Agents and Actions, vol. 19, 5/6 (1986)

0065-4299/86/060368-03 $ 1.50+0.20/0

How specific is the arachidonic acid-induced mouse ear oedema for lipoxygenase (LO)- and cyclooxygenase (CO)-inhibitors?

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Introduction

Arachidonic acid (AA) applied topically to the ears of mice produces an acute transient inflammatory reaction with immediate erythema and oedema formation (maximum at 1 h) and elevated tissue concentrations of prostaglandins (PGs) and leukotrienes (LTs) [1].
From inhibitor studies it has been concluded that this model may be especially suitable for the detection of LO-inhibitors *in vivo* [2]. We have tested different pharmacological classes of compounds to re-evaluate the specificity of this model. The same compounds were also tested in the croton oil-induced ear oedema model in mice and rats.

Materials and methods

AA-induced mouse ear oedema [2]

Male mice (Tif: MAGf/SPF, 24–28 g body weight, 8 animals per group) were anaesthetized and AA (Sigma, 4 mg/25 µl acetone) was administered to the inner surface of the right ear using a micropipette. Drugs were administered topically 30 or 180 min prior to AA in 25 µl of ether: pyridine: water (75:20:5). One hour after AA ear pieces of uniform size were punched out (diameter 7 mm) and weighed. Dose response curves and ED_{50}-values were estimated graphically. Statistics: Student's t-test.

Croton oil-induced ear oedema in mice and rats [3]

Male mice (Tif: MAGf/SPF, 24–28 g body weight, 8 animals per group) or male rats (Tif: RAIF/SPF, 110–130 g body weight, 5 animals per group) were anaesthetized and cutaneous inflammation was induced by applying a solution of 3% croton oil (Fluka) in ether: pyridine: water (72:20:5) to the inner surface of the right ear of mice (25 µl) or rats (50 µl). Test compounds were dissolved in the inflammation-inducing irritant. Six hours later uniform ear pieces (mouse: diameter 7 mm, rat: diameter 9 mm) were punched out and weighed and ED_{50}-values were estimated accordingly.
Compounds used were: Phenidone, hydroquinone, diphenyldisulfide and mepyramine from Fluka, nordihydroguaiaretic acid (NDGA) and salbutamol from Sigma, all other compounds were synthesized in Ciba-Geigy Labs.

Results and discussion

CO-inhibitors, LO-inhibitors, dual CO/LO-inhibitors as well as corticosteroids, β_2-stimulators and H_1-antagonists were tested in the AA-induced ear oedema model. ED_{50}-values are given in Table 1.
In the standard model (drug administration 30 min prior to AA) by far the most potent compounds were β_2-stimulators. The ED_{50} of formoterol was < 0.1 µg/ear. The LO-inhibitor Takeda AA 861 as well as several CO-inhibitors

Table 1
Effect of topically applied compounds on arachidonic acid- and croton oil-induced ear oedema.

Drug	ED_{50} (μg/ear)			
	Arachidonic acid oedema (1 h)		Croton oil oedema (6 h)	
	Mouse Drug: −30 min	Mouse Drug: −180 min	Mouse Drug: simult.	Rat Drug: simult.
Steroids				
Hydrocortisone	> 200	8	50	150
Dexamethasone	> 200	3	3	2
Clobetasol-17-propionate	> 200		3	1
CO-inhibitors				
Indomethacin	120	~200	> 1000	2000
Flurbiprofen	160		> 1000	4000
Naproxen	> 1000		> 1000	2800
CO/LO-inhibitors				
Phenidone	170	8–200*	> 1000	> 6000
NDGA	600		> 1000	2000
BW 755 C	> 1000		> 1000	> 4000
Benoxaprofen	> 1000		> 1000	1500
Lo-inhibitors				
Hydroquinone	> 1000		> 1000	> 4000
AA 861	200	40–200*	> 1000	2300
Diphenyldisulfide	> 1000		> 1000	> 4000
β_2-stimulators				
Formoterol	0.06	0.2	5	20
Salbutamol	1.0		80	200
H_1-Antagonists				
Ketotifen	130	800	> 1000	> 2000
Mepyramine	> 1000		> 1000	> 2000

* Plateau: 40–50% inhibition.

(indomethacin, flurbiprofen) and dual CO/LO-inhibitors (phenidone, NDGA) were moderately active ($ED_{50} > 100 \leqq 600$ μg/ear). Other LO-inhibitors (diphenyl/disulfide, hydroquinone), CO/LO-inhibitors (BW755 C, benoxaprofen) and naproxen (CO-inhibitor) were even less inhibitory ($ED_{50} > 1000$ μg/ear). The antihistamine ketotifen exhibited moderate activity ($ED_{50} \sim 130$ μg/ear), whereas mepyramine was nearly inactive ($ED_{50} > 1000$ μg/ear). Corticosteroids (hydrocortisone, dexamethasone, clobetasol- 17-propionate) were inactive under acute test conditions (at 200 μg/ear) but potent inhibitors when administered 3 hours prior to AA ($ED_{50} < 10$ μg/ear). Pretreatment (3 hours) enhanced the activity of phenidone and AA 861. The oedema was inhibited by 40 to 50% with concentrations of 8 and 40 μg/ear respectively, but even at high concentrations (1000 μg/ear) not more than 60% inhibition was obtained. Indomethacin had about the same activity as in the standard model, formoterol was about 4 times less active, but still the most potent compound.

Comparing the results in the AA-induced – and the croton oil-induced oedema models, we have found that some but not all tested CO-, LO- and dual CO/LO-inhibitors were moderately active in the AA-induced oedema (ED_{50} 120–600 μg/ear), whereas all of them were either inactive or only slightly active at high concentrations (1000 μg/ear) in the croton oil oedema models in mice and rats. In these standard models high croton oil concentrations (3%) were used to induce maximal inflammatory responses. Under these experimental conditions only corticosteroids and β_2-stimulators were potent inhibitors. However, recent results from Tubaro *et al.* [4] and ourselves (unpublished data) indicate that the croton oil mouse

ear oedema, when induced by low irritant concentrations (0.3%) can be effectively suppressed by CO-inhibitors (indomethacin, flurbiprofen), as well as by LO-inhibitors (AA 861) and dual CO/LO-inhibitors (NDGA) at concentrations ($ED_{50} \geqq 200 < 1000$ µg/ear), which are in the same range as those shown to be inhibitory in the AA-induced oedema.

In conclusion, several LO- and dual CO/LO-inhibitors, which are nearly inactive in the standard models of croton oil induced ear inflammation, exhibit moderate activity in the AA-induced mouse ear oedema when applied topically. These data confirm the recent results of Young *et al.* [2] and Carlson *et al.* [5]. However, potent CO-inhibitors (indomethacin, flurbiprofen) are at least comparably active in this model indicating that the AA-induced inflammation is promoted via products of the cyclooxygenase- and the lipoxygenase-pathway. Additionally, we found moderate activity of the H_1-antagonist ketotifen, Carlson *et al.* of chlorpheniramine, promethazine and atropine [5].

By far the most potent compounds in the AA-induced as well as in the croton oil-induced ear inflammation are β_2-stimulators and corticosteroids.

These results indicate that topically applied AA is not only the substrate for local PG- and LT-production, but is also a local irritant inducing complex proinflammatory reactions which can be effectively inhibited by mechanisms other than LO- and CO-enzyme inhibition.

References

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