Inhibitory Effect of Alkaloids Extracted from the Stem Bark of *Hunteria zeylanica* on 5-Lipoxygenase Activity in Vitro

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The effects of alkaloid extract from the stem bark of *Hunteria zeylanica* Gard. (H. zeylanica) on the activities of cyclooxygenase and 5-lipoxygenase in A23187-stimulated rat mast cells were investigated. *H. zeylanica* alkaloid extract (0.3—300 μg/ml) inhibited leukotriene C₄ (LTC₄) production by 5-lipoxygenase in a concentration-dependent manner and it blocked the production by 50% at 300 μg/ml. On the other hand, the extract had no effect on prostaglandin D₂ (PGD₂) production by cyclooxygenase. Neither (−)-eburnamine nor pleiometinine, major constituents of *H. zeylanica* alkaloid extract, inhibited the production of PGD₂ and LTC₄ in the A23187-stimulated mast cells. The inhibition of arachidonic acid metabolism via 5-lipoxygenase pathway may be due to minute amounts of other components as stated in the Discussion.

**Key words** *Hunteria zeylanica*; alkaloid extract; 5-lipoxygenase; inhibition; rat mast cell

_Hunteria zeylanica* Gard. (H. zeylanica) is a glabrous tree of the family Apocynaceae. The latex of this plant has been used as a folk medicine for treatment of the sores of yaws. It was also used as a substitute for Kemuning (*Murraya paniculata*, Rutaceae) to reduce boils and skin irritations. We previously found that the *H. zeylanica* alkaloid extract inhibited not only vascular permeability increased by acetic acid but also acute inflammation in experimental animal models, such as carrageenin-induced paw edema in rats and croton oil- and arachidonic acid-induced ear edema in mice. These findings suggest that the anti-inflammatory action of the extract is partly due to inhibition of 5-lipoxygenase and/or cyclooxygenase activity.

Mast cell is one of the major types of cells that produce various mediators such as leukotrienes and cyclooxygenase products involved in many inflammatory reactions, and the production can be stimulated by trigger substances such as A23187, a calcium ionophore. In the mast cells, prostaglandin D₂ (PGD₂) and leukotrien C₄ (LTC₄) can be produced from arachidonic acid as the major prostanoit and leukotrienes by cyclooxygenase and 5-lipoxygenase, respectively. Thus, in the present study, to determine the mechanism of anti-inflammatory activity of the extract in more detail, we examined its effects and those of its constituents, (−)-eburnamine and pleiometinine, on the generation of PGD₂ and LTC₄, which respectively represented the activities of cyclooxygenase and 5-lipoxygenase in the rat mast cells stimulated by A23187 in vitro.

**MATERIALS AND METHODS**

**Materials** Authentication of the stem bark of *H. zeylanica* Gard. was achieved by comparison with herbarium specimens in the Department of Biology.

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heparin, pH 6.80. The mast cells were harvested after massaging the animal trunk, and the cell suspension obtained was layered over 38% BSA and centrifuged. The pellets were washed and suspended in Ca2+-free MCM. Cell viability was determined by toluidine blue staining\(^1\) and the mast cell count was adjusted to 2 × 10\(^5\) cells/ml.

**PGD\(_2\)** and **LTC\(_4\)** **Production in Rat Mast Cells Stimulated by A23187, a Calcium Ionophore** Purified mast cells (2 × 10\(^5\) cells/ml) in Ca\(^2+\)-free MCM were incubated with 32 \(\mu\)M arachidonic acid and 1 mM CaCl\(_2\) in the presence and absence of test agents at 37°C for 15 min, and then 5 \(\mu\)M A23187 was added to stimulate the activities of cyclooxygenase and 5-lipoxygenase. After 30 min, the reaction was terminated by placing the tubes in an ice bath and centrifugation followed. PGD\(_2\) and LTC\(_4\) produced from arachidonic acid by cyclooxygenase and 5-lipoxygenase, respectively, were determined by radioimmunoassay with commercially available kits (Amersham UK). Vehicle control containing dimethyl sulfoxide (DMSO), ethanol and Ca\(^2+\)-free MCM was used in the experiments.

**Drugs** The following drugs were used: aspirin (Iwaki Seiyaku, Tokyo, Japan), fine powder gelatin, sodium chloride, potassium chloride, calcium chloride, dextrose (Nacalai Tesque Inc., Kyoto, Japan), arachidonic acid, A23187, bovine albumin fraction V, nordihydroguaiaretic acid (NDGA) (Sigma Chem. Co., St. Louis, MO, U.S.A.), heparin sodium salt, dimethyl sulfoxide, ethanol (Wako Pure Chem., Ltd., Osaka, Japan), toluidine blue (Polysciences Inc., U.S.A.). All drug solutions were prepared immediately before the experiments.

**Statistical Analysis** The data were analyzed using a parametric one-way analysis of variance (ANOVA) followed by Dunnett’s test. A difference was considered statistically significant at \(p < 0.05\).

### RESULTS

**Effect of *H. zeylanica* Alkaloid Extract on Cyclooxygenase Activity in Rat Mast Cells** The *H. zeylanica* alkaloid extract had no effect on the cyclooxygenase activity, whereas the reference drug, aspirin, inhibited the activity in a concentration-dependent manner (IC\(_{50}\) = 55 \(\mu\)g/ml or 305 \(\mu\)M) (Fig. 1). Neither (−)-eburnamine nor pleiominutine, constituents of the alkaloid extract, affected the A23187 stimulation of PGD\(_2\) production in rat mast cells (Table 1).

**Effect of *H. zeylanica* Alkaloid Extract on 5-Lipoxygenase Activity in Rat Mast Cells** The *H. zeylanica* alkaloid extract caused a concentration-dependent inhibition of 5-lipoxygenase activity in rat mast cells as shown in Fig. 2. This alkaloid extract, at 300 \(\mu\)g/ml, inhibited the 5-lipoxygenase-mediated LTC\(_4\) production by about 50%. NDGA, a selective inhibitor of lipoxygenase, markedly inhibited the 5-lipoxygenase activity (IC\(_{50}\) = 2.8 \(\mu\)g/ml or 9.3 \(\mu\)M). In contrast, aspirin produced relatively weak inhibition of the LTC\(_4\) synthesis. However, neither (−)-eburnamine nor pleiominutine affected generation of LTC\(_4\) by 5-lipoxygenase (Table 1).

### DISCUSSION

We previously demonstrated that *H. zeylanica* alkaloid extract had an anti-inflammatory effect on the arachidonic acid-induced ear edema in mice.\(^7\) In this inflammation model, lipoxygenase products play an important role since a 5-lipoxygenase inhibitor, but not a cyclooxygenase inhibitor, inhibits the ear edema caused by arachidonic acid.\(^14,15\) In the present study, we also found that the alkaloid extract inhibited the activity of 5-lipoxygenase but had no effect on cyclooxygenase in rat mast cells *in vitro*; these results supported the *in vivo* model of ara-
chidonic acid-induced ear edema previously mentioned.

Since only the 5-lipoxygenase but not the cyclooxygenase activity appeared to be inhibited at the same concentration of alkaloid extract, *H. zeylanica* alkaloid extract appears to selectively inhibit the 5-lipoxygenase. Moreover, this observation makes it unlikely that the alkaloid extract is toxic to mast cells. These results therefore indicated that inhibition of the arachidonic acid metabolism via 5-lipoxygenase activity may be to some degree involved in the anti-inflammatory activity of the *H. zeylanica* alkaloid extract.

The purified alkaloids, (-)-eburnamine and pleiomutinine which were 3.3 and 2.3% of the total *H. zeylanica* alkaloid extract (unpublished data), respectively, failed to inhibit 5-lipoxygenase-mediated LTC\textsubscript{4} production, suggesting that these two compounds are not the active components in the extract's inhibition of this activity. Other alkaloids such as pleiocarpamine, (+)-isoeburnamine, tubotaiwine, yohimbol, (+)-eburnamenine, (+)-eburnamonine, etc. were also isolated but their yield from the stem bark of *H. zeylanica* was small. Since the inhibitory effect of the alkaloid extract on 5-lipoxygenase activity did not show the maximal inhibition comparable to NDGA, a selective 5-lipoxygenase inhibitor,\textsuperscript{16} it is speculated that the active compound(s) presented in the *H. zeylanica* alkaloid is a relatively minute component(s) of the total alkaloid. It is of interest to investigate further the active constituent(s) responsible for such activities.

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**REFERENCES AND NOTES**

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