Natural Product Sciences
2(1) : 24 ~ 28 (1996)

Antiinflammatory and Analgesic Effects of Higenamine, a Component of Aconiti Tuber

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Abstract – The antiinflammatory and analgesic activities of higenamine were evaluated by measuring edema volume and pain threshold in adjuvant arthritic rats and acetic acid-induced writhing test in mice. Higenamine, with consecutive oral administrations at doses of 10 and 50 mg/kg/day, showed significant antiedemic effect and elevation of pain threshold during the secondary lesion of adjuvant arthritis. Higenamine also showed a significant inhibition of acetic acid-induced writhing syndrome with a single oral administration (200 mg/kg). From these results, it is postulated that higenamine might possess both of centrally and peripherally mediated analgesic properties.

Key words – Higenamine, Aconiti Tuber, antiinflammatory action, analgesic action, adjuvant arthritis, acetic acid-induced writhing.

Introduction

Aconiti Tuber (Aconite root) is the root of *Aconitum* spp. (Ranunculaceae) which has long been considered as one of the most important herbal drugs in oriental traditional medicine. It is used as cardiotonic, diuretic, and is used to improve blood circulation. It is also used as analgesic and anesthetic agents in the treatment of neuralgic and rheumatic affections (Bensky and Gamble, 1986, Tang and Eisenbrand, 1992). On the other hand, it has been considered very toxic so that only processed Aconiti Tubers have been used in traditional medicines. Aconiti Tuber contains various aconitine-type diterpene alkaloids, e.g. aconitine, mesaconitine, hypaconitine, etc., which are very toxic. Most of the diterpene alkaloids were found either decomposed or transformed to much less toxic substances and/or less water soluble lipo-alkaloids during various process (Kitagawa *et al.*, 1982, 1984; Park *et. al.*, 1990).

Higenamine is one of the constituents of Aconiti Tuber which was first isolated as a cardiotonic component from this plant. It is considerably unstable in basic medium, however fairly stable in acidic condition even at quite high temperature and is assumed to be rather stable during the usual processing of Aconiti Tuber (Kosuge and Yokota, 1976; Yamaguchi, 1958). The pharmacological studies of higenamine, up to date, have been mostly focused on the cardiovascular and hemodynamic circulatory systems. Higenamine exhibited cardiotonic effect, both chronotropic and inotropic *in vitro*, to increase the cardiac output and heart rate and to decrease the blood pressure and the systemic vascular resistance (Chang *et al.*, 1981; Kim *et al.*, 1986). Anti-platelet, an-

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ti-thrombotic, and anti-septic effects of higenamine were also reported recently (Yun-Choi and Kim, 1994).

The analgesic and antiinflammatory effects of higenamine are presented in this paper.

Experimental

Materials – Higenamine HBr was synthesized from 3,4-dimethoxyphenethylamine and 4-methoxyphenyl acetic acid as previously described (Chang *et al.*, 1984 ; Yun-Choi and Kim, 1994). *Mycobacterium butyricum*, desiccated (Difco code No.0640) was purchased from Difco Lab., Detroit, Michigan, U.S.A., and aspirin from Aldrich Chem. Co. Other reagents were the first grade commercially available.

Animals – Male Sprague-Dawley rats weighing 130-180 g and male ICR mice weighing 20-25 g were used. The animals were maintained at constant temperature and environments with 12 hr day/night cycle before experiments, given lab. chows (Samgang Lipid Co.) and tap water *ad lib.*

Induction of adjuvant arthritis – Antiedemic and analgesic activities were estimated according to the method of Walz *et al.*, (1971) in adjuvant arthritic rats. Adjuvant arthritic lesion was produced with a single intradermal injection of 0.1 mg of *Mycobacterium butyricum* suspended in 0.1 ml of white paraffin oil (Shinyo Pure Chem. Co. Ltd) into a foot pad of the left hind paw of rats under ether anesthesia. Edema volume and pain threshold of the injected foot were measured from the third day following adjuvant injection at three or four day intervals up to the 26th day, using plethysmometer (Ugo Basil) by the method of Winter *et al.*, (1963) and according to the method of Randall and Selitto (1957) using analgesy meter (Ugo Basil), respectively. Test compounds and acetylsalicylic acid as a reference drug suspended in 0.5% CMC-Na solution were administered (2 ml/kg/day, *p.o.*) once a day throughout the experimental period.

Acetic acid-induced writhing test – The effect of test compounds on writhing syndrome was estimated according to the method of Whittle (1964). Mice were administered orally with test compounds suspended in 0.5% CMC-Na one hr prior to the induction of writhing syndrome, which was induced by injection of 0.7% acetic acid in saline solution (10 ml/kg, *i.p.*). Ten min. after the administration of acetic acid, the number of writhing syndrome was counted for 10 min. A reduction in the writhing number as compared to control group was considered as evidence for the presence of analgesic effect which was expressed as percent inhibition of writhings.

Statistics – Data were statistically analyzed for significant differences between drug treated and control animals using student-*t* test. Pooled standard errors were calculated from the analysis of variance and $p<0.05$ was considered to be significant.

Results and Discussion

The effect of consecutive oral administrations of higenamine on the edema formation in adjuvant arthritis in rats was evaluated and the result was shown in Fig. 1. With a single intradermal injection of Freund's complete adjuvant (*Mycobacterium butyricum*) into a hindpaw foot pad, the edema volumes in the control rats were shown to increase in the distinct two phase i.e., the injected leg became inflammed and reached maximum during the first three or five days (primary lesion). Then after a delay of approximately nine or ten days, secondary lesions (from day 18 up to day 26) were observed indicating that there produced both the primary and the secondary lesions during the adjuvant arthritic responses. Higenamine, at a dose of 10 and 50 mg/kg/

day orally, did not significantly inhibit edema during the development of the primary lesion, but exhibited statistically significant inhibition of edema during the formation of the secondary lesion. Aspirin as a reference antiinflammatory drug, exhibited similar tendencies of inhibitory activity in arthritic edema formation. From the above results it can be postulated that the antiedemic efficacy of higenamine might appear in the secondary arthritic process of which immune responses are considered to be dominant.

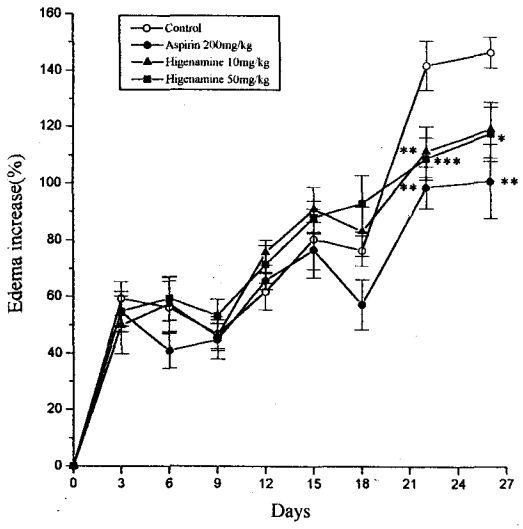

Fig. 1. Effect of higenamine on edema volume in adjuvant arthritis in rats.

Rats were administered orally with test compounds once a day throughout the experimental periods. Adjuvant arthritis was produced by a single *i.d.* injection of 0.1 mg of *Mycobacterium bulyricum* in 0.1 ml of white paraffin oil into a foot pad of left hind paw of rats under ether anesthesia. Edema volume was determined by plethysmometer at three or four day intervals from day 3 up to day 26. Significantly different from control as *p<0.05, **p<0.01 and ***p<0.001.

Adjuvant arthritis in rats is demonstrated to be a suitable model for measuring activities of antiinflammatory as well as analgesic agents due to its similarities to human arthritic symptoms, and thus not only edema volume but also pain threshold of the injected legs can be measured concommitantly (Walz *et al.*, 1971). Based on these experimental evidences and hypothesis, the effect of higenamine on the pain threshold of the inflammed paws in the course of the formation of adjuvant arthritic edema were measured throughout the experimental periods at three or four day intervals up to 26th day after Freund's complete adjuvant injection and the results were summarized in Table I.

With consecutive oral administrations of higenamine, the pain threshold was significantly elevated on the 9th day and then on the 18th day up to 26th day. Aspirin, as a reference drug, at a dose of 200 mg/kg/day exhibited similar tendencies of pain threshold elevation with higenamine on 12th day and then 18th day up to 26th day.

A significant analgesic effect was also observed in tests for acetic acid induced writhing syndrome in mice. As shown in Table II, higenamine was found to inhibit significantly the acetic acid-induced writhing response by 40% at 200 mg/kg, orally. The percent inhibition of writhing produced by

Table I. Effect of administration of higenamine on the primary lesion of adjuvant arthritis in rats.

Treatment	Dose (mg/kg/day, *p.o.*)	Pain threshold (g, mean±S.E.)							
		3	6	9	12	15	18	22	26 day
Control	0.5% CMC	135.7± 5.3	121.4± 6.7	107.1± 6.8	94.3± 6.5	117.1±12.5	117.1± 6.8	97.1±9.5	100.0± 8.2
Aspirin	200	151.4±14.6	118.3± 4.1	122.0±13.1	150.0±10.0**	132.0±16.0	166.0±10.8**	158.0±9.1**	142.0±11.7**
Higenamine	10	122.9±16.0	105.0± 5.5	146.7± 8.1**	108.3± 7.5	113.3±12.1	145.0± 9.6*	123.3±5.6*	118.3± 9.1
	50	145.7±15.1	140.0±17.3	147.1± 7.8**	125.7± 8.4*	121.4±14.6	132.9±16.0	137.1±5.7**	125.7± 7.8*

Rats were treated as described in Fig. 1 and pain threshold was determined at three or four day intervals by analgesy meter (Ugo Basil) from day 3 up to day 26. Data represent mean±S.E. of six or seven animals. Significantly different from control as *p<0.05 and **p<0.01.

Tabel II. Effect of higenamine on acetic acid induced writhing syndrome in mice.

Treatment	Dose (mg/kg, *p.o.*)	No. of mice	No. of writhing	Inhibition (%)
Control	0.5% CMC-Na	10	25±4	-
Aspirin	200	8	11±4*	56
Higenamine	200	8	15±2*	40

Mice were administered orally with test compounds suspended in 0.5% CMC-Na one hr prior to the injection of 0.7% acetic acid (10 ml/kg, *i.p.*). Number of writhing was counted for 10 min from 10 min after the induction of writhing syndrome. Significantly different from control as *$p<0.05$.

higenamine was a little weaker than those by aspirin at the same dose level.

The present results demonstrated that higenamine exhibited more potent analgesic effect during the secondary lesion of adjuvant arthritis rather than during the primary lesion. Walz *et al.* (1971) demonstrated that topical application (as 6% soln.) and oral doses of aspirin (200 mg/kg) in adjuvant arthritic rats produced significant antiedemic effect and only when administered orally it elevated pain threshold significantly during the primary lesion, which was considered to be due to the effect on the central nervous system (Goodman and Gilman, 1965). These results are in agreement with those of ours and pain threshold elevation produced by higenamine might be partly due to the central effect. Inhibition of acetic acid-induced writhing syndrome is generally considered to be peripherally mediated (Singh and Majumdar, 1995). From the fact that higenamine exhibited not only elevation of the pain threshold but also inhibition of writhing syndrome at relatively high dose, it may be concluded that higenamine possessed analgesic properties of mainly centrally mediated and weakly peripherally mediated. More precise mechanism of antiinflammatory and analgesic action of higenamine in relation to chemical mediators remains to be elucidated.

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(Accepted June 19, 1996)