The Journal of Applied Pharmacology, 14, 36-39(2006)

Inhibition of Collagenase by Anti-inflammatory Synthetic Flavones

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(Received October 18, 2005; Accepted December 5, 2005)

Abstract – Some flavones/flavonols were previously found to inhibit collagenase. To establish a therapeutic potential for skin inflammation, twenty-three synthetic flavone derivatives were examined for their inhibitory potential against collagenase from *Clostridium histolyticum*. From the results, it was found that most of them having various hydroxyl, methoxyl, methylsulfuryl and/or chloro substitution(s) on A- and B-rings were not efficient collagenase inhibitors. Among the synthetic flavones tested, only two synthetic derivatives, 3',4'-dihydroxyflavone and 5-hydroxy-4'-methoxyflavone, weakly inhibited bacterial collagenase (13 - 29% inhibition at 50 - 100 μM).

Keywords □ flavonoid, collagenase, matrix metalloproteinase, skin inflammation

INTRODUCTION

Flavonoids are one of large entities of plant constituents. They possess anti-inflammatory activity in vitro and in vivo (Gabor, 1986; Lewis, 1989). The most prominent points of their anti-inflammatory cellular actions are believed to be the effects on the enzymes related to eicosanoid metabolism and the effects on other proinflammatory molecules including matrix metalloproteinase (MMP) (Middleton *et al.*, 2000; Kim *et al.*, 2004). MMPs are the zinc-containing serine proteases that degrade various matrix components of the interstitial area. More than 20 different forms of MMPs have been found. Among them, matrix metalloproteinase-1 (MMP-1) is a collagenase (collagenase-1, interstitial collagenase, EC 3.4.24.7), which mainly breaks down collagen types I and III, the major intercellular matrix proteins in the dermal area of mammalian skin. Since unbalanced turn-over or rapid breakdown of collagen molecules occurs in several skin inflammatory diseases and photo-aged skin (Fisher and Voorhees, 1998), MMP-1 inhibitor may be useful to control some inflammatory disorders, especially skin inflammation.

In this respect, flavonoids were previously examined for their inhibitory activity on different types of MMPs. And certain kinds of flavonoids including delphinidine, kaempferol, quercetin and catechins were demonstrated to inhibit MMP activities such as MMP-2, -3 and -12 (Nagase *et al.*, 1998; Ende and Gebhardt, 2004; Melzig *et al.*, 2001; Sartol *et al.*, 2002). Particularly, certain flavonoids such as baicalein, quercetin and hyperoside were revealed to be potent inhibitors of elastase (MMP-12) (Melzig *et al.*, 2001). However, MMP-1 inhibitory flavonoids have been rarely demonstrated. For example, (-)-epicatechin gallate and (-)-epigallocatechin gallate from tea exhibited collagenase inhibition at high concentrations (Makimura *et al.*, 1993). And recent investigation by us has revealed that some flavonoids including several flavones/flavonols possessed the inhibitory activity against collagenase from *Clostridium histolyticum*, although not potent (Sin and Kim, 2005). To extend this observation and to establish a therapeutic value for skin inflammation, the collagenase inhibitory potential of various synthetic flavones were examined using bacterial collagenase as an enzyme source in the present study.

MATERIALS AND METHODS

Chemicals and Reagents

Collagenase (clostridiopeptidase A, EC 3.4.24.3) from *Clostridium histolyticum* type H, PZ-peptide (4-phenylazobenzyloxycarbonyl-Pro-Leu-Gly-Pro-D-Arg monohydrate), 1,10-phenanthroline (MMP inhibitor), chrysin and apigenin were purchased from Sigma-Aldrich (St. Louis, MO). The synthetic flavones used in this study were obtained according to the previously described (Dao *et al.*, 2003; Park *et al.*, 2005).

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Collagenase assay

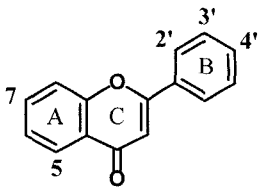
Collagenase activity was measured using the previously described procedure (Sawabe *et al.*, 1998) with slight modification. In brief, an assay tube contained collagenase (5 μg) and PZ-peptide (0.5 mg) in 0.1 M Tris buffer (pH 7.1) containing 20 mM $CaCl_2$ in the presence or absence of test compounds (total volume of 1.7 ml). The tube was incubated at 37°C for 30 min, and 25 mM citric acid solution (1 ml) was added to terminate the reaction. After mixing with ethylacetate (5 ml), the absorbance of the organic layer was measured at 320 nm. Test compounds including flavonoids were initially dissolved in DMSO and diluted in the same buffer to appropriate concentrations. Percent inhibition was calculated according to the following formula:

% inhibition = $(Abs_{control}-Abs_{sample}) \times 100/Abs_{control}$, where $Abs_{control}$ and Abs_{sample} represented (Abs of control with collagenase - Abs of control without collagenase) and (Abs in the presence of test compound with collagenase - Abs in the presence of test compound without collagenase), respectively. All measurements were duplicated and the arithmetic means were represented.

RESULTS AND DISCUSSION

Our previous investigation using various natural flavonoid derivatives including flavanones, flavones, isoflavones and flavonols has shown that some flavones and flavonols possessed collagenase inhibitory activity in somewhat different potencies. Especially, flavonols such as kaemferol, quercetin and myricetin showed considerable inhibition against collagenase from *Clostridium histolyticum* (IC_{50} of quercetin was 286 μM.) (Sin and Kim, 2005). The present study was carried out to clearly establish the inhibitory activity of various synthetic flavone derivatives. For this purpose, twenty-three synthetic flavones were evaluated for their inhibitory activity against collagenase from *C. histolyticum*. When the synthetic flavones having B-

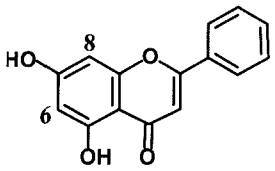
Table I. Inhibition of collagenase by synthetic flavones



	5	7	2'	3'	4'	% inhibition at 50 μM	% inhibition at 100 μM
F-1	H	H	H	H	OCH_3	-[a)]	-
F-2	H	H	H	OH	OH	13	14
F-3	OH	H	H	H	OCH_3	-	29
F-4	OCH_3	H	H	H	OCH_3	-	-
F-5	H	OH	H	H	H	-	NT
F-6	H	OH	OH	H	OH	14	-
F-7	H	OCH_3	H	H	H	-	-
F-8	H	OCH_3	H	H	OCH_3	-	-
F-9	H	OCH_3	H	OH	OH	-	-
F-10	H	OCH_3	OCH_3	H	OCH_3	-	-
F-11	H	OCH_3	H	Cl	Cl	-	-
F-12	OH	OCH_3	H	H	H	-	-
F-13	OH	OCH_3	H	H	OCH_3	-	-
F-14	OH	OCH_3	H	OH	OH	-	-
F-15	OCH_3	OH	H	H	H	-	-
F-16	OCH_3	OH	H	H	OCH_3	-	-
F-17	OCH_3	OCH_3	H	H	H	-	-
F-18	OCH_3	OCH_3	H	H	OCH_3	-	-
Chrysin	OH	OH	H	H	H	-[b)]	11[b)]
Apigenin	OH	OH	H	H	OH	-[b)]	15[b)]

[a)]Less than 10% inhibition, [b)]Data from Sin and Kim (2005), NT: Not tested.

Table II. Inhibition of collagenase by 5,7-dihydroxy-6,8-substituted flavones



	6	8	% inhibition at 50 μM	% inhibition at 100 μM
F-19 (wogonin)	H	OCH_3	-[a)]	-
F-20	H	SCH_3	-	-
F-21	OCH_3	OCH_3	-	-
F-22	SCH_3	SCH_3	-	-
F-23 (oroxylin A)	OCH_3	H	-	-

[a)]Less than 10% inhibition.

ring 2',3',4'-substitution(s) were examined, most of them did not show the meaningful inhibition at the concentrations up to 100 μM (Table I). Only two synthetic flavones, 3',4'-dihydroxyflavone (F-2) and 5-hydroxy-4'-methoxyflavone (F-3) were weakly inhibitory against collagenase at 100 μM. At 50 μM, F-2 and 2',4',7'-trihydroxyflavone (F-6) were weakly active. But, F-6 did not show the meaningful inhibition at 100 μM. Next, 5,7-dihydroxyflavones having A-ring 6,8-substitution(s) were examined. But, all of them did not possess the significant inhibitory activity against collagenase at the concentrations up to 100 μM (Table II). From the structure-activity comparison, it was found that methoxyl substitution at C-5 and/or C-7 was not favorable compared to hydroxyl moiety at the same positions (F-12, F-15 and F-17 vs. chrysin). Methoxyl or methylsulfuryl substitution at C-6 and/or C-8 reduced or abolished the inhibitory activity against collagenase (F-19 - F-23 vs. chrysin). Considering that the flavonols were stronger collagenase inhibitors than the flavones (Sin and Kim, 2005), it is not surprising that the various synthetic flavones tested here are not efficient collagenase inhibitors. However, it is possible that they may reduce collagen breakdown by down-regulating collagenase (MMP-1) induction, since many flavonoids were previously found to modulate proinflammatory gene expression (Kim *et al.*, 2004). In particular, certain flavonoids such as genistein, quercetin, nobiletin and baicalein were demonstrated to reduce MMP-1 induction (Kang *et al.*, 2003; Song *et al.*, 2001; Lin *et al.*, 2003; Choe *et al.*, 2003).

In conclusion, all results from the present investigation have clearly indicated that the synthetic flavones tested in this study are not efficient inhibitors of MMP-1 (collagenase). Nonetheless, it may be still possible to reduce collagen breakdown by down-regulating MMP-1 induction. This point is to be further elucidated.

ACKNOWLEDGMENTS

This work was supported by grant No. R01-2004-000-10134-0 from the Basic Research Program of the Korea Science & Engineering Foundation.

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