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EFFECTS OF BAICALEIN ON LEUKOTRIENE C₄ BIOSYNTHESIS IN HUMAN LEUKOCYTE

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We have studied the effects of baicalein on leukotriene C₄ biosynthesis induced by calcium ionophore A 23187 in human peripheral polymorphonuclear leukocytes. Baicalein inhibited calcium ionophore-induced leukotriene C₄ biosynthesis in human polymorphonuclear leukocytes. The concentration of baicalein required for 50% inhibition (IC₅₀) of leukotriene C₄ formation was 6.00×10^{-7} M at a calcium ionophore concentration of 1 $\mu\text{g/ml}$; at 0.1 $\mu\text{g/ml}$ it was 2.05×10^{-7} M.

KEYWORDS — human polymorphonuclear leukocyte; leukotriene C₄; calcium ionophore; baicalein; Scutellaria baicalensis

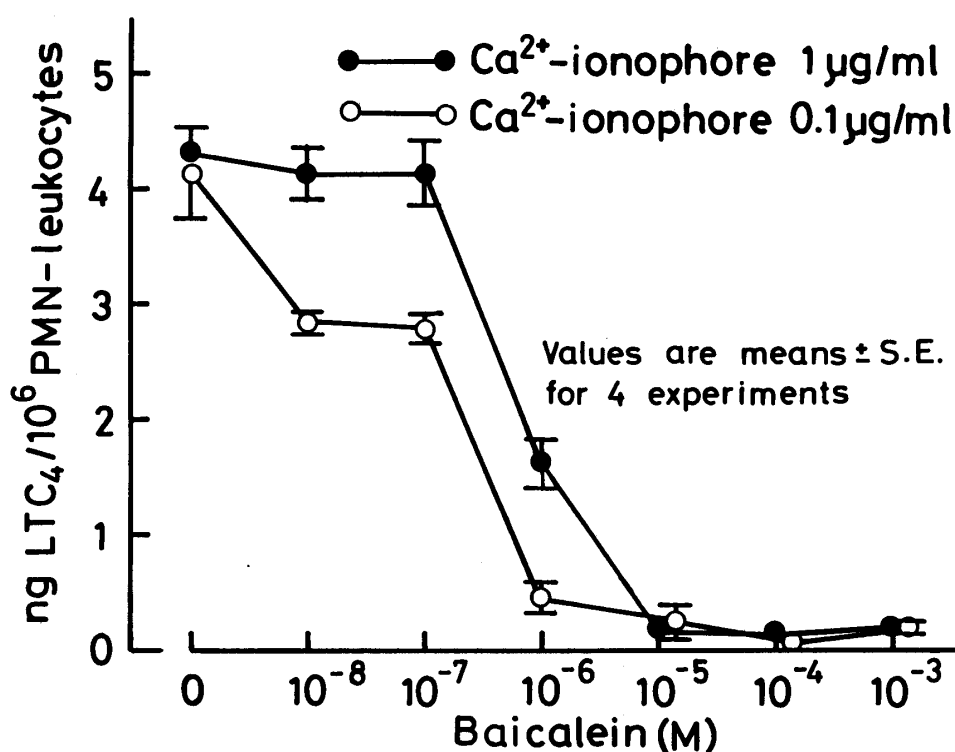
Since ancient times, the roots of Scutellaria baicalensis have been used to treat allergic and inflammatory diseases in China and Japan.

Leukotrienes are significantly involved in immunoregulation and in a variety of diseases, including asthma, inflammation and various allergic conditions. In the presence of 5-lipoxygenase, free arachidonic acid is converted into 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid (5-HPETE), which is then reduced to 5-HETE or oxygenated to the unstable intermediate leukotriene A₄ (LTA₄).¹⁾ LTA₄ can be further converted enzymatically to LTB₄, LTC₄ and LTD₄.^{2,3)} The slow reacting substance of anaphylaxis (SRS-A) is produced in immediate hypersensitivity reactions.¹⁾ It is composed of a mixture of LTC₄, LTD₄ and a small amount of LTE₄.⁴⁾

In the present work, we studied the inhibitory effects of baicalein obtained from Scutellariae Radix on calcium ionophore-induced LTC₄ biosynthesis in human polymorphonuclear leukocytes. Polymorphonuclear leukocytes, isolated from healthy human venous blood by sedimentation through dextran and Ficoll-metrizamide,⁵⁾ were suspended in Dulbecco's phosphate buffered saline (PBS) (1.0 mM CaCl₂·2H₂O, 2.68 mM KCl, 1.47 mM KH₂PO₄, 0.5 mM MgCl₂·6H₂O, 137 mM NaCl and 8.06 mM Na₂HPO₄·12H₂O, pH 7.4). These cells, identified as more than 97% polymorphonuclear leukocytes by Giemsa staining and light microscopy, were more than 95% viable as measured by the trypane blue exclusion test. The LTC₄ induced by calcium ionophore was assayed according to the method of Conroy *et al.*⁶⁾ Briefly, human polymorphonuclear leukocytes (1 × 10⁷ cells/ml) (280 μl , final; 2.8 × 10⁶ cells/tube) were preincubated with the indicated amount of baicalein and 10 mM L-cysteine for 5 min at 37°C. And then, calcium ionophore was added to give a final concentration of 0.1 $\mu\text{g/ml}$

or 1.0 $\mu\text{g/ml}$ in a final volume of 0.4 ml and the mixture was incubated for 15 min at 37°C. The reaction was stopped by adding 0.6 ml of ice-cold Dulbecco's PBS (pH 7.4). The incubation fluid after centrifugation at 1000 x g and 4°C for 15 min was used for the measurement of LTC_4 . The LTC_4 was determined by using a Leukotriene C_4 (^3H) Radioimmunoassay (RIA) Kit (New England).

As shown in the figure, baicalein inhibited the calcium ionophore-induced LTC_4 biosynthesis in human polymorphonuclear leukocytes dose-dependently. The IC_{50} values of baicalein for the LTC_4 biosynthesis induced by calcium ionophore were $6.00 \times 10^{-7}\text{M}$ at 1.0 $\mu\text{g/ml}$ of calcium ionophore and $2.05 \times 10^{-7}\text{M}$ at 0.1 $\mu\text{g/ml}$ of calcium ionophore.



Recently, it was reported that baicalin and its metabolites such as baicalein and baicalein-6,7-di-O-glucuronide appeared in blood when baicalin was administered orally.⁷⁾ Therefore, it seems likely that baicalein also appears in blood when baicalein and the extracts of *Scutellariae Radix* are administered orally. We are now investigating the concentrations of baicalein in blood caused by the oral administration of baicalein, baicalin and the extracts of *Scutellariae Radix*.

Koda *et al.*⁸⁾ reported that baicalein and baicalin inhibited the production of SRS-A in the lungs of sensitized guinea pigs. Furthermore, we previously reported that baicalein was a potent inhibitor of both 12- and 5-lipoxygenase in rat platelet and rat peritoneal leukocyte homogenates.^{9,10)} Therefore, the inhibitory effects of baicalein on calcium ionophore-induced LTC_4 biosynthesis may be due to the inhibition of the 5-lipoxygenase system. On the other hand, 12-HPETE (12-lipoxygenase product) was reported to activate 5-lipoxygenase in circulating leukocytes and

lung macrophages with subsequent release of bronchoconstricting leukotrienes.¹¹⁾ Therefore, the inhibition of 12-lipoxygenase by baicalein is also favorable for the prevention of leukotriene biosynthesis. Baicalein can be obtained in high yield from the root of *Scutellaria baicalensis*. These results show that baicalein could be developed in drugs to treat asthma and allergic diseases.

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