as in the rat and rabbit. It was found that AD-1590 prevented the fever caused by human LP at doses as low as 0·1–0·2 mg kg^{-1} i.v.; i.e. AD-1590 showed approximately the same antipyretic potency as in the rabbit.

In general, it is considered that LPS causes the production and release of LP (Atkins & Bodel 1974), and this LP causes fever through the production and release of the E-series prostaglandins within the hypothalamus (Rosendorff & Mooney 1971; Stitt 1973; Philipp-Dormston & Siegert 1974; Dinarello & Bernheim 1981). In accord with the results of animal experiments, prostaglandins of the E-series seem to act as mediators of fever during infectious disease in man (Philipp-Dormston & Siegert 1975). NSAIDs like aspirin, which inhibit the synthesis of prostaglandins, reduce both fever and the increased PGE_2 activity (Flower & Vane 1972; Feldberg et al 1973; Dey et al 1974; Clark & Cumby 1975). AD-1590, as well as being an aspirin-like NSAID, inhibited both the LPS- and LP-induced fevers without affecting normal body temperature. It also has anti-inflammatory and analgesic activities at least equivalent to indomethacin, and its potency as an inhibitor of prostaglandin synthetase in-vitro is 2–3 times that of indomethacin (Nakamura et al 1983). We therefore suggest that the mechanisms of AD-1590's antipyretic activity are similar to those of other NSAIDs.

From these results, it is concluded that AD-1590 shows a potent antipyretic activity in the rabbit and monkey as well as in the rat and is a potent antagonist of LPS-fever.

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