Note

Effects of Baicalein, a Flavonoid, and Other Anti-inflammatory Agents on Glyoxalase-I Activity

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Flavonoids, widely distributed in edible plants, have various biochemical and physiological activities¹⁻³⁾ including an anti-inflammatory effect.⁴⁾ In a previous article, we showed that flavonoids such as quercetin and fisetin are potent inhibitors of glyoxalase-I⁵⁾ [S-lactoylglutathione methylglyoxal-lyase (isomerizing) EC 4.4.1.5]. It was proposed that the enzyme is involved in inflammation, and some glyoxalase-I inhibitors suppress histamine release, one of the major inflammatory steps (Gillespie,⁶⁾ 1981). We wanted to know whether or not glyoxalase-I, in turn, would be inhibited by ordinary anti-inflammatory agents including baicalein and some other flavonoids extracted from *Scutellariae Radix* (the roots of *Scutellaria baicalensis* GEORGI, "Ogon" in Japanese), a traditional Chinese anti-inflammatory drug.⁷⁾

The flavonoids were obtained through the courtesy of Dr. Takashi Yamagishi of the Hokkaido Institute of Public Health. Glyoxalase-I was extracted from bovine liver and purified as described elsewhere.⁸⁾ The retinoids were gifts from Japan Roche. Indomethacin, dexamethazone (Wako), glycyrrhizic acid, and glycyrrhetic acid (Nakarai) were obtained from the commercial sources indicated.

The reaction was done in a cuvette which was kept at 37°C in a temperature-controlled cuvette holder attached to a Shimadzu spectrophotometer UV-240. The reaction mixture contained 2,850 μ l of 0.1 M sodium phosphate buffer, pH 6.8, 50 μ l of 1.0 M methylglyoxal, 50 μ l of 65 mM glutathione, and 30 μ l of 2.5 mM test substance dissolved in either dimethylsulfoxide or ethanol. After preincubation for 5 min, 20 μ l of enzyme solution was added and the absorbance at 240 nm due to lactoylglutathione was recorded every 5 sec for the initial 1 min. The amount of enzyme giving an increase of 0.14 in absorbance per minute in the absence of flavonoid (=control) was used.

Table I gives the inhibition ratings of the enzyme activity by various agents. Glycyrrhetic acid inhibited the enzyme very strongly, while glycyrrhizic acid, i.e. glycyrrhetic acid diglucuronide, and indomethacin were fairly

strongly inhibitory. Indomethacin inhibits cyclo-oxygenase activity in prostaglandin synthesis from arachidonic acid, which is supposedly one of the anti-inflammatory mechanisms of the drug. In contrast, salicylic acid acetate, known as aspirin, which also inhibits cyclo-oxygenase, did not inhibit glyoxalase-I. A synthetic

TABLE I. EFFECTS OF ANTI-INFLAMMATORY AGENTS ON GLYOXALASE-I ACTIVITY

The enzyme activity was estimated by measuring the absorbance increase at 240 nm due to lactoylglutathione formation. The final concentration of each test substance was 25 μ M, except that the wogonin concentration was 10 μ M owing to its low solubility. The experimental details are described in the text.

Test substance	Inhibition (%)
<i>Anti-inflammatory agent</i>	
Glycyrrhizic acid	33
Glycyrrhetic acid	64
Salicylic acid acetate	1
Indomethacin	39
Dexamethazone	14
<i>Flavonoid</i>	
Quercetin	44
Baicalein	80
Baicalin	9
Wogonin	19
Wogonin glucuronide	10
<i>Retinoid</i>	
Retinoic acid	5
Isoretinoic acid	18

FIG. 1. Inhibition of Glyoxalase-I Activity by Baicalein and Glycyrrhetic acid as a Function of Concentration.

The reaction conditions were the same as those described in the legend to Table I except that the concentrations of the inhibitors were varied. Each point indicates the average of triplicate measurements. Baicalein (●); glycyrrhetic acid (▲).

glucocorticoid, dexamethazone, was weakly inhibitory.

Among the flavonoids tested in this study, baicalein was the most potent glyoxalase-I inhibitor. Baicalin (= baicalein glucuronide) was less active than baicalein. Glycosidation seemed to decrease the inhibitory activities of aglycons as described previously.⁵⁾ Wogonin and its glucuronide, which are other component flavonoids found in anti-inflammatory *Scutellariae Radix*, were far less inhibitory than baicalein.

Although retinoids are not anti-inflammatory, we examined their inhibitory activities because they suppress tumor promotion caused by phorbol ester, an inflammatory agent. However, they were only weak inhibitors of glyoxalase-I.

The concentrations of baicalein and glycyrrhetic acid which give half maximal inhibition were 6 and 16 μM , respectively (Fig. 1). These values are some of the lowest reported so far. Since the value for quercetin, the most potent inhibitor in the previous study,⁵⁾ was 36 μM , baicalein and glycyrrhetic acid can be classified as very potent inhibitors.

The mode of the inhibition by both compounds was of the mixed type (data not shown).

Quercetin, a potent inhibitor of glyoxalase-I, suppresses tumor promotion⁹⁾ as well as inflammation.¹⁰⁾ Baicalein, another anti-inflammatory agent,¹¹⁾ has been demonstrated to inhibit glyoxalase-I strongly in this study. This suggests that baicalein would be a possible candidate as an anti-promotor in tumor formation.

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