Arch. Pharm. Res. Vol. 17, No. 3, pp. 166-169, 1994

Reduced Anti-inflammatory Activity of Acetylsalicylic Acid Maltol Ester, Aspalatone

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(Received December 27, 1993)

The anti-inflammatory activity of acetylsalicylic acid maltol ester (aspalatone), a potential antithrombotic agent, was studied using the several experimental animal models of inflammation. By oral administration, aspalatone was found to possess the weak anti-inflammatory activity in models of an acute inflammation, in which aspalatone showed approximately one-third to one-fourth of the anti-inflammatory activity of aspirin. Aspalatone (200 mg/kg/day) and aspirin (50 mg/kg/day), however, did not show the inhibitory activity against granuloma formation and adjuvant-induced arthritis.

Key words: Anti-inflammatory activity, aspalatone, aspirin, maltol, acute inflammation, chronic inflammation

INTRODUCTION

Acetylsalicylic acid (aspirin), a classical nonsteroidal anti-inflammatory drug (NSAID), has been reported to have antithrombotic activity and a long-term regular dosing of aspirin was found to be beneficial to prevent artherosclerosis and heart attack (Pedersen and Fitzgerald, 1984; Livio *et al.*, 1989). However, the serious side-effects such as peptic ulcerogenicity limit a long-term use of aspirin for this purpose. In an attempt to develope a safer and more effective antithrombotic aspirin-derivative, aspirin was conjugated with maltol, a potent antioxidant, forming stable metal-chelates (Han *et al.*, 1985). Aspalatone (acetylsalicylic acid maltol ester) was demonstrated to be a potential antithrombotic agent with low ulcerogenicity (Han *et al.*, 1993). On the other hand, Han *et al.* (1988) reported that aspalatone possessed reduced anti-inflammatory activity when determined with carrageenan (CGN)-induced paw edema method. This study was carried out to further investigate the anti-inflammatory activity of aspalatone using several inflammation models.

MATERIALS AND METHODS

Aspalatone was chemically synthesized as previously described (Han *et al.*, 1993). Aspirin, maltol, indomethacin, arachidonic acid (AA) and λ-carrageenan (CGN) were purchased from Sigma Chem. Co. (USA). Prednisolone was a product of Upjohn Co. *Mycobacterium butyricum* was obtained from Difco Co. Male ICR mice and Sprague-Dawley (SD) rats were maintained in our animal facility, feeding with mouse pellet lab. chow and water *ad libitum* under the conditions of 22± 1℃, 12 h/12 h (L/D) cycle.

Mouse Ear Edema Inhibition

According to the slightly modified procedure (Kim *et al.*, 1993) of an original ear edema test (Tonneli *et al.*, 1963), 2.5% AA (acetone) was topically applied to both ears of mice (20-22 g) and ear thicknesses increased were measured using a dial thickness gauge (Lux Scientific Instrument) 1 hr after AA treatment. Test compounds dissolved in 0.5% Tween 80 (0.2 ml) were orally administered 1 hr prior to AA treatment. The control group received same amount of vehicle.

CGN-Induced Paw Edema Inhibition

Following the procedure of Sedgwick and Willoughby (1989), 1% CGN (0.1 ml) was injected to left hind paws of rats (100-120 g) and edema increased was measured using water displacement technique 5 hr after CGN-treatment. Test compounds dissoved in 0.5% Tween 80 (1 ml) were orally administered 1 hr prior to CGN-injection. The control group received same amount of vehicle.

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CGN-Induced Pleurisy Inhibition

According to the procedure of Schrier *et al.* (1984), 1% CGN (0.2 ml) was injected to rats intrapleurally. After 5 hrs, rats were sacrificed. Pleurisy volumes were measured and total cells infiltrated were counted using the previously described procedure of Kim *et al.* (1993). Test compounds were orally administered 1 hr prior to CGN injection.

Cotton Pellet Granuloma Inhibition

Cotton pellet granuloma test was carried out according to the procedure of Kim *et al.* (1987). Cotton pellets (35±1 mg, Richmond Dental Co., USA) were implanted under the each axilla of rats (100-120 g). Rats were sacrificed 7 days after pellet implantation. The removed pellets were dried and weighed. Test compounds were orally administered each day for 7 days.

Adjuvant-Induced Arthritis (AIA) Inhibition

According to the procedure of Kubo *et al.* (1984), suspensions of Mycobacterium butyricum (1 mg/rat) were injected into the right hind paws of rats (100-120 g). The volumes of both paws were measured. Inhibition of the contralateral paw volume (secondary lesion) was regarded as anti-arthritic activity, and nodules appeared on tails were checked. Test compounds were orally administered once a day starting from 1 day after injection of the adjuvant.

Statistical Analysis

Student t-test was used throughout the experiments for evaluating the statistical analysis and considered as statistically significant when P values were less than 0.01.

RESULTS AND DISCUSSION

The anti-inflammatoty activity of aspalatone was studied using the several experimental animal models of inflammation. For an acute inflammatory model, arachidonic acid (AA)-induced mouse ear edema test, CGN-induced rat paw edema test and rat pleurisy test were employed. In AA-induced mouse ear edma test, aspalatone showed approximately one-third to one-fourth of the anti-inflammatory activity of aspirin when orally administered (Table I). The ED_{50} values for aspirin and aspalatone were calculated as 86 and 320 mg/kg, respectively. Maltol showed an inhibition of ear edema with similar potency to aspalatone. In CGN-induced paw edema test, the similar order of potency for aspirin and aspalatone was found, while maltol did not show the anti-inflammatory activity at doses of up to 400 mg/kg (Table II). By extrapolation, the ED_{50} values of aspirin and aspalatone were calculated as 430 mg/kg and 1,030 mg/kg, respectively. These results confirmed the early study by Han *et al.* (1988) which showed a reduced anti-inflammatory activity of aspalatone. In CGN-induced pleurisy test (Table III), aspalatone at 400 mg/kg, p.o., slightly, but not significantly reduced the number of cells infiltrated while aspirin significantly reduced the number of cells infiltrated at 100 mg/kg, p.o.

In order to investigate the activity of aspalatone in other types of inflammatory models, granuloma formation and adjuvant-induced arthritis (AIA) test were em-

Table I. Arachidonic acid (AA)-induced ear edema inhibition in mice

Group[a]	Dose[b] (mg/kg)	Thickness incresed (mm)	Inhibition (%)
Control	–	0.19± 0.02	–
Indomethacin	100	0.06± 0.02**[c]	67
Aspirin	20	0.14± 0.02**	29
	50	0.13± 0.03**	33
	100	0.10± 0.02**	50
Aspalatone	100	0.14± 0.03*	29
	200	0.11± 0.03**	43
	400	0.08± 0.02**	56
Maltol	100	0.15± 0.04	22
	200	0.11± 0.02**	41
	400	0.09± 0.02**	56

[a]All compounds were dispensed in 0.5% Tween 80, except maltol dissolved in 0.5% CMC., [b]Orally administered, [c]Significantly different from control group (n=10), Values are mean± S.E.
*: P<0.01, **: P<0.001.

Table II. CGN-induced paw edema inhibition in rats

Group	Dose (mg/kg)	Edema increased (ml)	Inhibition (%)
Control	–	0.54± 0.03	–
Indomethacin	100	0.30± 0.05**[a]	48
Aspirin	20	0.48± 0.05	11
	50	0.43± 0.04	22
	100	0.37± 0.13*	32
Aspalatone	100	0.48± 0.07	11
	200	0.42± 0.04*	23
	400	0.36± 0.12**	34
Maltol[b]	100	0.53± 0.05	6
	200	0.58± 0.14	–
	400	0.50± 0.05	11
	800	0.55± 0.07	3

[a]Significantly different from control (n=8), Values are mean± S.E.
*: P<0.01, **: P<0.001, [b]Dissolved in 0.5% CMC, the control was 0.57± 0.07 ml.

Table III. CGN-induced pleurisy inhibition in rats

Group	Dose (mg/kg)	Pleurisy Vol. (ml)	Total cells ($\times 10^7$)	Inhibition (%)
Control	–	6.2± 0.11	0.58± 0.18	–
CGN	–	7.6± 0.13	8.35± 0.41	–
Indomethacin	20	6.8± 0.12	2.80± 0.43**[a]	66
Aspirin	100	7.3± 0.12	3.90± 0.13**	53
Aspalatone	400	7.4± 0.11	6.90± 0.15	17
Maltol[b]	400	7.4± 0.11	5.15± 1.02*	38

[a] Significantly different from CGN-treated group (n=8), Values are mean± S.E.
*: P<0.01, **: P<0.001, [b] Dissolved in 0.5% CMC, The control CGN group was 7.6± 0.2 ml.

Table IV. Cotton pellet granuloma inhibition in rats

Group	Dose (mg/kg/day)	Granuloma wt. (mg)	Inhibition (%)
Control	–	36.8± 7.2	–
Indomethacin	10	21.4± 3.5**	42
Prednisolone	10	14.4± 5.2**	61
Aspirin	50	39.3± 7.7	–
Aspalatone	200	35.9± 6.6	–
Maltol[a]	200	39.2± 5.5	–

[a] Dissolved in 0.5% CMC., The control was 37.2± 6.9 mg, Significant different from control (n=8), Values are mean± S.E.
**: P<0.001.

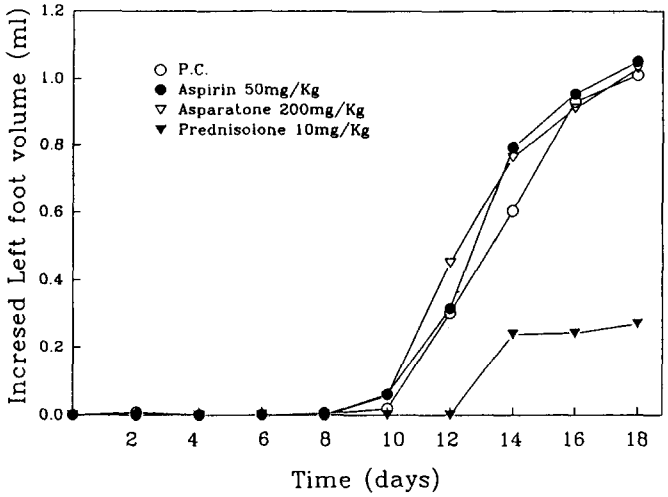

Fig. 1. Effects of aspalatone on adjuvant-induced arthritis in rats.
Compounds were orally administered once a day for 18 days. Control (○), aspirin (●, 50 mg/kg/day), aspalatone (▽, 200 mg/kg/day), prednisolone (▼, 10 mg/kg/day)

ployed. In cotton pellet granuloma inhibition test, aspirin and aspalatone did not show the activity at a dose of 50 mg/kg/day for aspirin or 200 mg/kg/day for aspalatone, by oral administration (Table IV). By local impregnation directly to pellets, both aspirin and aspalatone at the doses of up to 8 mg/pellet did not show the significant inhibition of granuloma formation (Data not shown). For studing the chronic anti-inflammatory activity, adjuvant-induced arthritis (AIA) test in rats was carried out. As expected, both aspirin (50 mg/kg/day) and aspalatone (200 mg/kg/day) did not inhibit the contralateral paw volume and the formation of nodules on a tail, while prednisolone (10 mg/kg/day) inhibited the paw volume (Fig. 2) as well as the formation of nodules (data not shown).

All of these results indicated that aspalatone showed the reduced anti-inflammatory activity compared to aspirin in acute inflammatory models tested, while aspirin and aspalatone did not show the anti-inflammatory activity in granulomatic inflammation and adjuvant-induced arthritis at the doses tested.

ACKNOWLEDGEMENTS

We thank Mr. Dong Young Choi for the technical assistant. This study was supported by a research grant from the Ministry of Health and Social Affairs (1993) and greatly acknowledged.

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