The Journal of Applied Pharmacology, 15, 7-15(2007)

Molecular Events on Experimental Skin Inflammation and Modulation by Topical Anti-inflammatory Flavonoids

Hyun Pyo Kim

College of Pharmacy, Kangwon National University, Chunchon 200-701, Korea

(Received February 27, 2007; Accepted March 16, 2007)

Abstract – There have been various animal models of skin inflammation. These models have been used for establishing anti-inflammatory activity of the topical agents including cosmetics. Here, the molecular mechanisms of most widely-used animal models of skin inflammation including contact irritation, acute and chronic inflammation, and delayed-type hypersensitivity are summarized. Against these animal models, varieties of plant flavonoids showed anti-inflammatory activity. The action mechanisms of anti-inflammation by topical flavonoids are presented. A therapeutic potential of flavonoids is discussed.

Key words □ skin, inflammation, flavonoid

INTRODUCTION

Various animal models of skin inflammation have been used to establish the effects of topical anti-inflammatory agents and to develop new formulation against human skin inflammatory diseases. Several pharmacological characteristics of animal skin inflammation were previously summarized (Bouclier *et al.*, 1990). Role of adhesion molecules, chemotactic factors, and cytokines in inflammatory and neoplastic skin diseases up to 1990 was reviewed (Nickoloft *et al.*, 1990). Here, the molecular events of most widely-used animal models of skin inflammation are summarized and topical anti-inflammatory activity of flavonoid compounds is discussed. Various animal models of human psoriasis were previously reviewed (Schon, 1999; Mizutani *et al.*, 2003). They include models of spontaneous mutation, transgenic mice, knockout mice, T-cell transfer and xenotransplantation. But, in this review, the explanations of these models are out of scope.

Animal skin inflammation - Simple irritation

Contact irritation by simple irritants was observed in Balb/c mouse ear. The irritants used in this study were arachidonic acid (AA), capsaicin, 12-*O*-tetradecanoylphorbol 13-acetate (TPA), dinitrochlorobenzene (DNCB), lactic acid, retinoic acid and sodium lauryl sulfate. They produced the ear edema when measured 2 h after topical application (Wille *et al.*, 1998). The thickness increased on the treated-ears was 0.02 - 0.08 mm. Even in these simple irritations, the molecular mechanisms and outcome are quite different depending on the irritants used. AA and TPA induced the characteristic skin inflammatory responses described below. Sodium lauryl sulfate impaired the epidermal barrier function by detergent effect. Platelet activating factor (PAF) injection subcutaneously to mouse skin produced the increased vascular permeability, which was inhibited by indomethacin and reversed by prostaglandin E_2 (PGE_2), but not by nitric oxide (NO) (Fujii *et al.*, 1995). This finding was supported by the previous observation that PAF-induced vascular permeability was not affected by NO in guinea-pig skin (Teixeira *et al.*, 1993).

We have also used the animal model of contact irritation to examine anti-irritant effects of plant flavonoids. In our experiment, phenol (10%, 20 μl/ear) induced the ear edema on ICR mouse ear (0.08 mm). When skin biopsies were histologically compared, phenol-induced dermatitis on ICR mouse skin was mainly a dermal edema (Lim *et al.*, 2004). Phenol treatment caused a down-regulation of the constitutive genes such as cyclooxygenase-1 (COX-1) and fibronectin while the expression of the proinflammatory inducible gene, COX-2, increased slightly. But the biological meaning of this result is not known at present. These animal models may be successfully used in screening anti-irritants for the cosmetic products.

*Corresponding author
Tel: +82-33-250-6915, Fax: +82-33-255-9271
E-mail: hpkim@kangwon.ac.kr

Animal models of AA- and TPA-induced skin inflammation

Young *et al.* (1984) have found that a topical application of AA to Sim mouse ear provoked an edematic change (peak, 40 - 60 min) and the lipoxygenase (LOX)/COX inhibitors inhibited strongly while COX inhibitors like indomethacin showed much weaker activity. The peak edematic change was coincided with vasodilation measured by Evans blue extravasation. PGE_2 may cause vasodilation of erythema, while LOX products may be responsible for vascular leakage and edema consequent on cellular infiltrate (Wedmore and Williams, 1981). Almost no hyperplasia of epidermis was observed. The peak edematic response was well matched with our previous study, but in ours, indomethacin showed potent inhibition (80%) at 2 mg/ear of ICR mice (Kim *et al.*, 1993). This result was repeatedly confirmed by Sanchez and Moreno (1999a) demonstrating that indomethacin (500 μg/ear) potently inhibited AA-induced ear edema (64%) in Swiss mice. The differences of the results obtained may be partly due to the difference of the mice strains used. But, indomethacin seems to be an exception against this concept because of its powerful action. It was also demonstrated that leukotriene C_4 (LTC_4) and PGE_2 were present in the edematous ear produced by AA application (Opas *et al.*, 1985; Chang *et al.*, 1986). LTC_4 and other LOX products were found to mainly mediate AA-induced ear edema, while PGE_2 facilitated ear swelling (Inoue *et al.*, 1988). The levels of LTB_4 decreased rapidly 3 min after AA application. LOX inhibitors, especially 5-LOX inhibitors, strongly inhibit mouse AA-induced ear edema.

The common thought about AA-induced edema is that AA was used as a substrate for COX/LOX in the skin to produce eicosanoids that induce edema and infiltration of many inflammatory cells. But, the several lines of evidence have recently shown that there are some other pathways such as induction of proinflammatory molecules including COX-2 in 1 h. Although there is possibility that these induced molecules may not be the major molecules contributing to the edematic response in 1 h, the induction is apparent. Intradermal injection of 5-hydroperoxyeicosatetraenoic acid (5-HPETE) into ICR mouse ear provoked moderate increase of PGE_2 and LTB_4 with marked increase of $LTC_4/D_4/E_4$. Otherwise, intradermal injection of AA provoked the marked increase of PGE_2 with moderate increase of LTB_4 (Tsuji *et al.*, 1998). These results may reflect the induction of eicosanoid metabolizing enzymes such as COX-2 by AA treatment. Actually, COX-2 induction was found in AA-treated mouse ear (Sanchez and Moreno, 1999a). We have also observed COX-2 and IL-1β induction by AA treatment to ICR mouse skin in 1 h (Chi *et al.*, 2003). Therefore, it is evident that AA application to mouse skin modulates some gene expressions even in 1 h, and the agents to affect this pathway may have a positive effect against AA-induced skin inflammation.

On the other hand, an application of TPA known as protein kinase C (PKC) activator to mouse skin or certain cells in culture results in a host of biological and biochemical alterations, including stimulation of AA metabolism, accumulation of neutrophils and inflammatory responses (Raick, 1973). Single topical dose of TPA to mouse skin resulted in an edematous response that reached a maximum level around 6 h. PMN infiltration reached a maximum level by 25 h (De Young *et al.*, 1989). Single application of TPA (2.0/2.5 nmol) to hairless mouse dorsal skin induced skin histological changes including hyperplasia and inflammatory cell infiltration for 24 - 48 h (Reynolds *et al.*, 1997). The kinds and characteristics of eicosanoids in TPA- and AA-treated dermal area were somewhat varying depending on the experimental designs. Single treatment of TPA to Balb/c mouse ear increased PGE_2 and LTB_4 concentration at 6.5 and 24 h (Raederstorff *et al.*, 1996). Meanwhile, Sanchez and Moreno (1999a, 1999b) have found that LTB_4 and 6-keto-$PGF_{1\alpha}$ increased in TPA-ear edema, whereas PGE_2 and LTB_4 moderately increased in AA-ear edema. They also found that COX-2 was over-expressed in Swiss Webster mice ear by TPA- (6 h) or AA- (1 h) application revealed by Western blotting analysis. Dexamethasone and manoalide down-regulated TPA-induced COX-2 expression, but ketoprofen and indomethacin did not. Dexamethasone pretreated 2 h before AA-application reduced COX-2 expression with concomitant reduction of ear edema, while indomethacin and baicalein did not. In addition, TPA-induced several gene expression of the skin was observed in CD-1 mice. Single treatment of TPA on dorsal site of mice (4 h) induced COX-2, transforming growth factor-β1 (TGF-β1), tumor necrosis factor-α (TNF-α), c-Myc, c-Fos, c-Jun genes with weak induction of COX-1. In control animals, moderately high levels of COX-1, c-Myc and c-Jun expression were observed (Jang and Pezzuto, 1998; Kennard *et al.*, 1995). It was also demonstrated that COX-2 promoter activity was found to be regulated by PKC, extracellular signal-regulated kinase 1 (ERK1) and c-Jun (Wang and Smart, 1999). Further, TPA-mediated activation of COX-2 was accompanied by an induction of c-Jun and activator protein-1 (AP-1) activity via cAMP response element (Maldve *et al.*, 2000). In other experiment, single TPA treatment (6 h) on C57BL/6 mouse dorsal skin committed the

diverse gene expression changes. These changes were tracked using cDNA microarray technique, showing the increased expression of many genes such as ornithine decarboxylase and cathepsin L with the decreased expression of many genes including procollagen genes (Schlingemann *et al.*, 2003).

Animal model of chronic skin inflammation-Multiple treatment of TPA

One of the animal models of chronic skin inflammation is the experimental inflammation provoked by multiple application of TPA (Stanley *et al.*, 1991). Multiple treatment of TPA to the same skin site produced the quite different biological responses, priming and activation. In this model, the peak PMN influx was observed on day 3. Immunohistochemical studies have shown that the number of PMNs decreased drastically with multiple applications of TPA, whereas the number of monocytes/macrophages increased profoundly (Alford *et al.*, 1992). This suggests that a transition from acute to chronic inflammation has occurred in some period during TPA treatment. This model appears to be selective for agents that are known to be effective in the treatment of chronic skin inflammation. Anti-inflammatory steroids and LOX inhibitors are active. Unlike single-dose TPA model, COX inhibitors and antihistamines were not active.

In our experiment of 3-day model, several proinflammatory genes were found to be induced. They include COX-2, IL-1β, etc., and prednisolone greatly reduced the expression of these proinflammatory molecules (Chi *et al.*, 2003). The biological outcome of 3-day TPA treatment model and 7-day TPA treatment model (or longer treatment) are somewhat different in respect of proinflammatory gene expression. In 3-day multiple TPA treatment, COX-2 was highly induced, whereas COX-2 level moderately increased in 7-day multiple TPA treatment. Instead, IL-1β seems to be important in 7-day model since the expression level was prominent in this model (Lim *et al*, 2006a). Another difference is the degree of epidermal hyperplasia. In 7-day TPA treatment model, there was a massive epidermal hyperplasia compared to the moderate degree of epidermal proliferation in 3-day model (2 - 5 keratinocyte layers). The detailed molecular aspects of 3-day and 7-day models are to be elucidated further.

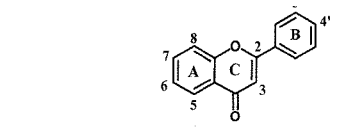
Animal skin inflammation of delayed-type hypersensitivity

Delayed-type hypersensitivity (DTH) reaction, type IV allergic response, comprises the induction phase induced by treatment of certain antigen and the elicitation phase by repeated application of same antigen to the sensitized animals. Tarayre *et al.* (1990) demonstrated that COX inhibitors except indomethacin had no effect on several DTH reactions in mice, suggesting that COX pathway may not participate in DTH reaction. Instead, several proinflammatory gene expression/production play an important role. PPD injection to human skin increased the expression of several adhesion molecules like intercellular adhesion molecule-1 (ICAM-1) (Norris *et al.* 1991). Several cytokines were induced in the induction phase of contact sensitivity (Enk and Katz, 1992). Using Northern blotting, Piguet *et al.* (1991) have found that TNF-α was critically involved within basal keratinocytes and dermal infiltrate in hapten-induced irritant and contact hypersensitivity reactions in mice by treatment of trinitrochlorobenzene (TNCB). Anti-TNF-α antibody drastically reduced delayed hypersensitivity reaction. IL-1α mRNA was also detected. Shornick *et al.* (1996) demonstrated the importance of IL-1β in contact hypersensitivity using IL-1β-deficient mice. And Morita *et al.* (1996) demonstrated the importance of NO in contact hypersensitivity with the increased production of IL-2 and IFN-γ. Five repeated treatment of dinitrofluorobenzene (once a weak) increased phospholipase A_2-IIE (PLA_2-IIE) gene expression in Balb/c mouse ear (Murakami *et al.*, 2002).

cDNA microarray technique was applied and sequential gene expression pattern of various chemokines was demonstrated (Mitsui *et al.*, 2003). In our experiment, the several proinflammatory genes including COX-2, IL-1β, ICAM-1 and iNOS were induced in the elicitation phase of TNCB-treated DTH response in mice. The lesion of activation phase reaction weakly induced the gene families of COX-2 and IL-1β (Lim *et al.*, 2004). Depending on the antigens used, the patterns of the inducible proinflammatory genes expressed were different, but TNF-α, IL-1β, and IFN-γ are most important to produce DTH response in this animal model.

Bioavailability and in vivo metabolism of flavonoids

Flavonoids (Fig. 1) are ingested daily as food component, and they are sometimes administered as a form of plant extracts in Chinese medicine. The total daily flavonoid uptake by normal person was estimated to be 23 mg/man - 1 g/man, depending on the experimental designs including subjects and analytical procedures employed (Kuhnau, 1976; Hertog *et al.*, 1992). However, the biological/pharmacological activities exerted by flavonoid intake more significantly depend on their bioavailability and metabolism in the body. For instance, oral intake of fried onion (equivalent to 64 mg quercetin), apple



	3	5	6	7	8	3'	4'
Flavone	H	H	H	H	H	H	H
Apigenin	H	OH	H	OH	H	H	OH
Luteolin	H	OH	H	OH	H	OH	OH
Wogonin	H	OH	H	OH	OCH_3	H	H
Nobiletin	H	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3
Flavonol	OH	H	H	H	H	H	H
Kaempferol	OH	OH	H	OH	H	H	OH
Quercetin	OH	OH	H	OH	H	OH	OH

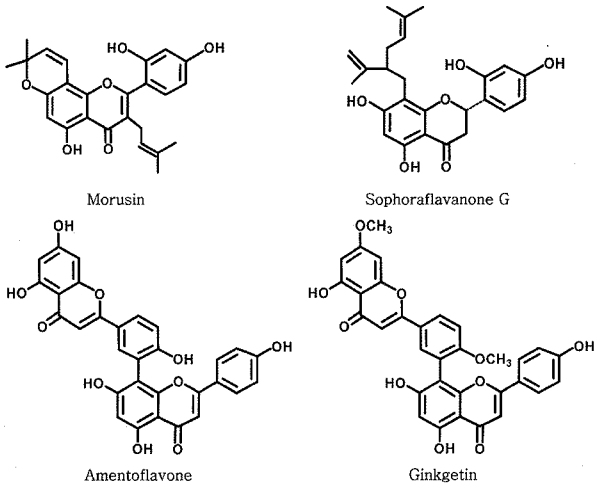


Fig. 1. Chemical structures of some flavonoids mentioned in this study

(100 mg quercetin) or rutin (100 mg quercetin) resulted in approximately 0.8 - 3 μM of peak plasma concentrations as total quercetin metabolites after 0.7 - 7 h (Hollman *et al.*, 1997). The similar results were also observed that the time to reach peak plasma concentration of oral quercetin intake was 1.9 h while the peak time for rutin was 6 h (Erlund *et al.*, 2000). These studies indicate that the time to reach peak plasma concentration is varying depending on the types of flavonoid molecules, aglycone or glycoside. Therefore, the time to measure biological activity by oral flavonoid intake is an important determinant to obtain the optimal results. A review summarizing recent findings of absorption and bioavailability of flavonoids is available (Ross and Kasum, 2002). Some flavonoids orally administered are well absorbed into the body in certain conditions, but these cases are not common. Most flavonoids showed very limited bioavailability by oral treatment. Even if their bioavailabilities are high, the actual original molecules in circulation were hardly found (Chen *et al.*, 2005).

The orally administered flavonoids undertook rapid and intensive metabolism mostly in intestine and liver (Murota and Terao, 2003). The major metabolites of flavonoids were found as flavonoid glucuronides and flavonoid sulfates. Only a small portion was detected as the original molecule in the plasma. The metabolism is largely determined by the hydroxylation pattern. It was reported that the flavonoids having 5,7- and 3',4'-hydroxylations were easily hydrolyzed and the ring was cleaved more easily by intestinal microorganisms (Paganga and Rice-Evans, 1997). After intake of quercetin-rich diet, quercetin glucuronides, quercetin sulfates and their *O*-methylated derivatives appeared in the plasma reaching maximum concentrations, approximately 0.1 - 10 μM (Moon *et al.*, 2001; Day *et al.*, 2001; Wittig *et al.*, 2001). When chrysin (400 mg/man) was orally administered, the concentrations of chrysin and chrysin metabolites found in the plasma were 3 - 16 ng/ml plasma (approximately 0.1 μM), and chrysin sulfate was detected 30 fold higher than chrysin glucuronide while chrysin itself was rarely found in the plasma (Walle *et al.*, 2001). In other animal study, naringenin glucuronide and sulfate were detected with 4 - 8% bioavailability following oral treatment to rabbits (Hsiu *et al.*, 2002). In one human study, plasma isoflavonoid (genistein) concentration in Japanese men having a higher intake of genistein as soy products was approximately 0.3 μM (Adlercreutz *et al.*, 1993).

All these previous reports clearly demonstrate that flavonoids are absorbed by oral administration and are present in the circulation regardless of their metabolic forms. But, as recently reviewed by Manach *et al.* (2005) and Williamson and Manach (2005), flavonoid uptake through the intestine is not high and mostly glucuronide/sulfate metabolites are detected in the plasma. The maximum plasma concentrations seem to be approximately 10 μM by oral intake (Manach and Donovan, 2004). It should be mentioned that the active form of flavonoid molecules to most cells and biological systems are generally believed to be flavonoid aglycones, but not their glycosides, glucuronides or sulfates. Furthermore, considering the hydrophilic nature of the metabolites and their low biological activities compared to those of flavonoid aglycones, the estimate of maximum plasma concentration would be even lower than 10 μM. Therefore, the most sensitive biological systems in the body may be only affected by oral flavonoid intake. Some flavonoids possess regulatory effects on the expression of many proinflammatory molecules. But the biologically meaningful effects on these molecules possibly appear at higher than 10

μM of flavonoids, suggesting that oral flavonoids as a part of regular food intake are not likely to exert the considerable anti-inflammation including COX/LOX inhibition and down-regulation of proinflammatory molecule expression. In pharmacological treatment of flavonoids, however, the situation is somewhat different. By oral administration, massive doses of certain flavonoids, (ex) more than 5 g/man, may exceed plasma concentration of 10 μM for prolonged time. In this condition, many biological systems are possibly affected and anti-inflammatory activities would appear.

Otherwise, the high concentrations of flavonoids may be obtained more easily by local treatment. Especially, topical application through skin is one of the plausible routes of flavonoid administration in human, and flavonoids may be efficiently used for skin inflammatory disorders topically if proper formulation is developed. And, as shown by Li and Birt (1996), certain flavonoids such as apigenin penetrate well into the skin and they are not rapidly transformed to inactive metabolites in the epidermal and dermal area. This is the scientific rationale for topical therapy with flavonoids and the potential use as skin anti-inflammatory agents needs to be studied.

Proinflammatory mediators of skin inflammation and flavonoids

Eicosanoids are prominent mediators of inflammation, and they are associated with skin inflammatory disorders. PLA_2 is involved in several skin inflammatory disorders and the inhibitors of this enzyme showed an inhibitory activity against an animal model of chronic skin inflammation, multiple exposal of TPA (Burke, 2001). LOX products are known to be deeply associated with human psoriasis (Fogh and Kragbelle, 2000; Iversen and Kragbelle, 2000). Importantly, the proinflammatory cytokines including IL-1β and TNF-α are pivotal factors to provoke chronic skin inflammatory disorders such as atopic dermatitis (AD). Therefore, the compounds that possess modulating activity on these proinflammatory molecules may have potential to treat skin inflammatory disorders. In this respect, flavonoids among natural products may be significant for further study since they possess the favorable modulating effects on these proinflammatory molecules.

Flavonoids show multiple pharmacological actions on inflammatory responses. First, some flavonoids possess antioxidative action, which may be beneficial to reduce or eliminate oxidative stress during inflammatory insults. The chemical species flavonoids may interfere with are H_2O_2, O_2^-, NO, etc. Second, some derivatives possess direct modulatory activities of the enzymes involved in inflammatory process. The target enzymes are PLA_2, COX-1, COX-2, 5-LOX, 12-LOX, iNOS, etc. In third, most importantly, certain derivatives have a capacity to modulate the expression levels of inducible enzymes/proteins. They include COX-2, iNOS, IL-1α, TNF-α, etc. (Kim *et al.*, 2004). All these properties of flavonoids may participate in treating some chronic inflammatory diseases such as rheumatoid arthritis and psoriasis. In particular, a higher anti-inflammatory activity exerted by topical flavonoids as mentioned earlier is suitable to treat skin inflammatory disorders. Thus, flavonoids are potential candidates of new anti-inflammatory agents against skin inflammation.

Anti-inflammatory flavonoids against skin inflammation

For a long time, certain plant extracts having flavonoid components as major constituents have been used topically as anti-inflammtory drugs in Chinese medicine or in cosmetic preparations. The typical examples are Scutellaria extract and Chamomile extract. The major flavonoid components in these plant extracts are baicalein, wogonin and their glycosides in the former, while apigenin and luteolin are the major flavonoids in the latter. As plant constituents, the major forms of flavonoids are not the aglycones, but the various types of glycosides. The flavonoid glycosides are generally more hydrophilic, so that they are not easily penetrable through the skin barrier. Therefore, it is advised that the aglycone types of flavonoids may be preferentially used for topical formulation to give a better effect.

Against several animal models of skin inflammation, various flavonoid molecules were demonstrated to possess anti-inflammatory activity by topical application. For instance, quercetin was found to inhibit TPA-induced tumor promotion by topical application and this inhibition might be related with its inhibitory activity of LOX (Kato *et al.*, 1983). Della Loggia *et al.* (1986) demonstrated that apigenin and luteolin showed the inhibitory activity against mouse croton oil-induced ear edema, while their glycosides showed much less activity as expected. The same group also reported that kaempferol and quercetin possessed topical anti-inflammatory activity against mouse croton oil-induced ear edema. The order of potency was kaempferol >> quercetin >> esculetin (Della Loggia *et al.*, 1988). These earlier investigations clearly demonstrated that certain flavones/flavonols possessed anti-inflammatory activity against animal skin inflammation by topical application.

Using TPA-induced mouse ear edema assay, various types of flavones, flavonols and their glycosides were found to possess

an anti-inflammatory activity by topical application and some structure-activity relationships were deduced (Yasukawa *et al.*, 1989). A C-2,3-double bond was crucial and 5,7,4'-hydroxylations were favorable. In the same types, flavonols were more active than the respective flavones. In addition, some flavonoids such as isoliquilitigenin (Yamamoto *et al.*, 1991), baicalein (Hara *et al.*, 1992), quercetagetin, hispidulin and scutellarein (Gil *et al.*, 1994) were also reported to inhibit TPA-induced mouse ear edema by topical application. The latter three compounds possess PLA_2 inhibitory activity.

We have examined the inhibitory activity of different types of natural and synthetic flavonoids in order to elucidate anti-inflammatory activity of various flavonoids depending on the routes of administration and to find structure-activity relationships. Our results have shown that certain flavones/flavonols including their glycosides possessed more or less anti-inflammatory activity on croton oil- and AA-induced mouse ear edema by oral and topical administration (Kim *et al.*, 1993; Lee *et al.*, 1993). But, the potencies were far less than those of the reference drugs, indomethacin and hydrocortisone. From the structure-activity relationships, it was found that 5,7-hydroxyl groups were important to show inhibition against croton oil-induced ear edema. Against this model, the flavonols having C-3 OH showed a higher activity than the flavones without C-3 OH. 3',4'-Hydroxylations were also favorable. Some of these chemical structures were coincided with the structural requirements previously claimed by Della Loggia *et al.* (1988) and Yasukawa *et al.* (1989). In comparison, most flavones/flavonols tested showed a higher inhibitory activity against AA-induced ear edema when topically applied than against croton oil-induced ear edema. And the flavone types showed a higher activity than the flavonols against AA-induced ear edema. These results may provide a scientific rationale for the topical use of Scutellaria and Chamomile extracts in herbal remedy.

It is interesting to note that against skin inflammation induced by intradermal injection of xanthine oxidase/hypoxanthine (O_2^- radical generator) in rats, apigenin-7-glucoside showed a dose-dependent inhibition by intradermal injection, indicating its antioxidant effect (Fuchs and Milbradt, 1993). Nobiletin from citrus inhibited TPA-induced skin inflammation in mice. This inhibitory activity was proved to be related with an inhibition of oxidative stress (Murakami *et al.*, 2000). Besides, several prenylated flavonoids showed meaningful anti-inflammatory activity by topical application. Morusin from *Morus alba* showed anti-tumorogenic activity by topical application (Yoshizawa *et al.*, 1989). Sophoraflavanone G from *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). Morus and Sophora species are important sources of the prenylated flavonoids. The cosmetics containing these plant extracts may be beneficial to maintain healthy and radiant skin.

One of the most prominent flavonoid inhibitors against skin inflammation is wogonin (5,7-dihydroxy-8-methoxyflavone) from Scutellaria radix. Wogonin was initially found to be anti-inflammatory against animal models of acute and chronic inflammation by oral administration (Kubo *et al.*, 1984). Later, wogonin was demonstrated to inhibit TPA-induced ear edema by topical application (Yasukawa *et al.*, 1989). Recently, this compound was revealed as a down-regulator of proinflammatory molecules such as iNOS, COX-2 and IL-1β expression from several cell lines (Kim *et al.*, 1999; Chi *et al.*, 2001a; Kim *et al.*, 2001). This compound is very unique among the flavonoid derivatives in that it is not only a down-regulator of proinflammatory molecules, but a COX-2 inhibitor (Chi *et al.*, 2001a). Especially, wogonin down-regulated COX-2 induction from the activated skin fibroblasts (NIH/3T3), suggesting the therapeutic effect against skin inflammation (Chi and Kim, 2005). In order to prove in vivo effect, wogonin was examined on TPA- (3-day) and AA-induced skin inflammation. When topically applied, wogonin inhibited ear edema of these models. The same compound inhibited COX-2 induction, leading to the reduced PGE_2 production (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). It also inhibited phenol-induced simple contact irritation as well as delayed hypersensitivity with suppression of several proinflammatory gene expression (Lim *et al.*, 2004).

Biflavonoids are flavonoid-dimers having a potential for topical anti-inflammatory agents. For example, amentoflavone from *Sellaginella tamariscina* showed significant anti-inflammatory activity by topical application against AA-induced ear edema in mice (Kim *et al.*, 1998a). Amentoflavone was also found to inhibit epidermal COX-1 (Kim *et al.*, 1998b). Particularly, ginkgetin from the leaves of *Ginkgo biloba* 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 the 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.*, 2006b).

Perspectives

As mentioned above, some flavonoids such as the prenylated ones, wogonin and some biflavonoids have a therapeutic potential against skin inflammation. They can modulate an edematic response as well as proinflammatory gene expression. 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 for chronic skin inflammatory disorders such as AD. Unlike SAIDs, they show relatively no or greatly reduced adverse effects, resulting in the safe use for a long period. A clinical trial is necessary to prove usefulness of flavonoid therapy in near future.

ACKNOWLEDGMENTS

This study was financially supported by the research grant No. R01-2004-000-10134-0 from the Basic Research Program of the Korea Science & Engineering Foundation and post-BK21 program.

REFERENCES

Adlercreutz, H., Markkanen, H. and Watanabe, S. (1993). Plasma concentrations of phyto-oestrogens in Japanese men. *Lancet* **342**, 1209-1210.

Alford, J. G., Stanley, P. L., Todderud, G. and Tramposch, K. M. (1992). Temporal alteration of leukocyte subsets into mouse skin inflamed with phorbol ester. *Agents Actions* **37**, 261-267.

Bouclier, M., Cavey, D., Kail, N. and Hensby, C. (1990). Experimental models in skin pharmacology. *Pharmacol. Rev.* **42**, 127-153.

Burke, J. R. (2001). Targeting phospholipase A_2 for the treatment of inflammatory skin diseases. *Curr. Opin. Investig. Drugs* **2**, 1549-1552.

Chang, J., Carlson, R. P., Oneill-Davis, L., Lamb, B., Sharma, R. N. and Lewis, A. J. (1986). Correlation between mouse skin inflammation induced by arachidonic acid and eicosanoid synthesis. *Inflammation* **10**, 205-214.

Chen, X., Yin, O. Q., Zuo, Z. and Chow, M. S. (2005). Pharmacokinetics and modeling of quercetin and metabolites. *Pharm. Res.* **22**, 892-901.

Cheon, B. S., Kim, Y. H., Son, K. H., Chang, H. W., Kang, S. S. and Kim, H. P. (2000). Effects of prenylated flavonoids and biflavonoids on lipopolysaccharide-induced nitric oxide production from the mouse macrophage cell line RAW 264.7. *Planta Med.* **66**, 596-600.

Chi, Y. S., Cheon, B. S. and Kim, H. P. (2001a). Effect of wogonin, a plant flavone from Scutellaria radix, on the suppression of cyclooxygenase-2 and induction of inducible nitric oxide synthase in lipopolysaccharide-treated RAW 264.7 cells. *Biochem. Pharmacol.* **61**, 1195-1203.

Chi, Y. S., Jong, H. G., Son, K. H., Chang, H. W., Kang, S. S. and Kim, H. P. (2001b). Effects of naturally occurring prenylated flavonoids on enzymes metabolizing arachidonic acid: Cyclooxygenases and lipoxygenases. *Biochem. Pharmacol.* **62**, 1185-1191.

Chi, Y. S. and Kim, H. P. (2005). Suppression of cyclooxygenase-2 expression of skin fibroblasts by wogonin, a plant flavone from Scutellaria radix. *Prostag. Leukot. Essent. Fatty Acids* **72**, 59-66.

Chi, Y. S., Lim, H., Park, H. and Kim, H. P. (2003). Effect of wogonin, a plant flavone from Scutellaria radix, on skin inflammation: in vivo regulation of inflammation-associated gene expression. *Biochem. Pharmacol.* **66**, 1271-1278.

Day, A. J., Mellon, F., Barron, D., Sarrazin, G., Morgan, M. R. and Williamson, G. (2001). Human metabolism of dietary flavonoids: identification of plasma metabolites of quercetin. *Free Radic. Res.* **35**, 941-952.

Della Loggia, R., Ragazzi, E., Tubaro, A., Fassina, G. and Vertua R. (1988). Anti-inflammatory activity of benzopyrones that are inhibitors of cyclo- and lipo-oxygenase. *Pharmacol. Res. Comm.* **20 (Suppl. V)**, 91-94.

Della Loggia, R., Tubaro, A., Dri, P., Zilli, C. and Del Negro, P. (1986). The role of flavonoids in the anti-inflammatory activity of *Chamomilla recutita*. In *Plant flavonoids in biology and medicines, 1986* (Cody et al. Eds.), pp. 481-484. Alan R. Liss, New York.

De Young, L. M., Kheifets, J. B., Ballaron, S. J. and Young, J. M. (1989). Edema and cell infiltration in the phorbol ester-treated mouse ear are temporally separate and can be differentially modulated by pharmacologic agents. *Agents Actions* **26**, 335-341.

Enk, A. H. and Katz, S. J. (1992). Early molecular events in the induction phase of contact sensitivity. *Proc. Nat'l. Acad. Sci. USA* **89**, 1398-1402.

Erlund, I., Kosonen, T., Alfthan, G., Maenpaa, J., Perttunen, K., Kenraali, J., Parantainen, J. and Aro, A. (2000). Pharmacokinetics of quercetin from quercetin aglycone and rutin in healthy volunteers. *Eur. J. Clin. Pharmacol.* **56**, 545-553.

Fogh, K. and Kragballe, K. (2000). Eicosanoids in inflammatory skin diseases. *Prostag. Other Lipid Mediat.* **63**, 43-54.

Fuchs, J. and Milbradt, R. (1993). Skin anti-inflammatory activity of apigenin-7-glucoside in rats. *Arzneim. Forsch.* **43**, 370-372.

Fujii, E., Irie, K., Uchida, Y., Ohba, K. and Muraki, T. (1995). Role of eicosanoids but not nitric oxide in the platelet-activating factor-induced increase in vascular permeability in mouse skin. *Eur. J. Pharmacol.* **273**, 267-272.

Gil, B., Sanz, M. J., Terencio, M. C., Ferrandiz, M. L., Bustos, G., Paya, M., Gunasegaran, R. and Alcaraz, M. J. (1994). Effects of flavonoids on *Naja naja* and human recombinant synovial phospholipase A_2 and inflammatory responses in mice. *Life Sci.* **54**, PL333-338.

Hara, H., Sukamoto, T., Ohtaka, H., Abe, K., Tatumi, Y., Saito, Y., Suzuki, A. and Tsukamoto, G. (1992). Effects of baicalein and α-tocopherol on lipid peroxidation, free radical scavenging activity and 12-*O*-tetradecanoylphorbol acetate-induced ear edema. *Eur. J. Pharmacol.* **221**, 193-198.

Hertog, M. G. L., Hollman, P. C. H. and Katan, M. B. (1992). Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J.*

Agri. Food Chem. **40**, 2379-2383.

Hollman, P. C. H., van Trijp, J. M. P., Mengelers, M. J. B., de Vries, J. H. M. and Katan, M. B. (1997). Bioavailability of the dietary antioxidant flavonol quercetin in man. *Cancer Lett.* **114**, 139-140.

Hsiu, S. L., Huang, T. Y., Hou, Y. C., Chin, D. H. and Chao, P. D. (2002). Comparison of metabolic pharmacokinetics of naringin and naringenin in rabbits. *Life Sci.* **70**, 1481-1489.

Inoue, H., Mori, T. and Koshihara, Y. (1988). Sulfidopeptide-leukotrienes are major mediators of arachidonic acid-induced mouse ear edema. *Prostaglandins* **36**, 731-739.

Iversen, L. and Kragballe, K. (2000). Arachidonic acid metabolism in skin health and disease. *Prostag. Other Lipid Mediat.* **63**, 25-42.

Jang, M. and Pezzuto, J. M. (1998). Effects of resveratrol on 12-*O*-tetradecanoylphorbol-13-acetate-induced oxidative events and gene expression in mouse skin. *Cancer Lett.* **134**, 81-89.

Kato, R., Nakadate, T., Yamamoto, S. and Sugimura, T. (1983). Inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-induced tumor promotion and ornithine decarboxylase activity by quercetin: possible involvement of lipoxygenase inhibition. *Carcinogenesis* **4**, 1301-1305.

Kennard, M. D, Kang, D. C., Montgomery, R. L. and Butler, A. P. (1995). Expression of epidermal ornithine decarboxylase and nuclear proto-oncogene in phorbol ester tumor promotor-sensitive and -resistant mice. *Mol. Carcinog.* **12**, 14-22.

Kim, D. W., Chi, Y. S., Son, K. H., Chang, H. W., Kim, J. S., Kang, S. S. and Kim, H. P. (2002). Effects of sophoraflavanone G, a prenylated flavonoid from *Sophora flavescens* on cyclooxygenase-2 and in vivo inflammatory response. *Arch. Pharm. Res.* **25**, 329-335.

Kim, H., Kim, Y. S., Kim, S. Y. and Suk, K. (2001). The plant flavonoid wogonin suppresses death of activated C6 rat glial cells by inhibiting nitric oxide production. *Neurosci. Lett.* **309**, 67-71.

Kim, H. K., Cheon, B. S., Kim, Y. H., Kim, S. Y. and Kim, H. P. (1999). Effects of naturally occurring flavonoids on nitric oxide production in the macrophage cell line RAW 264.7 and their structure-activity relationships. *Biochem. Pharmacol.* **58**, 759-765.

Kim, H. K., Namgoong, S. Y. and Kim, H. P. (1993). Anti-inflammatory activity of flavonoids: Mice ear edema inhibition. *Arch. Pharm. Res.* **16**, 18-24.

Kim, H. K., Son, K. H., Chang, H. W., Kang, S. S. and Kim, H. P. (1998a). Amentoflavone, a plant biflavone: A new potential anti-inflammatory agent. *Arch. Pharm. Res.* **21**, 406-410.

Kim, H. P., Mani, I., Iversen, L. and Ziboh, V. A. (1998b). Effects of naturally occurring flavonoids and biflavonoids on epidermal cyclooxygenase and lipoxygenase from guinea-pigs. *Prostag. Leukot. Essent. Fatty Acids* **58**, 17-24.

Kim, H. P., Son, K. H., Chang, H. W. and Kang, S. S. (2004). Anti-inflammatory plant flavonoids and cellular action mechanisms. *J. Pharmacol. Sci.* **96**, 229-245.

Kubo, M., Matsuda, H., Tanaka, M., Kimura, Y., Okuda, H., Higashino, M., Tani, T., Namba, K. and Arichi, S. (1984). Studies on Scutellaria radix. VII. Anti-arthritic and anti-inflammatory actions of methanolic extract and flavonoid components from Scutellaria radix. *Chem. Pharm. Bull.* **32**, 2724-2729.

Kuhnau J. (1976). The flavonoids: A class of semi-essential food components: their role in human nutrition. *World Rev. Nutr. Diet* **24**, 117-191.

Kwak, W-J., Han, C. K., Son, K. H., Chang, H. W., Kang, S. S., Park, B. K. and Kim, H. P. (2002). Effect of ginkgetin from *Ginkgo biloba* leaves on cyclooxygenases and in vivo skin inflammation. *Planta Med.* **68**, 316-321.

Lee, S. J., Son, K. H., Chang, H. W., Do, J. C., Jung, K. Y., Kang, S. S. and Kim, H. P. (1993). Anti-inflammatory activity of naturally occurring flavones and flavonol glycosides. *Arch. Pharm. Res.* **16**, 25-28.

Li, B. and Birt, D. F. (1996). In vivo and in vitro percutaneous absorption of cancer preventive flavonoid apigenin in different vehicles in mouse skin. *Pharmaceutical Res.* **13**, 1710-1715.

Lim, H., Park, H. and Kim, H. P. (2004). Inhibition of contact dermatitis in animal models and suppression of proinflammatory gene expression by topically applied flavonoid, wogonin. *Arch. Pharm. Res.* **27**, 442-448.

Lim, H., Son, K. H., Chang, H. W., Kang, S. S. and Kim, H. P. (2006a). Inhibition of chronic skin inflammation by topical anti-inflammatory flavonoid preparation, Ato Formula®. *Arch. Pharm. Res.* **29**, 503-507.

Lim, H., Son, K. H., Chang, H. W., Kang, S. S. and Kim, H. P. (2006b). Effects of anti-inflammatory biflavonoid, ginkgetin, on chronic skin inflammation. *Biol. Pharm. Bull.* **29**, 1046-1049.

Maldve, R. E., Kim, Y., Muga, S. J. and Fisher, S. M. (2000). Prostaglandin E_2 regulation of cyclooxygenase expression in keratinocytes is mediated via cyclic nucleotide-linked prostaglandin receptors. *J. Lipd Res.* **41**, 873-881.

Manach, C. and Donovan, J. (2004). Pharmacokinetics and metabolism of dietary flavonoids in human. *Free Radical Res.* **38**, 771-185.

Manach, C., Williamson, G., Morand, C., Scalbert, A. and Remesy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* **81(Suppl.)**, 230S-242S.

Mitsui, G., Mitsui, K., Hirano, T., Ohara, O., Kato, M. and Niwano, Y. (20003). Kinetic profiles of sequential gene expressions for chemokines in mice with contact hypersensitivity. *Immunol. Lett.* **68**, 191-197.

Mizutani, H., Yamanaka, K., Konishi, H. and Murakami, T. (2003). Animal models of psoriasis and pustular psoriasis. *Arch. Dermatol. Res.* **295**, S67-S68.

Moon, J-H., Tsushida, T., Nakahara, K. and Terao, J. (2001). Identification of quercetin 3-*O*-β-D-glucuronide as an antioxidative metabolite in rat plasma after oral administration of quercetin. *Free Radic. Biol. Med.* **30**, 1274-1285.

Morita, H., Hori, M. and Kitano, Y. (1996). Modulation of picryl chloride-induced contact hypersensitivity reaction in mice by nitric oxide. *J. Invest. Dermatol.* **107**, 549-552.

Murakami, A., Nakamura, Y., Torikai, K., Tanaka, T., Koshiba, T., Koshimizu, K., Kuwahara, S., Takahashi, Y., Ogawa, K., Yano, M., Tokuda, H., Nishino, H., Mimaki, Y., Sashida, Y., Kitanaka, S. and Ohigashi, H. (2000). Inhibitory effect of citrus nobiletin on phorbol ester-induced skin inflammation, oxidative stress, and tumor promotion in mice. *Cancer Res.* **60**, 5059-5066.

Murakami, M., Yoshihara, K., Shimbara, S., Lambeau, G., Singer, A., Gelb, M. H., Sawada, M., Inagaki, N., Nagai, H. and Kudo, I. (2002). Arachidonate release and eicosanoid generation by group IIE phospholipase A_2. *Biochem. Biophys. Res. Comm.* **292**, 689-696.

Murota, K. and Terao, J. (2003). Antioxidative flavonoid quercetin: implication of its intestinal absorption and metabolism. *Arch. Biochem. Biophys.* **417**, 12-17.

Nickoloft, B. J., Griffiths, C. E. M. and Barker, J. N. W. N.

(1990). The role of adhesion molecules, chemotactic factors, and cytokines in inflammatory and neoplastic skin disease-1990 update. *J. Invest. Dermatol.* **94**, 151S-157S.

Norris, P., Poston, R. N., Thomas, D. S., Thorhill, M., Hawk, J. and Haskard, D. O. (1991). The expression of endothelial leukocyte adhesion molecule-1 (ELAM-1), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) in experimental cutaneous inflammation: A comparison of ultraviolet B erythema and delayed hypersensitivity. *J. Invest. Dermatol.* **96**, 763-770.

Opas, E. E., Bonney, R. J. and Humes, J. L. (1985). Prostaglandin and leukotriene synthesis in mouse ears inflamed by arachidonic acid. *J. Invest. Dermatol.* **84**, 253-256.

Paganga, G. and Rice-Evans, C. A. (1997). The identification of flavonoids as glucosides in human plasma. *FEBS Lett.* **401**, 78-82.

Park, B. K., Heo, M. Y., Park, H. and Kim, H. P. (2001). Inhibition of TPA-induced cyclooxygenase-2 expression and skin inflammation in mice by wogonin, a plant flavone from Scutellaria radix. *Eur. J. Pharmacol.* **425**, 153-157.

Piguet, P. F., Grau, G. E., Hauser, C. and Vassali, P. (1991). Tumor necrosis factor is a critical mediator in hapten-induced irritant and contact hypersensitivity reactions. *J. Exp. Med.* **173**, 673-679.

Raederstorff, D., Pantze, M., Bachmann, H. and Moser, U. (1996). Anti-inflammatory properties of docosahexaenoic and eicosapentaenoic acids in phorbol-ester-induced mouse ear inflammation. *Int. Arch. Allergy Immunol.* **111**, 284-290.

Raick, A. N. (1973). Ultrastructural, histological and biochemical alterations produced by 12-*O*-tetradecanoylphorbol-13-acetate on mouse epidermis and their relevance to skin tumor promotion. *Cancer Res.* **33**, 269-286.

Reynolds, N. J., McCombie, S. W., Shankar, B. B., Bishop, W. R. and Fisher, G. J. (1997). SCH 47112, a novel staurosporine derivative, inhibits 12-*O*-tetradecanoylphorbol-13-acetate-induced inflammation and epidermal hyperplasia in hairless mouse skin. *Arch. Dermatol. Res.* **289**, 540-546.

Ross, J. A. and Kasum, C. M. (2002). Dietary flavonoids: Bioavailability, metabolic effects and safety. *Ann. Rev. Nutr.* **22**, 19-34.

Sanchez, T. and Moreno, J. J. (1999a). Role of prostaglandin H synthase isoforms in murine ear edema induced by phorbol ester application on skin. *Prostag. Other Lipid Mediat.* **57**, 119-131.

Sanchez, T. and Moreno, J. J. (1999b). Role of leukocyte influx in tissue prostaglandin-H synthase-2 overexpression induced by phorbol ester and arachidonic acid in skin. *Biochem. Pharmacol.* **58**, 877-879.

Schlingemann, J., Hess, J., Wrobel, G., Breitenbach, U., Gebhardt, C., Steinlein, P., Kramer, H., Furstenberger, G., Hahn, M., Angel, P. and Lichter, P. (2003). Profile of gene expression involved by the tumor promoter TPA in murine epithelial cells. *Int. J. Cancer* **104**, 699-708.

Schon, M. P. (1999). Animal models of psoriasis - What can we learn from them?. *J. Invest. Dermatol.* **112**, 405-410.

Shornick, L. P., De Togni, P., Mariathasan, S., Goellner, J., Strauss-Schoenberger, J., Karr, R. W., Ferguson, T. A. and Chaplin, D. D. (1996). Mice deficient in IL-1β manifest impaired contact hypersensitivity to trinitrochlorobenzene. *J. Exp. Med.* **183**, 1427-1436.

Stanley, P. L., Steiner, S., Havens, M. and Tramposch, K. M. (1991). Mouse skin inflammation induced by multiple topical application of 12-*O*-tetradecanoylphorbol-13-acetate. *Skin Pharmacol.* **4**, 262-271.

Tarayre, J. P., Barbara, M., Aliaga, M. and Tsne-Versailles, J. (1990). Comparative actions of immunosuppressants, glucocorticoids and non-steroidal anti-inflammatory drugs on various models of delayed hypersensitivity and in a non-immune inflammation in mice. *Arzneim. Forsch.* **40**, 1125-1131.

Teixeira, M. M., Williams, T. J. and Hellewell, P. G. (1993). Role of prostaglandins and nitric oxide in acute inflammatory reactions in guinea-pig skin. *Br. J. Pharmacol.* **110**, 1515-1521.

Tsuji, F., Miyake, Y., Horiuchi, M. and Mita, S. (1998). Involvement of leukotriene B_4 in murine dermatitis models. *Biochem. Pharmacol.* **55**, 297-304.

Walle, T., Otake, Y., Brubaker, J. A., Walle, U. K. and Halushka, P. V. (2001). Disposition and metabolism of the flavonoid chrysin in normal volunteers. *Br. J. Clin. Pharmacol.* **51**, 143-146.

Wang, H. Q. and Smart, R. C. (1999). Overexpression of protein kinase Cα in the epidermis of transgenic mice results in striking alterations in phorbol ester-induced inflammation and COX-2, MIP-2 and TNF-α expression but not tumor promotion. *J. Cell Sci.* **112 (Pt 20)**, 3497-3506.

Wedmore, C. V. and Williams, T. J. (1981). Control of vascular permeability by polymorphonuclear leukocytes in inflammation. *Nature* **289**, 646-650.

Wille, J. J., Kydonieus, A. F. and Kalish, R. S. (1998). Inhibition of irritation and contact hypersensitivity by ethacrynic acid. *Skin Pharmacol. Appl. Skin Physiol.* **11**, 279-288.

Williamson, G. and Manach, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *Am. J. Clin. Nutr.* **81(Suppl.)**, 243S-255S.

Wittig, J., Herderich, M., Graefe, E. U. and Veit, M. J. (2001). Identification of quercetin glucuronides in human plasma by high-performance liquid chromatography-tandem mass spectrometry. *Chromatography B Biomed. Sci. Appl.* **753**, 237-243.

Yamamoto, S., Aizu, E., Jiang, H., Nakadate, T., Kiyoto, I., Wang, J. C. and Kato, K. (1991). The potent anti-tumor promoting agent isoliquiritigenin. *Carcinogenesis* **12**, 317-323.

Yasukawa, K., Takido, M., Takeuchi, M. and Nakagawa, S. (1989). Effect of chemical constituents from plants on 12-*O*-tetradecanoylphorbol-13-acetate-induced inflammation in mice. *Chem. Pharm. Bull.* **37**, 1071-1073.

Yoshizawa, S., Suganuma, M., Fugiki, H., Fukai, T., Nomura, T. and Sigimura, T. (1989). Morusin, isolated from root bark of *Morus alba* L., inhibits tumor promotion of teleocidin. *Phytotherapy Res.* **3**, 193-195.

Young, J. M., Spires, D. A., Bedord, C. J., Wagner, B., Ballaron, S. J. and De Young, L. M. (1984). The mouse ear inflammatory response to topical arachidonic acid. *J. Invest. Dermatol.* **82**, 367-371.