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Amentoflavone, a Plant Biflavone : A New Potential Anti-inflammatory Agent

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Biflavonoid is one of unique classes of naturally-occurring bioflavonoids. Certain biflavonoids including amentoflavone were previously reported to have inhibitory effect on the group II phospholipase A_2 activity. Amentoflavone was also found to inhibit cyclooxygenase from guinea-pig epidermis without affecting lipoxygenase. In this study, anti-inflammatory and analgesic activities of amentoflavone were evaluated. When amentoflavone was administered intraperitoneally, it showed a potent anti-inflammatory activity as determined by amelioration of croton-oil induced mouse ear edema. It also showed a potent anti-inflammatory activity in the rat carrageenan paw edema model (ED_{50}=42 mg/kg) compared to the activity of prednisolone (35 mg/kg) and indomethacin (10 mg/kg). However, amentoflavone did not show a significant inhibitory activity against rat adjuvant-induced arthritis, a chronic inflammatory model. In addition, amentoflavone was found to possess a potent analgesic activity in the acetic acid writhing test (ED_{50}=9.6 mg/kg) compared to the activity of indomethacin (3.8 mg/kg). These results suggest that amentoflavone may be a potential lead for a new type of anti-inflammatory agents having dual inhibitory activity of group II phospholipase A_2 and cyclooxygenase.

Keywords : Amentoflavone, Flavonoid, Biflavonoid, Anti-inflammatory activity, Analgesic activity, Phospholipase A_2, Cyclooxygenase

INTRODUCTION

Flavonoids are widely distributed polyphenol compounds in plant kingdom and known to possess a variety of biological/pharmacological activities *in vitro* and *in vivo* (Havsteen, 1982). A search for anti-inflammatory/immunoregulatory flavonoids as potential therapeutic agents has been continued. There have been numerous reports describing anti-inflammatory flavonoids as active principles of medicinal plants using various experimental animal models (Gabor, 1986; Lewis, 1986). In order to establish structural-activity relationship of flavonoids, various flavonoid derivatives including flavones, flavonols, isoflavones and their glycosides were evaluated for their *in vivo* anti-inflammatory activity. The results indicated that C-ring 2,3-double bond was crucial and B-ring hydroxylation pattern was important (Kim *et al.*, 1993; Lee *et al.*, 1993). Recently, Panthong *et al.* (1994) and Gil *et al.* (1994) reported an anti-inflammatory activity of several hydroxylated and methoxylated flavones/flavonols *in vivo*. However, a few flavonoid derivatives possessed a potent anti-inflammatory activity enough for a therapeutic trial. In a continuous search for anti-inflammatory flavonoids, several biflavonoids such as amentoflavone, ochnaflavone, ginkgetin and morelloflavone were found to possess a potent inhibitory activity on group II phospholipase A_2 from platelets and synovial fluids originally by Chang *et al.* (1994), and followed by Gil *et al.* (1997). Interestingly, amentoflavone also inhibited cyclooxygenase from guinea-pig epidermis comparable to indomethacin without affecting lipoxygenase (Kim *et al.*, 1998). Since group II phospholipase A_2 and cyclooxygenase are known to be involved in most inflammatory conditions, amentoflavone was tested for an anti-inflammatory activity *in vivo* using several animal models in this investigation. From this study, amentoflavone is revealed to possess a potent anti-inflammatory activity by intraperitoneal injection on animal models of acute inflammation as well as a potent analgesic activity, but it showed a weak antiarthritic activity against chronic inflammation, adjuvant-induced arthritis in rats.

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MATERIALS AND METHODS

Chemicals

Amentoflavone (Fig. 1) was isolated and identified from *Selaginella tamariscina* and *Ginkgo biloba* leaves according to the procedures of Kang *et al.* (1990) and Shin and Kim (1991). Purity was checked by TLC using two different solvent systems. And it showed a single spot. Croton-oil, arachidonic acid (99%), λ-carrageenan (CGN) and mineral oil were purchased from Sigma Chem. Co. (St. Loius, MO). *Mycobacterium butyricum* was a product of Difco Lab. (Detroit, MI). All other reagents used in this study were highest grade chemicals available.

Animals

Male ICR mice and Sprague-Dawley (SD) Rats were obtained from Korea Experimental Animal Co. (Seoul, Korea). Animals were used after 7~10 days of acclimation in our animal facility (KNU). Animals were maintained with mice lab. chow (Purina Korea) and free access of water at conditions of 22±1°C and 12/12 hr (L/D) cycle.

Preparation of compounds

Drugs and amentoflavone were suspended in 0.5% CMC solution as fine particles and administered to animals via oral or intraperitoneal route. For topical application, compounds were dissolved in acetone in appropriate concentrations.

Anti-inflammatory activity

For evaluating an anti-inflammatory activity against acute inflammation, mouse ear edema and rat paw edema tests were used according to the previously published procedures of Kim *et al.* (1993) and Winter *et al.* (1962). Briefly, 2.5% croton-oil dissolved in acetone was topically applied to both ears (25 ul/ear) of mice (20±2 g). Drugs and amentoflavone were applied topically to ears of mice or intraperitoneally injected 30 min prior to croton-oil treatment, or administered orally 1 hr prior to croton-oil treatment. Ear thickness was measured 5 hrs after croton-oil treatment by dial thickness gauge (Fowler Precision Instrument, USA). Thickness increased after croton-oil treatment was regarded as an edema formed. In arachidonic acid (AA) induced ear edema test, 2% AA dissolved in acetone was applied topically to ears of mice. Indomethacin and amentoflavone were applied as same as in croton-oil treatment. Ear thickness was measured 1 hr after AA treatment. For rat paw edema test, 1% CGN dissolved in pyrogen free saline (0.05 ml) was injected to right hind paw of rats (100±10 g). After 5 hrs, swelling of the treated paw was measured using plethysmometer (Ugo Basile, Italy). Compounds were administered intraperitoneally 30 min prior to CGN injection. For testing an anti-inflammatory activity against chronic inflammation, rat adjuvant-induced arthritis (AIA) was used following the procedure of Kim *et al.* (1997). In brief, arthritis was provoked by injection of *Mycobacterium butyricum* (0.6 mg/rat) dissolved in mineral oil to right hind paw of rats. Compounds were intraperitoneally injected everyday. The swelling of non-treated left hind paw between 12 and 18 days after injection of adjuvant was regarded as an arthritic inflammation.

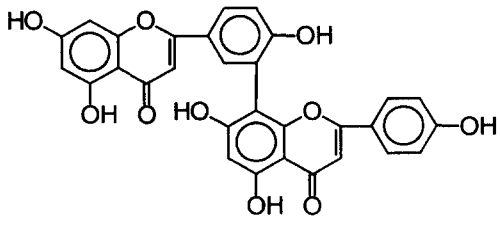

Fig. 1. Chemical structure of amentoflavone.

Analgesic activity

Acetic acid induced writhing test was carried out to measure an analgesic activity of amentoflavone according to the slightly modified procedure of Bentley *et al.* (1983). Indomethacin and amentoflavone were administered intraperitoneally to mice (20±2 g) 30 min prior to acetic acid injection. One hundred microliter of acetic acid (0.7%) was administered intraperitoneally and numbers of writhing were counted for 10 min starting 10 min after administration of acetic acid solution.

Statistics

All data were represented as arithmatic mean±S.E.M. ED_{50} values were caluculated by linear regression analysis. One-way ANOVA test was used for evaluating a statistical significance and P values less than 0.01 was considered as stastically significant.

RESULTS

Amentoflavone was evaluated for anti-inflammatory and analgesic activities using several animal models. For testing an anti-inflammatory activity against acute inflammation, mouse ear edema and rat paw edema tests were used. In croton-oil induced mouse ear edema, amentoflavone showed a potent inhibitory activity (Fig. 2a). By intraperitoneally, ED_{50} values of amentoflavone and prednisolone were revealed to be 74 mg/kg and 30 mg/kg, respectively. Topical and oral administration of amentoflavone gave much less activity than that of intraperitoneal injection compared

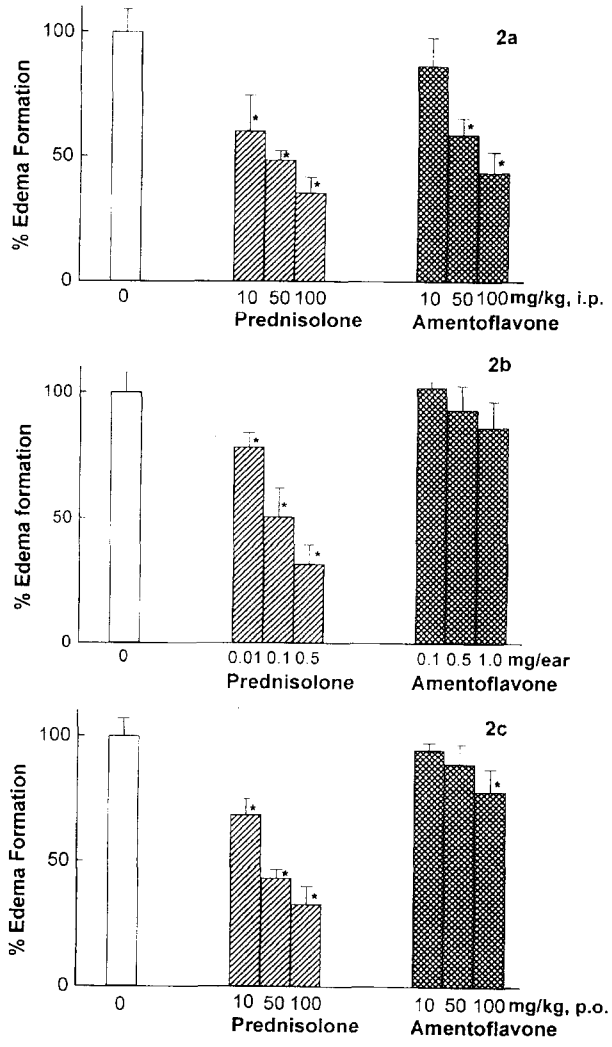

Fig. 2. Inhibition of croton-oil induced mouse ear edema. Each column and bar represented mean±S.E.M., *: P<0.01, significantly different from control

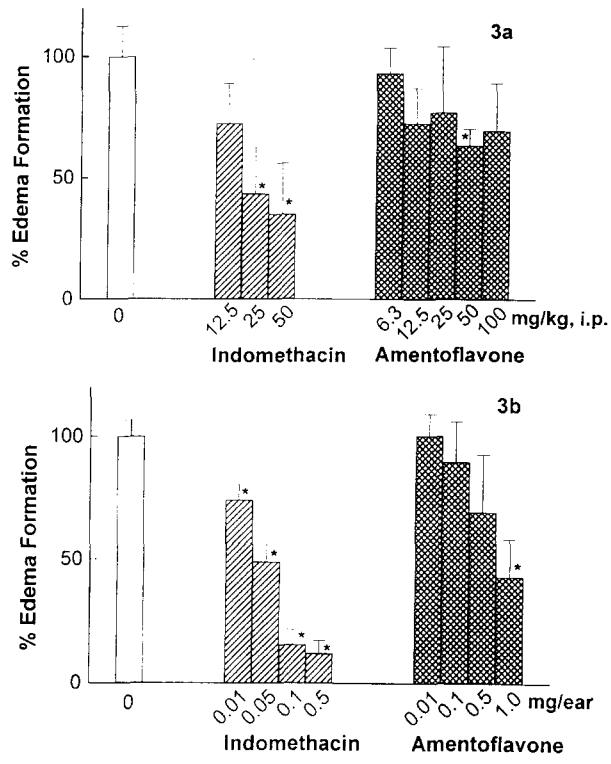

Fig. 3. Inhibition of arachidonic acid induced mouse ear edema. Each column and bar represented mean±S.E.M., *: P< 0.01, significantly different from control

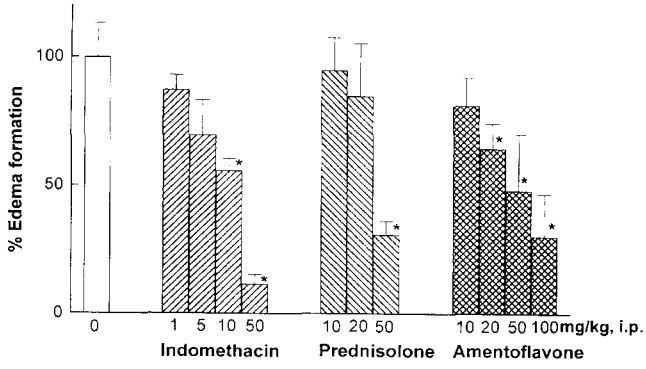

Fig. 4. Inhibition of λ-carrageenan paw edema in rats. Each column and bar represented mean±S.E.M., *: P<0.01, significantly different from control.

with the anti-inflammatory activity of prednisolone (Fig. 2b and 2c). When amentoflavone was tested in AA-induced ear edema, it also showed inhibitory activity, although it did not reach the half-maximal activity up to the doses of 100 mg/kg via intraperitoneal route of administration (Fig. 3a). Topical application showed 47 fold less activity than that of indomethacin (Fig. 3b). Amentoflavone, in addition, potently inhibited rat CGN paw edema, an another animal model of acute inflammation (Fig. 4). ED_{50} values of amentoflavone, prednisolone and indomethacin were 42 mg/kg, 35 mg/kg and 10 mg/kg, respectively, by intraperitoneal injection. In contrast, amentoflavone showed only a slight inhibitory activity aganist chronic inflammatory model, rat adjuvant induced arthritis at doses of 5-100 mg/kg/day (less than 20% inhibition at 16 days, data not shown). For evaluating an analgesic activity, acetic acid writhing test was used. Amentoflavone showed a potent analgesic activity comparable to indomethacin as shown in Fig. 5. ED_{50} values of amentoflavone and indomethacin were revealed to be 9.6 mg/kg and 3.8 mg/kg, respectively.

DISCUSSION

Biflavonoid is one of structurally unique classes of bioflavonids. Previously, several biological activities of biflavonoids were reported such as inhibitions of phosphodiesterase (Ruckstuhl *et al.*, 1979), lens aldose reductase (Iwu *et al.*, 1990; Felicio *et al.*, 1995), mast

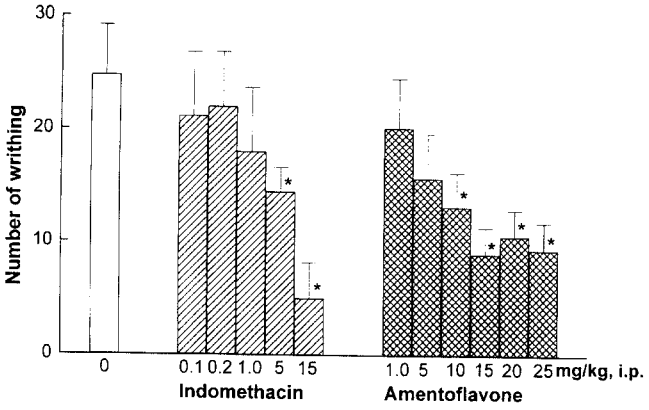

Fig. 5. Inhibition of acetic acid induced writhing in mice. Each column and bar represented mean±S.E.M., *: P<0.01, significantly different from control.

cell histamins release (Amella *et al.*, 1985) and lymphocyte proliferation (Lee *et al.*, 1995). However, a few *in vivo* studies was described. During a continual study for finding anti-inflammatory natural products expecting less side effects, we have found that amentoflavone, a biflavone, inhibited enzyme activity of phospholipase A_2 and cyclooxygenase (Chang *et al.*, 1994; Kim *et al.*, 1998). These findings prompted us to study *in vivo* anti-inflammatory and analgesic activities. Here we showed a potent anti-inflammatory activity against acute inflammation and an analgesic activity in contrast to the previous results of Della Loggia *et al.* (1996) describing a weak anti-inflammatory activity of amentoflavone by topical application in mouse croton-oil induced ear edema. It is thought that the discrepancy of the potency of anti-inflammatory activity between the results of Della Loggia *et al.* (1996) and ours may be due to the low bioavailability of amentoflavone. Amentoflavone showed a potent anti-inflammatory activity by intraperitoneal route against mouse croton-oil ear edma and rat CGN paw edema, while it gave the reduced activity by topical and oral routes. Additionally, it was also found that amentoflavone inhibited AA-induced mouse ear edema. True phospholipase A_2 inhibitor is assumed not to be active in this model. Morelloflavone, a plant biflavonoid of putative phospholipase A_2 inhibitor, did not show an inhibition on this model (Gil *et al.*, 1997). Therefore, our results strongly indicated that amentoflavone showed an activity in AA-induced ear edema, not by phospholipase A_2 inhibition, but by cyclooxygenase inhibition, at least in part, as previously described (Kim *et al.*, 1998). Amentoflavone also possessed a potent analgesic activity comparable to indomethacin. It is hoped that a further study to improve bioavailability may lead to new anti-inflammatory agent.

In conclusion, present investigation has shown the anti-inflammatory and analgetic activities of amentoflavone, of which the potency may be enough for a clinical trial. And all of our results suggest that amentoflavone, as a putative dual inhibitor of phospholipase A_2 and cyclooxygenase, may be a potential lead for a new type of anti-inflammatory agent from natural products in future.

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