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Flavonoid and Skin Inflammation

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Abstract - There have been various inflammatory skin disorders in humans including atopic dermatitis, eczema and psoriasis. Although some drugs have been used for these disorders, there is an urgent need for safer and more effective topical anti-inflammatory agents. Plant flavonoids possess anti-inflammatory activity and some of them have multiple pharmacological mechanisms, inhibition of eicosanoid metabolizing enzymes, histamine release and/or down-regulation of proinflammatory gene expression. These properties of flavonoids may be suitable for treating chronic skin inflammatory disorders. Especially, wogonin, some prenylated flavonoids and biflavonoids have a strong potential as new anti-inflammatory agents by topical application.

Keywords: flavonoid, skin, inflammation, eicosanoid, proinflammatory gene expression.

Plant flavonoids have been used as anti-inflammatory agents, especially on skin inflammation. They are used in a form of plant extracts as cosmetics and some topical drugs. They improve skin condition and help to maintain healthy skin. In order to prove their anti-inflammatory action, various flavonoids have been examined on animal models of skin inflammation. Here, some characteristics of animal models of skin inflammation and anti-inflammatory activity of flavonoids are presented mainly based on the experimental results from our laboratory. A therapeutic potential of flavonoids is also discussed.

Animal models of skin inflammation

Topical application of irritants produces contact irritation. As irritants, phenol, arachidonic acid (AA), 12-*O*-tetradecanoylphorbol 13-acetate (TPA), and dinitrochlorobenzene (DNCB) were used and they provoked mainly an edema. Even in these simple animal models, molecular mechanisms and outcomes are quite different depending on the irritants used. For example, phenol (10%, 20

μl/ear) induced a dermal edema of ICR mouse ear and caused down-regulation of the constitutive genes such as cyclooxygenase-1 (COX-1) and fibronectin while the expression of proinflammatory inducible gene, IL-1β, increased slightly (Lim *et al.*, 2004).. Topical application of AA to Sim mouse ear produced edema (peak, 40 - 60 min) and lipoxygenase (LOX)/COX inhibitors inhibited strongly (Young *et al.*, 1984; Chang *et al.*, 1985). Epidermal hyperplasia was not observed. Single topical TPA application to mouse skin produced an edematous response (De Young *et al.*, 1989) and epidermal hyperplasia (Reynolds *et al.*, 1997). Single TPA treatment to Balb/c mouse ear increased PGE_2 and LTB_4 concentration at 6.5 and 24 h (Raederstorff *et al.*, 1996). TPA treatment changed the expression pattern of many genes and the agents affecting these gene expressions may change inflammatory response of TPA-induced skin inflammation. On the other hand, multiple TPA treatment on animal skin produces chronic type inflammation. In our experiment of 3-day multiple TPA treatment, several proinflammatory genes such as COX-2, IL-1β, etc. were induced (Chi *et al.*, 2003). The biological outcomes of 3-day TPA treatment model and 7-day TPA treatment model (or longer treatment) are somewhat different in respect of patterns of proinflammatory gene expression. Using these animal models, anti-inflammatory activity of flavonoids was examined.

Anti-inflammatory flavonoids against skin inflammation

In Chinese medicine and in some cosmetics, certain flavonoid preparations in a form of plant extracts have been used topically to control skin inflammation. Topically applied flavonoids must penetrate into epidermal and dermal area through skin barrier for exerting their anti-inflammatory effects per se. In this respect, flavonoid aglycones may be effective because flavonoid glycosides are more hydrophilic rendering skin penetration more difficult. If possible, aglycone-concentrated fraction from certain plant extracts would be better to use although major forms of flavonoids in plants are various types of flavonoid glycosides in general. Initially, topical treatment of quercetin inhibited TPA-induced tumor promotion in mouse skin and LOX inhibitory action might contribute to this effect (Kato *et al.*, 1983). Up to date, various flavonoids were demonstrated to show in vivo anti-inflammatory effects by topical application (Table I). From these in vivo studies, some structure-activity relationships could be deduced. Yasukawa *et al.* (1989) have drawn a conclusion that C-2,3-double bond and 5,7,4'-hydroxylations in flavonoid chemical structures were important among 33 flavonoid derivatives tested. Our studies have also shown that certain flavones/flavonols including their glycosides possessed anti-inflammatory activity in croton oil- and AA-induced mouse ear edema by topical application among 20 flavonoids tested (Kim *et al.*, 1993; Lee *et al.*, 1993). 5,7-Hydroxyl groups were important to show inhibition

against croton oil-induced ear edema. In this model, flavonols having C-3 OH showed a higher activity than flavones without C-3 OH. 3',4'-Hydroxylations were also favorable, while flavonoid glycosides showed much less active as expected.. On the other hand, most flavones/flavonols tested showed a higher inhibitory activity against AA-induced ear edema.

It is significant to note that several prenylated flavonoids also showed meaningful anti-inflammatory activity by topical application. Prenyl residues in flavonoids make the molecules more hydrophobic rendering easy penetration through skin barrier. Furthermore, some prenylated flavonoids possess COX/LOX inhibitory activity (Chi *et al.*, 2001b). Morusin from *Morus alba* was found to possess anti-tumorigenicity by topical application (Yoshizawa *et al.*, 1989). In particular, sophoraflavanone G from the roots of *Sophora flavescence* inhibited skin inflammation of mouse croton oil-induced ear edema by topical application at 10 - 250 μg/ear, possibly via inhibition of eicosanoid formation (Chi *et al.*, 2001b; Kim *et al.*, 2002). This prenylated flavonoid is a dual inhibitor of COX/LOX.

One of important flavonoids for topical anti-inflammatory agents is wogonin (5,7-dihydroxy-8-methoxyflavone) from Scutellaria radix since it has not only high pharmacological activity, but also has unique properties. Wogonin was initially found to inhibit TPA-induced ear edema by topical application (Yasukawa *et al.*, 1989). Later, this compound was revealed as a down-regulator of proinflammatory molecules such as iNOS, COX-2 and IL-1ß expression, and the same compound directly inhibits COX-2 (Kim *et al.*, 1999; Chi *et al.*, 2001a). Recently, wogonin was also found to down-regulate COX-2 induction from the activated skin fibroblasts (NIH/3T3), suggesting therapeutic effect against skin inflammation (Chi and Kim, 2005). In addition, in vivo mechanism studies have been carried out. When examined on TPA- (3-day) and AA-induced skin inflammation, wogonin by topical application inhibited ear edema of these models. The same compound inhibited COX-2 induction, leading to reduction of PGE_2 formation (Park *et al.*, 2001). By RT-PCR analysis, it was revealed that wogonin down-regulated other proinflammatory gene expression such as IL-1β on mouse skin (Chi *et al.*, 2003). Wogonin also inhibited phenol-induced simple contact irritation as well as delayed hypersensitivity with suppression of several proinflammatory gene expressions (Lim *et al.*, 2004).

Another potential flavonoid family for treating skin inflammatory conditions is biflavonoid, flavonoid-dimer. For example, biflavonoids from *Ginkgo biloba* leaves showed anti-inflammatory activity by topical application (Della Loggia *et al.*, 1996). Especially, liposome-encapsulated form gave higher anti-inflammatory activity. Amentoflavone from *Sellaginella tamariscina* also showed significant anti-inflammatory activity by topical application against AA-induced ear edema in

mice (Kim *et al.*, 1998a), possibly by inhibiting epidermal COX-1 (Kim *et al.*, 1998b). Particularly, ginkgetin from *G. biloba* leaves draws special attention since it could strongly inhibit iNOS expression (Cheon *et al.*, 2000). Later, the same compound was found to inhibit COX-2 expression and showed considerable topical anti-inflammatory activity against TPA-induced inflammation in mice (Kwak *et al.*, 2002). Recently, Western and RT-PCR analysis showed that ginkgetin treatment significantly inhibited edematic response and several proinflammatory gene expressions on chronic skin inflammation model in mice (Lim *et al.*, 2006).

Flavonoids and skin matrix metalloproteinase-1 (MMP-1)

Extracellular matrix including collagen network is degraded by MMPs. Especially, MMP-1 (mammalian collagenase-1) highly induced in some inflammatory conditions is most important to degrade type I and III collagen, main collagen molecules in skin. Thus inhibition and/or down-regulation of MMP-1 may lead to anti-inflammation and possibly preventing wrinkle formation.

We have found that some flavonoids, especially flavonol derivatives including kaempferol, quercetin and myricetin, showed considerable inhibition of collagenase from *Clostridium histolyticum* over the concentration ranges of 25 - 500 μM (Sin and Kim, 2005). Similar results were obtained by other group (Sim et al., 2007). We recently investigated the effects on mammalian MMP-1 activity and MMP-1 expression (Lim and Kim, 2007). When naringenin (flavanone), apigenin, wogonin (flavone), kaempferol and quercertin (flavonol) were examined, flavonols showed higher inhibition on human recombinant MMP-1, while flavones showed much reduced inhibition. And flavones and flavonols clearly inhibited MMP-1 induction from TPA-induced human skin fibroblasts. These flavonoids inhibited AP-1 activation. But the inhibitory mechanisms are different depending on the chemical structures of flavonoids. Quercetin inhibited ERK and p38 MAPK activation, while kaempferol inhibited p38 and JNK activation. Flavones did not affect these three MAPKs.

Conclusions

Previously, topical application of certain flavonoids was demonstrated to suppress edematic response and to inhibit eicosanoid production. By modern techniques employed, flavonoids are now revealed to regulate proinflammatory gene expression from several animal models of skin inflammation. The target genes include COX-2, iNOS, IL-1, TNF-α, MMP-1, etc. Since it is necessary to develop new agents having different cellular mechanism(s) from clinically used SAIDs or NSAIDs, topical flavonoid may become a useful therapeutic alternative for chronic skin inflammatory disorders such as atopic dermatitis. Unlike SAIDs,

they show relatively no or greatly reduced adverse effects, resulting in safe use for a long period. A clinical trial is necessary to prove usefulness of flavonoid therapy in near future. All these efforts may lead to successful development of new anti-inflammatory agents for skin disorders.

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Table I. Topically-treated flavonoids showing anti-inflammatory effects on animal skin

Flavonoids	Inflammagens	Animal models	References
Apigenin, luteolin, kaemferol, quercetin and glycosides	croton oil	ear edema	Della Loggia *et al.* (1986 & 1988)
Various flavones and flavonols, wogonin	TPA	ear edema	Yasukawa *et al.* (1989)
Isoliquiritigenin	TPA	ear edema	Yamamoto *et al.* (1991)
Baicalein	TPA	ear edema	Hara *et al.* (1992)
Various flavones and flavonols and glycosides	croton oil & AA	ear edema	Kim *et al.* (1993), Lee *et al.* (1993)
Quercetagetin, hispidulin, scutellarein and glycosides	TPA	ear edema	Gil *et al.* (1994)
Amentoflavone, sciadopytisin, ginkgetin, isoginkgetin	croton oil	ear edema	Della Loggia *et al.* (1996)
Morelloflavone	TPA	ear edema	Gil *et al.* (1997)
Amentoflavone	AA	ear edema	Kim *et al.* (1998a)
Nobiletin	TPA	dorsal skin edema	Murakami *et al.* (2000)
Sophoraflavanone G	croton oil	ear edema	Kim *et al.* (2002)
Wogonin	TPA, multiple TPA, AA, DNFB	edema	Park *et al.* (2001), Chi *et al.* (2003), Lim *et al.* (2004)
Ginkgetin	TPA & multiple TPA	edema	Kwak *et al.* (2002), Lim *et al.* (2006)
Gnaphaliin, pinocembrin, tiliroside	TPA & multiple TPA	ear edema	Sala *et al.* (2003)
Sigmoidin A, B	TPA	ear edema	Njamen *et al.* (2004)