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Histopathological Comparison of Animal Models of Skin Inflammation and Inhibition of the Inflammatory Responses by Plant Flavonoid, Wogonin

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Abstract – Wogonin (5,7-dihydroxy-8-methoxyflavone), an anti-inflammatory plant flavonoid, was previously demonstrated to modulate the several parameters of animal skin inflammation. This compound inhibited edematic response as well as proinflammatory gene expression. In this investigation, the histopathological changes of the lesions from different types of experimental skin inflammation were compared and the potential therapeutic effect of topically applied wogonin was evaluated. From the results, it was found that multiple TPA treatment drastically increased ear edema accompanied with epidermal hyperplasia and inflammatory cell infiltration, while phenol treatment provoked only edematic response in the dermal area. Wogonin somewhat differently inhibited these animal models of skin inflammation.

Keywords □ flavonoid, wogonin, skin inflammation, histology

INTRODUCTION

Animal models of skin inflammation have been used for elucidating cellular and pathological mechanisms of human skin inflammatory disorders as well as for establishing anti-inflammatory potential of various compounds (Bouclier *et al.*, 1990). The models widely used include skin inflammation of acute, subchronic, chronic types and delayed hypersensitivity. Depending on the types, there are considerable differences of the nature and progress of inflammation such as inflammatory cells infiltrated, hyperplasia, edematic response, and duration of inflammation, etc. These characteristics of the inflammatory models could be occasionally detected by a histological comparison of skin biopsies. Thus, for investigating potential effect of topical anti-inflammatory agents, it is meaningful to compare the histopathological changes using skin biopsies from the lesions of experimental animal models of skin inflammation.

Known as anti-inflammatory agents, plant flavonoids have been used for skin inflammatory diseases from ancient time as major constituents of plant extracts such as Scutellaria radix and Chamomilla extract. The major types of flavonoids are baicalein, wogonin and their glycosides in Scutellaria radix, while apigenin and luteolin are predominant in the latter. Various flavonoids were previously demonstrated to possess anti-inflammatory activity *in vitro* and *in vivo* (Middleton *et al.*, 2000; Kim *et al.*, 2004). Among the flavonoid derivatives examined so far, wogonin (Fig. 1) has been found to be the most potent in suppressing cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expression from bacterial lipopolysaccharide (LPS)/cytokine-treated mouse macrophages or macrophage-like cell line such as RAW 264.7 cells (Wakabayashi, 1999; Chi *et al.*, 2001). This compound was also proved to suppress COX-2 induction and reduce prostaglandin E_2 production on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-treated mouse skin by topical application (Park *et al.*, 2001). A further study revealed that wogonin potently inhibited COX-2 and tumor necrosis factor-α expression with less effect

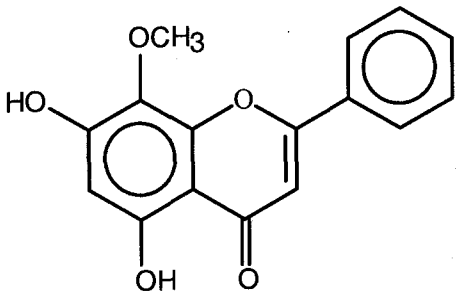

Fig. 1. Chemical structure of wogonin (5,7-dihydroxy-8-methoxyflavone).

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on intercellular adhesion molecule-1 and interleukin-1β expression in a sub-chronic skin inflammation model provoked by multiple TPA treatment (Chi *et al.*, 2003). These previous investigations strongly suggest that wogonin may have a potential for new topical anti-inflammatory agent against skin inflammation through the novel mechanism of suppressing proinflammatory gene expression.

The purposes of present investigation were to evaluate histopathological changes of the lesions from different types of experimental skin inflammation, and to find potential therapeutic effect of topically applied wogonin as a model flavonoid.

MATERIALS AND METHODS

Chemicals

Phenol, TPA and hematoxylin and eosin (H&E) staining solution were obtained from Sigma-Aldrich Co. (St. Louis, MO). Picryl chloride was a product of Nacalai Tesque Inc. (Japan) and prednisolone was purchased from Upjohn Co. (Kalamazoo, MI). Wogonin was isolated from the methanol extract of Scutellaria radix according to the previously described procedure (You *et al.*, 1999). The purity of wogonin was determined by HPLC analysis and proved to be > 95% (w/w).

Animals

Male specific pathogen-free ICR mice (18-22 g) were obtained from Orient Co. (Emsung, Korea). Animals were maintained and acclimatized with laboratory chow (Purina Korea) and water *ad libitum*, at least 7 days prior to experiments under the conditions of 21±1°C, 40-60% relative humidity and 12-h/12-h (light/dark) cycle.

Animal models of skin inflammation and treatment of wogonin

Phenol-induced contact dermatitis was used for an animal model of acute skin irritation (Wille *et al.*, 1998). Briefly, 10% phenol in acetone (20 μl) was topically smeared on the inner and outer surfaces of right ear of a mouse (five mice/group). Test compounds in an oil-based vehicle (20 μl/ear) were topically applied to the same site 5 min after phenol treatment, while control group received the same amount of vehicle. Two hours later after phenol treatment, animals were sacrificed by cervical dislocation. Ears were excised.

For a subchronic model of skin inflammation, TPA-induced dermatitis (multiple treatment, 3-days) was employed. On the day of experiment (day 1), TPA (3 μg/20 ml acetone) was applied to mouse ear according to the previously described (Chi *et al.*, 2003). Test compounds were topically applied to the same site (20 μl/ear) at 1 and 12 h after TPA treatment. Control group received TPA and vehicle. On next day, same treatment regimen was carried out with TPA and test compounds. On day 3, TPA was applied, and one hour later, test compounds were treated. Ears were removed after five hours.

For inducing delayed type hypersensitivity reaction, hair of abdomen of mice was cut and 7% picryl chloride in acetone (100 μl/mouse) was smeared to sensitize the animals (Tarayre *et al.*, 1990). Seven days later, delayed hypersensitivity was induced by application of 1% picryl chloride in acetone (20 μl/ear) to the right ears of the sensitized mice. After 24 h, ear biopsies were obtained. Test compounds in vehicle (20 μl/ear) were applied to the right ears of mice 1 h after initial treatment with sensitizer or acetone. The same amounts of test compounds in vehicle (20 μl/ear) were treated again on the same site 1 h after final picryl chloride treatment.

Histology and measurement of ear thickness

Three randomly selected ear samples per group were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned and stained with H&E. Ear thickness was measured with micrometer under microscope and ranges in mm from fifteen randomly selected sites from three ear samples were represented.

RESULTS

Histological samples were prepared from ear biopsies (Fig. 2) and ear thickness of the intact lesions was measured under the microscope (Table I). As expected, ear thickness of inflammagen/allergen-treated groups increased at least twice. Especially, multiple TPA treatment for 3 days provoked a highest increase of ear thickness. Wogonin and prednisolone exhibited similar pattern of inhibition of ear edematic response. They showed considerable inhibition against TPA- and picryl chloride-induced ear edema, while only a weak reduction was observed against phenol-induced ear edema.

When pathological changes were compared in these histological samples, it was found that phenol treatment produced profound increase of ear thickness, mostly in the dermal area (2a and 2b in Fig. 2). Epidermal hyperplasia and infiltration of inflammatory cells to the lesion were hardly observed, indicating that phenol treatment induced edematic inflammation

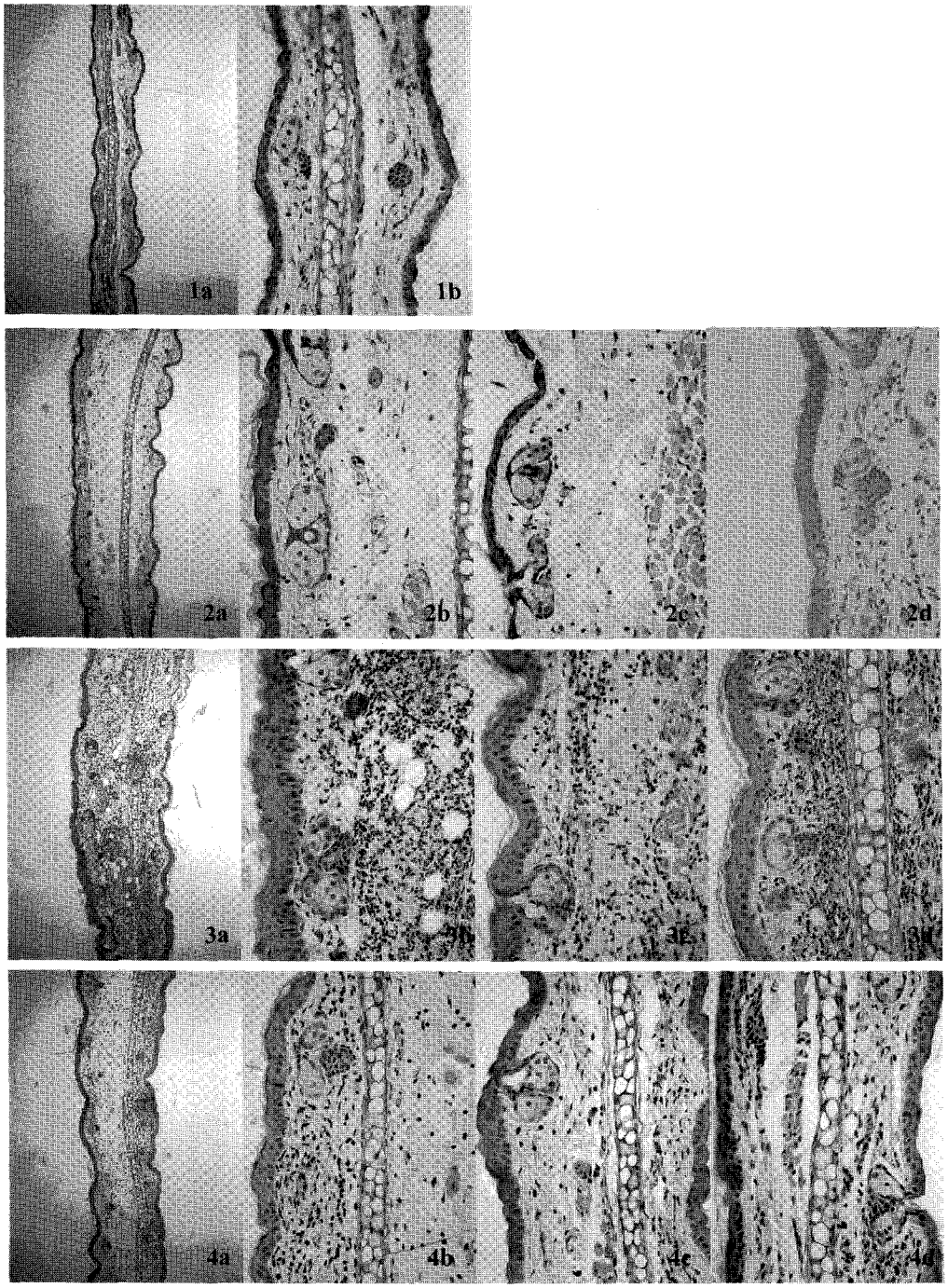

Fig. 2. Representative photomicrographs of H&E stained mouse ear cross sections. (1) No treatment, (2) phenol-induced dermatitis, (3) multiple TPA-induced dermatitis, (4) picryl chloride-induced delayed hypersensitivity, (a) (×100), (b) (×400), (c) wogonin-treated (×400), (d) prednisolone-treated (×400). Note: Phenol-treated dermatitis induced a simple dermal edema while TPA treatment and picryl chloride-treated delayed hypersensitivity induced epidermal hyperplasia and infiltration of inflammatory cells in the lesions.

mainly by exudating fluid. On the other hand, multiple TPA treatment for 3 days resulted in the characteristic epidermal hyperplasia (3a and 3b). The number of epidermal keratinocyte layer increased to 2 - 4 from 1 - 2 layers in the nontreated control. Moreover, massive infiltration of neutrophils to the lesion was marked, accompanied with ear thickness increase. In the case of delayed hypersensitivity reaction, slight epidermal hyperplasia was observed (1 - 3 layers) with edematic response

Table I. Ear thickness of histological biopsies

Group	Ear thickness (mm)[a]
No treatment	0.10 - 0.20
Phenol	0.25 - 0.47
Phenol + wogonin[b]	0.20 - 0.50
Phenol + prednisolone[b]	0.25 - 0.45
TPA	0.30 - 0.60
TPA + wogonin	0.20 - 0.45
TPA + prednisolone	0.22 - 0.40
Picryl chloride	0.25 - 0.45
Picryl chloride + wogonin	0.20 - 0.35
Picryl chloride + prednisolone	0.15 - 0.35

[a]Ear thickness was measured by micrometer under microscope. The represented data are the ranges (mm) from fifteen randomly selected sites of three histological samples per group.
[b]Wogonin was treated at 200 μg/ear/treatment and prednisolone was applied at 50 μg/ear/treatment.

in 24 h after elicitation with picryl chloride (4a and 4b). In contrast to the lesion of TPA-treatment, the inflammatory cells infiltrated were mixtures of polymorphonuclear leukocytes and monocytes/lymphocytes. Under these conditions, wogonin more or less inhibited inflammatory response of skin inflammation when topically applied (2c - 4c). This compound considerably inhibited epidermal hyperplasia as well as neutrophil infiltration in TPA-induced dermatitis, while almost no significant pathological change was seen in phenol-induced dermatitis. Wogonin also inhibited inflammatory cell infiltration in picryl chloride-induced delayed hypersensitivity. Prednisolone showed similar pattern of inhibition with wogonin against these three animal models of skin inflammation.

DISCUSSION

The present investigation has clearly shown the histopathological changes of animal models of skin inflammation and anti-inflammatory activity of wogonin. Depending on animal models of skin inflammation and the end-point time used to measure the response, there were considerable differences in the nature and characteristics of skin inflammation. For example, phenol treatment produced simple irritation reaction in the skin, peaking at approximately 2 h, while delayed hypersensitivity gave maximum edema at 24 h after elicitation with picryl chloride (Lim *et al.*, 2004). Multiple TPA treatment, on the other hand, gradually increased ear edematic response and daily treatment for three consecutive days produced considerable increase. Our results were well correlated with the previous findings that multiple TPA treatment led to the epidermal hyperplasia as well as ear edema (Park *et al.*, 2001; Giannaras *et al.*, 2005). In this study, all treatment schedules of wogonin and prednisolone were to find curative effect, so that test compounds were applied after inflammagen/allergen was treated.

Under the same experimental conditions, wogonin was previously reported to inhibit phenol-induced ear edema only slightly, while stronger inhibition was observed against multiple TPA-treated inflammation and delayed hypersensitivity (Chi *et al.*, 2003; Lim *et al.*, 2004). These previous findings were well correlated with the present study clearly demonstrating that the topical application of wogonin considerably inhibited pathological changes of TPA-induced inflammation and delayed type hypersensitivity, but not those of phenol-induced skin inflammation. And these results suggest that wogonin may be more favorable to treat subchronic and allergic skin inflammatory disorders, but not to treat acute skin inflammation.

From the present study, it is worthy to mention that the patterns of inhibition of pathological changes by wogonin and prednisolone were found to be very similar. However, the cellular action mechanisms showing anti-inflammatory activity by these agents are quite different. Our previous study revealed that prednisolone lost its suppressive activity on iNOS induction from LPS-induced RAW 264.7 cells by the addition of a steroid receptor antagonist (Ru-486), but wogonin did not (Chi *et al.*, 2001). This finding and others (Goppelt-Struebe, 1997) indicate that prednisolone suppresses proinflammatory gene expression by inhibition of transcription factor activation partly via a steroid-receptor mediated process. On the other hand, flavonoids including wogonin, luteolin and quercetin may reduce proinflammatory gene expression by inhibition of transcription factor activation probably via inhibition of protein kinases involved in the signal transduction pathway (Chang *et al.*, 2001; Xagorari *et al.*, 2002; Wadsworth *et al.*, 2001). Thus, it is expected that wogonin may not show steroid-like side effects in human use. Wogonin has a potential as new anti-inflammatory agent against skin inflammatory disorders.

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