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ScienceDirect
Chinese Journal of Natural Medicines 2018, **16**(2): 0105–0112
Chinese Journal of Natural Medicines

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

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Available online 20 Feb., 2018

[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-1β (IL-1β) 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-κB (NF-κB), 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 might be related to down-regulation of PGE2, TNF-α, and IL-1β. The results also showed that escin exerted 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-κB and AP-1.

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

[CLC Number] R965 **[Document code]** A **[Article ID]** 2095-6975(2018)02-0105-08

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

[Received on] 14-May-2017
[Research funding] This work was supported by the National Natural Science Foundation of China (No. 81602108).
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These authors have no conflict of interest to declare.

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-κB, 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 ºC, 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 ºC. 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-κB) 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-anti-rabbit 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-1β were both obtained from Lianke Biotechnology Co., Ltd. (Cat: EK382P for TNF-α and EK301BP for IL-1β, 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 cm × 1.5 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 acetone−saline (7 : 3) for 48 h, and the OD_{610} 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):