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Anti-inflammatory effect of external use of escin on cutaneous inflammation: possible involvement of glucocorticoids receptor

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[ABSTRACT] Escin, as an internally applied anti-inflammatory agent, has been widely used in the treatment of inflammation and edema resulting from trauma or operation in the clinic. However, the effect of its external use on cutaneous inflammation and edema remains unexplored. In the present study, the anti-inflammatory and anti-edematous effects of external use of escin were studied in carrageenan-induced paw edema and histamine-induced capillary permeability in rats, paraxylene-induced ear swelling in mice, and cotton pellet-induced granuloma in rats. Effects of external use of escin gel on prostaglandin E2 (PGE2), tumor necrosis factor- α (TNF- α), and interleukin-1 β (IL-1 β) were determined by ELISA. The anti-inflammatory mechanism was explored by detecting the expression of glucocorticoid receptor (GR) with Western blotting and Real-time PCR analyses, with further exploration of nuclear factor- κ B (NF- κ B), p38 mitogen-activated protein kinase (P38MAPK) and activator protein-1 (AP-1) expressions. We demonstrated that external use of escin showed significant anti-inflammatory effects on acute and chronic inflammation in different animal models and its anti-inflammatory effects by promoting the expression of GR, with the possible mechanism being inhibition of the expressions of GR-related signaling molecules such as NF- κ B and AP-1.

[KEY WORDS] Escin; Cutaneous inflammation; Glucocorticoid; Nuclear factor-κB; Activator protein-1

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Introduction

Escin, extracted from the seeds of Aesculus *wilsonii Rehd*, is a natural mixture of triterpene saponins ^[1]. Accumulating researches ^[2-4] have shown that escin delivered by intravenous administration exerts potent anti-inflammatory and anti-edematous effects. For example, Jiang *et al.* ^[5] have found that escin has protective effect on liver injury induced

by endotoxin, and the underlying mechanisms are associated with its anti-inflammatory and anti-oxidation effects. Another research ^[6] has suggested that escin might have potent protective effect on Lipopolysaccharides-induced acute lung injury through inhibiting the inflammatory response. Furthermore, oral and intravenous administrations of escin show therapeutic effects in the murine models of allergic inflammation and dermatitis ^[7]. However, intravenous administration of escin is reported to lead to phlebitis, allergic reaction, local swelling, and other serious adverse reactions ^[8]. Theoretically, external use would help reduce the risk and severity of adverse drug reactions, but study on the anti-inflammatory effect of external use of escin is still rare.

Acute or chronic skin inflammation such as eczema and psoriasis is a common inflammatory disease, which significantly affects the quality of patients' life and has considerable



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socioeconomic impact ^[9]. The etiologies for skin inflammation are complex, including gene mutation, environmental triggering factors, skin barrier defects, and immune dysfunction ^[10]. Glucocorticoids (GCs), as a traditional anti-inflammatory drug, are used in skin inflammation, but long-term application may be associated with serious adverse effects, such as immune inhibition ^[11], and increasing risk of cardiovascular diseases ^[12]. In the present study, we investigated the anti-inflammatory effect of escin gel, a topical preparation of escin, on cutaneous inflammation in various animal models, including carrageenan-induced paw edema and histamine-induced vascular permeability in rats, paraxylene-induced ear swelling in mice, and cotton pellet-induced granuloma in rats. The mechanisms of anti-inflammatory effects of external use of escin were investigated by detecting the expression of inflammation-related molecules in the skin tissues, including GR, p38MAPK, NF-*k*B, and AP-1.

Material and Methods

Animals

Specific pathogen free (SPF) grade Sprague Dawley (SD) rats (weighing 180–220 g) and Kunming mice (weighing 18–22 g) were purchased from the Laboratory Animal Center of Hubei Province (Wuhan, China). Animal experimental procedures were approved by the Laboratory Animal Ethical Committee of Wuhan University of Science and Technology (approval Number: 2015-60, approved on 1st Mar, 2015). All the animals were housed in the lab of temperature 24 ± 1 °C, humidity of 55% to 70% and kept by the accredited laboratory animal breeder. Free access to food and tap water were allowed.

Chemicals

Escin musk ointment gels (Escin gel, 20%) were supplied by Ma Ying Long Pharmaceutical Co., Ltd. (batch No. 130515, Wuhan, China) and stored in a freezer at -20 °C. Dexamethasone (10%) acetate ointment was supplied by Fujian Sanming Thai Pharmaceutical Co., Ltd. (batch No. 20130312, Fujian, China). Rabbit anti-GAPDH antibody was obtained from Tianjin Sanjian Pharmaceutical Co., Ltd. (batch No. KM9002, Tianjin, China). Rabbit anti-GR antibody was purchased from Cell Signaling Technology Co., Ltd. (batch No. 3660. Shanghai, China). Rabbit anti-P38 antibody was purchased from Epitomics (batch No. 1544-S, Hangzhou, China). Rabbit anti-p65 (NF- κ B) and c-jun (AP-1) antibodies were purchased from Santa Cruz Bio, Inc. (batch No. 10745; sc-1694, California, USA). The Goat-anti-mouse IgG secondary antibody [HRP (Horseradish Peroxidase) and Goat-antirabbit IgG secondary antibody [HRP (Horseradish Peroxidase)] were purchased from Abcam (Shanghai, China). PrimeScript™RT Master Mix Kit and SYBR®Premix Ex Taq™ (Tli RNaseH Plus) Kit were purchased from Takara Bio Co., Ltd. (batch Nos. RR036A, RR420A, Dalian, China). ELISA kits for PGE2 were obtained from Jianglai Biotechnology Co., Ltd. (Cat: HZ93761, Shanghai, China), and ELISA kits for TNF- α

and IL-1 β were both obtained from Lianke Biotechnology Co., Ltd. (Cat: EK382P for TNF- α and EK301BP for IL-1 β , Wuhan, China).

Carrageenan-Induced paw edema in rats

The paw edema model induced by carrageenan in rats was established according to Yuan *et al.* ^[1] with some modifications. Briefly, 50 rats were randomly divided into 5 groups (n = 10 per group) and pre-treated with either blank gel or corresponding drugs (escin 0.01, 0.02, and 0.04 g·kg⁻¹ or Dexamethasone 0.04 g·kg⁻¹ of body weight) *via* dermal administration once a day, for three days. Paw edema was induced by subcutaneously injection of 0.1 mL of 1% carrageenan solution 1 h after the last drug administration, followed by another drug administration immediately. Paw volume was measured with water volume method at 1 h before and at 1 and 4 h after carrageenan injection. For each animal, the swelling of edema was expressed as the increase in paw volume after carrageenan injection.

Paraxylene-induced ear swelling in mice

One hundred Kunming mice were randomly divided into 5 groups (n = 20 per group) and pre-treated with the same drugs as described above *via* dermal administration on the ears once a day, for three days. Ear swelling was induced by external daubing paraxylene 0.03 mL on right ear 1 h after the last drug administration. 30 min later, the mice were treated with corresponding drug again, and sacrificed at 3 h after paraxylene administration. Tissue samples were obtained from the same 8-mm regions of right and left ears, punched by diameter. The auricle swelling was expressed by the increased weight of right ear by subtracting the left ear.

Histamine-induced capillary permeability in rats

Analysis of capillary permeability induced by histamine in rats was performed according to Olajide's method with some modifications ^[13]. In brief, 80 rats of male and female were randomly divided into 5 groups (n = 8 each sex per group) and pre-treated with the same drugs as described above after the hairs in an area of $1.5 \text{ cm} \times 1.5 \text{ cm}$ being removed with sodium sulphide (8%). Vascular permeability test was initiated by subcutaneous injection of 0.05 mL freshly-prepared 0.01% histamine phosphate in 0.9% normal saline solution. Subsequently, the rats received external administration of corresponding drugs, followed by intravenously injected with 0.05 mL of 1% Evans blue in saline solution via a tail vein. The rats were decapitated at 30 min after the last administration. Blue stained tissue samples were cut down immediately and soaked in the solution of acetonesaline (7:3) for 48 h, and the OD₆₁₀ of leachate was detected. Cotton pellet granuloma in rats

The cotton pellet granuloma model was developed according to the method of Olajide's with some modifications ^[14]. In brief, cotton pellet granuloma was induced by intraperitoneal implantation of a sterilized cotton pellet weighing 10 mg in the groin region of each rat. On the second day, 80 male rats were randomly divided into 5 groups (n = 16 per group):



a vehicle group, a dexamethasone $(0.04 \text{ g}\cdot\text{kg}^{-1})$ group, and three escin (0.01, 0.02, and 0.04 $g \cdot kg^{-1}$) groups. Dermal use of corresponding drugs at the operating region once a day was continued for seven days. The pellets were removed at 3 h after the last administration, freed from extraneous tissues, dried overnight at 60 °C and weighed. The results were expressed as the net weight of granuloma in 100 gram of body weight. ELISA for PGE2, TNF- α , and IL-1 β analyses

The levels of PGE2, TNF- α and IL-1 β were determined according to LI's method ^[1]. In brief, the tissue samples were obtained from paw edema and capillary permeability models, followed by homogenizing with ice-cold saline for a 10%

(W/V) homogenate. The ELISA kits of PGE2 (Jianglai **Bio, Shanghai, China**), TNF- α (Lianke Bio, Wuhan, China) and IL-1 β (Lianke Bio, Wuhan, China) were use, according to manufacturer's instructions.

Real-time PCR

The tissue samples were obtained from paw edema and capillary permeability models and stored at a -80 °C freezer until analysis. The skin tissues were homogenized in TRIzol reagent (Ambin, Texas, USA), and centrifuged at 14 000 r·min⁻¹ for 15 min at 4 °C. Total RNA was extracted by chloroform and isopropanolanl, followed by reverse transcription to generate first-strand cDNAs using the PrimeScript™RT Master Mix Kit (Takara Co., Ltd., Dalian, China), according to the manufacturer's instructions. The primer sequences for GR, P38MAPK, NF- κ B and β -actin used in the present study were as follows: GR: F-5'-TAC CAC AGC TCA CCC CTA CC-3' and R-5'-AGC AGG GTC ATT TGG TCA TC-3'; P38MAPK: F-5'-GGT TTT GGA CTC GGA TAA GA-3' and R-5'-GTG GGA TGG ACA GAA CAG AAG-3'; NF-kB: F-5'-GGT GGA GTT TGG GAA GGA TTT G-3' and R-5'-TTT TCT CCG AAG CTG AAC AAA CAC-3'; and β -actin: F-5'-GAG GGA AAT CGT GCG TGA C-3' and R-5'-CTG GAA GGT GGA CAG TGA G-3'. Real Time PCR analysis was performed using SYBR®Premix Ex Taq™ (Tli RNaseH Plus) Kit (Takara Co., Ltd.), according to the manufacturer's instructions. The cycle threshold (Ct) values of the target genes were normalized to that of β -actin from the same sample. And the relative differences between the groups were calculated and expressed as relative increase. Each sample was tested in triplicate.

Western blotting analysis

The tissue samples were obtained from paw edema and capillary permeability models, and stored at -80 °C freezer until analysis. The expression of corresponding proteins was analyzed by Western blotting assay as described previously ^[15]. In brief, the skin tissues were homogenized in protein extraction reagent (Thermo Scientific, Massachusetts, USA). The samples with 50 µg of total protein were separated using 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels. The blots were subsequently transferred to nitrocellulose membranes, followed by incubating in blocking buffer (5% skimmed milk in 0.1% Tween 20/Tris-buffered saline solution) for 1 h. Further incubations with primary antibodies, dilution for anti-GAPDH antibody (Sanjian Pharmaceutical Co., Ltd., batch No. KM9002, Tianjin, China), was 1 : 10 000; dilution for other antibodies, GR antibody (Cell Signaling Technology Co., Ltd., batch No. 3660, Shanghai, China), P38MAPK antibody (Epitomics, batch No. 1544-S, Hangzhou, China), p65 (NF-κB) and c-jun (AP-1) antibodies (Santa Cruz Bio, Inc., batch No, 10745; sc-1694, California, USA) were 1 : 1 000; and secondary antibody (dilution, 1:10 000) were conducted in the antibody dilution (0.2% skimmed milk in 0.1% Tween 20/Tris-buffered saline solution) for 2 h. Immunoreactive bands were detected using enhanced chemiluminescence (ECL) and analyzed with image J (National Institutes of Health, USA). The results were expressed as relative densitometric units of the corresponding GAPDH control.

Statistical analysis

All the assay values are given as means \pm SD. Differences between the groups were compared with analysis of variance (ANOVA). A value of P < 0.05 was considered statistically significant.

Results

External use of escin inhibits carrageenan-induced hind paw edema in rats

Carrageenan intraplantar injection led to a time-dependent development of inflammation which peaked within 2-3 h and lasted for the next 6-8 h [16]. As shown in Fig. 1A, escin administration caused a significant and dose-related inhibition in paw edema. In the model control animals, intraplantar injections of carrageenan provoked an edema response that was more pronounced at 4 h. Paw edema was found significantly less in the rats that received 0.02 and 0.04 $g kg^{-1}$ of escin, with the inhibition rates being 33.7% and 37.2% at 1 h and 32.9%, and 36.1% at 4 h, respectively. Dexamethasone pre-treatment demonstrated greater inhibition on paw edema at both times with the inhibition 41.3% and 38%, respectively. The treatment with 0.01 g·kg⁻¹ of escin exerted significantly inhibitory effect until 4 h only.

External use of escin inhibits xylene induced ear edema in mice

The ear edema model is recognized as the assessment of anti-inflammatory steroids and has less sensitivity to non-steroidal anti-inflammatory agents ^[17]. External administration of escin (0.01, 0.02, and 0.04 $g \cdot kg^{-1}$), dose-dependently inhibited xylene induced ear edema in mice (Fig. 1B). The inhibitory effect produced by escin was significant at all doses with the inhibition rates being 38.7%, 42.1%, and 46.5%, respectively, greater than that produced by dexamethasone (27.0%).

External use of escin inhibits increased skin vascular permeability induced by injection of histamine

Capillary permeability model is usually used to study the drug effects on inflammatory permeability ^[14]. The effect of test drugs on vascular permeability in this experiment was expressed as optical density at 610 nm (OD_{610}). As shown in Fig. 1C, escin exhibited a dose-dependent decrease in OD_{610} , and the effects at the doses of 0.02 and 0.04 g·kg⁻¹ were significant, showing 23.8% and 27.3% inhibition, respectively, compared to that of the model control group. Treatment with dexamethasone caused a significant decrease in OD_{610} with an inhibition rate of 30.5%, greater than that produced by escin.

External use of escin inhibits cotton pellet induced granuloma

The cotton pellet-induced granuloma is widely used to assess model of the sub-chronic anti-inflammatory activity ^[18]. Our results from the present study revealed that external use of escin inhibited granulomatous phase of inflammation, as escin at 0.02 and 0.04 g·kg⁻¹ showed 23.8% and 28.8% inhibition of granuloma, although less effective than the inhibition (35.5%) induced by dexamethasone (Fig. 1D).



Fig. 1 Anti-inflammatory effects of escin *in vivo*. A: effects of escin on paw edema in rats; B: effects of escin on ear edema in mice; C: effects of escin on capillary permeability in rats; D: effects of escin on cotton granuloma in rats. Model: the group treated with blank gel; Dex: the reference drug group treated with Dexamethasone acetate cream. *P < 0.05 and **P < 0.01 vs model group



Fig. 2 Effects of escin on PGE2, TNF- α , and IL-1 β levels following intraplantar injection of carrageenan and histamine in rats. A1, B1, C1: PGE2, TNF- α , and IL-1 β levels in tissues of carrageenan induced paw edema in rats; A2, B2, C2: PGE2, TNF- α and IL-1 β levels in tissues of histamine induced vascular permeability in rats. [#]P < 0.05, ^{##}P < 0.01 vs normal control; ^{*}P < 0.05 vs model control



Escin gel reduces the levels of inflammatory factors

As modulators of inflammation, PGs have a major role in the early phase of inflammatory progress ^[19]. Previous studies have concluded that TNF- α and IL-1 β , major products of inflammation cells, contribute to the progression of inflammation ^[11]. In the present study, we analyzed the levels of PGE2, TNF- α , and IL-1 β to investigate whether external use of escin can affect the release of inflammatory factors. As shown in Fig. 2, both carrageenan and histamine injections significantly increased the levels of PGE2, TNF- α , and IL-1 β . Escin gel treatment dose-dependently decreased the PGE2 level. Similarly, escin gel treatment also induced a decline on TNF- α and IL-1 β levels, but only 0.04 g·kg⁻¹ of escin caused significant decrease, compared to the model control treatment. *GR is involved in the anti-inflammatory effect of escin gel*

GCs are widely used to treat both acute and chronic inflammation ^[11] which exert the anti-inflammatory effect by binding to glucocorticoid receptor (GR) and forming GC-GR complex. It's reported that escin injection can increase GR expression in the lungs, which lead to a potent protective effect on acute lung injury induced by endotoxin [6]. To investigate the potential involvement of GR in the anti-inflammatory progress of cutaneous use of escin, we detected the expression of GR in the skin from paw edema model and capillary permeability model in rats. As shown in Fig. 3, the gene expression of GR was significantly decreased after treatment with blank gel in both model groups, and escin treatment increased GR level obviously at the doses of 0.02 and $0.04 \text{ g}\cdot\text{kg}^{-1}$. The effects of escin in both models were almost the same. Similarly, the protein expression of GR (Fig. 4) showed a consistent trend with its gene expression. By detecting the expression of GR, we confirmed the involvement of GR in the inflammation progress and in the anti-inflammation progress of escin.



Fig. 3 Effects of escin on gene expression of GR in inflammatory tissues. A: Gene expression in the edematous tissue of paw edema in rats, relative to the β -actin; B: Gene expression in the inflammatory tissue of capillary permeability in rats, relative to the β -actin. The results are presented as means \pm standard error of the mean of three independent experiments. **P < 0.01, *P < 0.05 vs model group; ##P < 0.01, #P < 0.05 vs the normal control group



Fig. 4 Effects of escin on protein expression of GR in inflammatory tissues. A: GR expression in rat hind paw; B: Relative expression of GR in paw tissue, normalized to the GAPDH. C: GR expression in rat back skin; D: Relative expression of GR in capillary permeability skin, normalized to GAPDH. $^{#}P < 0.05$ vs normal control, $^{*}P < 0.05$ and $^{**}P < 0.01$ vs model control



Escin gel regulates the signaling molecules of GR

The anti-inflammatory effect of GR is partly mediated by mutual interference with transcription factors, such as NF- κ B and AP-1 ^[20], which appeal attention with regulation on the transcription of inflammatory mediators and cytokines ^[21]. P38MAPK, upstream the GR, could induce or activate other transcription factors that might sequester critical coactivator(s)

required for GR transcriptional activation, leading to inhibition of GR function by regulatory squelching ^[22]. In the present study, we further detected the signaling molecules to study the possible pathways by which escin exerted its anti-inflammatory effects. The gene expression of P38MAPK and NF- κ B was analyzed by Real-time PCR and protein expression of P38MAPK, NF- κ B, and AP-1 was analyzed by



Fig. 5 Effects of escin on gene expression of P38MAPK and NF-*k*B in inflammatory tissue. A: gene expression in the edematous tissue of paw edema in rats, relative to the β -actin; B: gene expression in capillary permeability tissues in rats, relative to the β -actin. The results are presented as means \pm standard error of the mean of three independent experiments. **P < 0.01, *P < 0.05 vs model group; ##P < 0.01, #P < 0.05 vs the normal control group



Fig. 6 Effect of escin on protein expression of P38MAPK, NF- κ B and AP-1 following intraplantar injection of carrageenan in rat hind paw. A: protein expressions of P38MAPK, NF- κ B and AP-1, relative to the GAPDH control. B: relative content of P38MAPK, NF- κ B and AP-1, normalized to GAPDH. A criterion of significance was accepted and marked as [#]P < 0.05 vs normal control; ^{*}P < 0.05 and ^{**}P < 0.01 vs model control



Fig. 7 Effects of escin on protein expression of P38MAPK, NF- κ B and AP-1 following intraplantar injection of histamine in rat back skin. A: protein expressions of P38MAPK, NF- κ B and AP-1, relative to the GAPDH control. B: relative content of P38MAPK, NF- κ B and AP-1, normalized to GAPDH. A criterion of significance was accepted and marked as [#]P < 0.05 vs normal control; ^{*}P < 0.05 and ^{**}P < 0.01 vs model control

Western blotting (Figs. 5, 6 and 7). Compared with the normal group, the two model groups both showed increased expression of NF- κ B, P38MAPK on the mRNA level, increased expression of NF- κ B, P38MAPK and AP-1 on the protein level. Treatment with escin (0.02 and 0.04 g·kg⁻¹) significantly inhibited the expressions of NF- κ B and AP-1 (P < 0.05 or P < 0.01). Similarly, the mRNA expression of NF- κ B was significantly declined after escin (0.02 and 0.04 g·kg⁻¹) administration (P < 0.05 or P < 0.01). However, no obvious change in both gene and protein expression of P38MAPK was observed after escin administration at all doses. These results indicated that the possible mechanism of escin was to inhibit NF- κ B and AP-1 signaling pathways.

Discussion

The present study aimed at investigating the effect of external use of escin, a traditional Chinese medicine, on inflammation. Previous studies have demonstrated that that oral administration and injection of escin significantly reduce inflammation responses ^[1, 3, 23]. However, no study has looked at the effect of external administration of escin. In the present study, we selected four classic animal models to induce acute and chronic inflammation, including carrageenan-induced hind paw edema in rats, paraxylene-induced ear swelling in mice, histamine-induced capillary permeability in rats, and cotton pellet induced granuloma in rats. Using these models, we demonstrated that escin, given externally, had dose-related effects on acute inflammation induced by carrageenan and xylene in rats. The vascular reaction is the central part of the inflammatory process which is the main cause of local exudation of liquid, protein, and cells ^[24]. In the present study, upon cutaneous administration of escin at the doses of 0.02 and $0.04 \text{ g} \cdot \text{kg}^{-1}$, the vascular permeability was significantly ameliorated. Similar effect was also observed in the granuloma models, which has been widely used to evaluate sub-chronic inflammation^[18]. Surprisingly, escin exhibited better efficacy than dexamethasone in the two models.

TNF- α and IL-1 β , the typical inflammatory cytokines, play key roles in the induction and maintenance of inflammation by up-regulating other pro-inflammatory cytokines such as IL-6 ^[25]. Prostaglandin E2 is found to be one of the most important biologically active prostaglandins in promoting cell proliferation and vasodilation ^[26], which are the typical pathological change in the early phase of acute inflammation. In the present study, the level of PGE2 was declined with escin gel treatment, indicating that escin gel may exert anti-exudative, anti-proliferative effect at least in part by down-regulating the release of PGE2. Further, we found that escin treatment decreased the content of TNF- α and IL-1 β in the inflammatory tissues. And these results were consistent with previous study that escin has little effect on the immune cells and their functions ^[3].

From the pharmacodynamic experiments *in vivo*, we could conclude that external use of escin could attenuate experimental cutaneous inflammation and the effect might be related to down-

regulation of PGE2, TNF- α , and IL-1 β directly or indirectly.

A previous study ^[27] has indicated that epidermal loss of GR provokes skin barrier defects and cutaneous inflammation. Escin is reported to have effect of increasing GR expression in lungs ^[6] and exert synergistic anti-inflammatory effect when combined with GCs ^[23]. It is thought-provoking whether escin exerts effects by increasing GR expression in skin. In the present study, we first demonstrated that GR was at least partly involved in the anti-inflammatory effect of escin, as GR expression was decreased at both gene and protein levels by subcutaneous injection of histamine and carrageenan, but increased, even higher than the normal level, by treating with escin or Dex.

Accumulating studies have indicated that several signaling molecules exist in up or down stream of GR, including NF- κ B, AP-1, and P38MAPK^[28-29]. NF-*k*B mediates the transcription and activation of many cytokine genes and cytokine-induced genes, which are involved in immune and inflammatory responses ^[13]. The signaling pathway of P38MAPK is involved in numerous pro-inflammatory cytokines and plays an important role in various inflammatory responses ^[29]. To explore the relative signaling pathways of GR that may be involved in the anti-inflammatory mechanism of escin, we further investigated the expression of NF-kB and P38MAPK in the inflammatory tissues. The results showed that treatment with escin significantly inhibited the expression of NF- κ B at both protein and gene levels, which indicated that escin might exert anti-inflammatory effect by inhibiting the expression of NF- κ B. However, no significant change was found in the expression of P38MAPK after treatment with escin, which at least confirmed that escin and dexamethasone increase expression of GR was not directly by suppressing P38MAPK expression and function.

The expression of inflammatory mediators and cytokines are also regulated by AP-1 at the transcription level ^[21]. With AP-1 and NF- κ B playing key roles in the inflammation and immune responses, the pathways to these transcription factors have caught most attention [30]. In the present study, we further analyzed the AP-1 expression with Western blotting analysis and found that the level of AP-1 was declined by treatment with escin in both paw edema and capillary permeability models, possibly because that escin inhibited the expression of AP-1 to ameliorate symptoms and responses of inflammation. Taken together, escin exerted anti-inflammatory effect possibly by increasing GR expression, and its mechanism might be related to the inhibition of GR-related signaling molecules expression of NF- κ B and AP-1, but not P38MAPK. This result also explained the inhibitory effect of escin on TNF- α , IL-1 β , and PGE2.

Conclusion

In the present study, we studied the anti-inflammatory, anti-edematous, anti-swelling, and anti-exudative effects of external use of escin, and preliminarily studied the mechanism of anti-inflammatory effect of escin. The results showed that escin, given external, exerted potent anti-inflammatory effect in various animal models and decreased the level of TNF- α and IL-1 β . Its mechanism was indicated to be through up-regulating the expression of GR and inhibiting the expression of GR-related signaling molecules, such as NF- κ B and AP-1.

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