

Escin Inhibits Type I Allergic Dermatitis in a Novel Porcine Model

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Key Words

Allergy · Type I hypersensitivity · Escin · Skin prick test · Pig · Compound 48/80 · Glucocorticoid receptor

Abstract

Background: Current standard medications for the treatment of allergic inflammation consist primarily of glucocorticoids and anti-histamines, but adverse side effects or insufficient responsiveness by patient subpopulations illustrate the need for safe and novel alternatives. Thus, there is a demand to develop a porcine model that is able to mimic mast cell-mediated type I hypersensitivity. Previously, we found that escin, a pharmacologically active mix of triterpene saponins from horse chestnut extracts, exerts anti-allergic effects in murine models and merits further investigation as an anti-allergic therapeutic. **Methods:** We developed a new porcine model of allergic dermatitis based on a clinical prick test protocol. Histamine clearly provoked erythema and swelling at the prick site, whereas the mast cell-degranulating compound 48/80 even more pronounced caused wheal and flare reactions known from the human prick response. This model was used to test the anti-allergic efficacy of orally applied escin. **Results:** Oral pretreatment of animals with escin strongly inhibited the allergic skin response induced

by compound 48/80 in a dose-dependent manner. Additional in vitro data from murine mast cells indicate an engagement of the glucocorticoid receptor pathway upon treatment with escin. **Conclusions:** This model provides a valuable and easy-to-set-up tool for preclinical studies of mast cell-inhibiting compounds. The successful implementation of this model supports the development of oral escin applications as a novel anti-allergic therapy.

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Introduction

Type I hypersensitivity is an increasing health threat in Western civilization [1]. It is characterized by excessive activation of immune cells, primarily mast cells, basophils, and T helper cells, in response to contact with diverse environmental agents, such as pollen, house dust mite excreta, or ingredients of food or cosmetics [2]. Fcε-receptors on mast cells or basophils are activated by cross-linking via allergens that are bound to IgE antibodies in sensitized individuals. Within minutes of allergen exposure, these activated cells release various preformed or rapidly synthesized mediators such as histamine and serotonin, which elicit vasodilation, increased vascular

permeability, edema, and acute functional changes in affected organs. Clinically, this early phase leads to edema, reddening, and pruritus in the case of skin reactions, or asthma attacks if airways are affected. Leukocytes are recruited into the affected tissues by mast cell-derived cytokines and chemokines, which accounts for the subsequent allergic inflammation [3–6].

Currently, glucocorticoids (GCs), anti-histamines, and mast cell stabilizers comprise the primary pharmacologic armamentarium. Although the benefit of these medications is indisputable, most of these treatments also cause substantial adverse side effects, particularly in the case of long-lasting and/or systemic applications [7–9]. Moreover, a subpopulation of asthmatic patients lacks or loses responsiveness to GCs [10]. Therefore, the development of novel anti-allergic drugs with a satisfactory safety and tolerability profile for long-term use is still a major challenge.

We previously described the anti-inflammatory and anti-allergic potential of the natural compound escin in murine models of allergy [11]. Escin, the main pharmacologically active component of horse chestnut (*Aesculus hippocastanum*) seed extracts, is a mixture of triterpene saponins (fig. 1). It has shown remarkable anti-edematous, anti-inflammatory, and venotonic properties in in vitro and in vivo settings, and is broadly used to treat chronic venous insufficiency (CVI). It has also been demonstrated to be effective in the therapeutic regimen against hemorrhoids, postoperative edemas, postoperative ileus, and acute impact injuries, with overall excellent tolerability [12–22]. Escin has also been investigated concerning its potential use in oncology [23–28].

In our studies, we found that escin has potent anti-allergic properties. We demonstrated its anti-allergic efficacy in models of passive cutaneous anaphylaxis and allergic airway inflammation after intraperitoneal application in mice [11], which indicated that escin treatment may prove useful in clinically relevant allergic conditions. To further evaluate this potential, we extended our studies to pigs as a species with immunological characteristics similar to those of humans. We tested the anti-allergic efficacy of escin after oral application, which is the clinically preferred route.

The skin of pigs shares many similarities with its human ortholog [29]. The use of pigs for an allergy model was previously described for experimentally induced allergic contact dermatitis, which is a model for primarily T cell-mediated type IV hypersensitivity [30]. The extensive characterization of this porcine model demonstrated the clinical and histological relevance for the human dis-

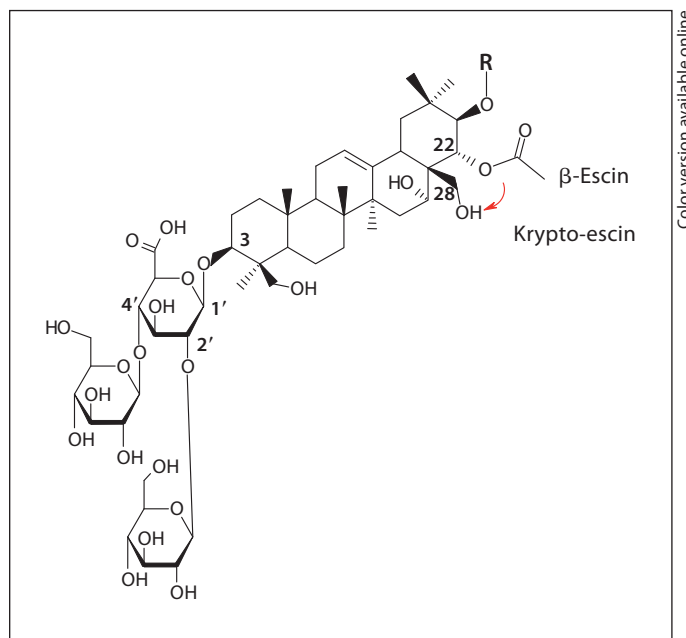


Fig. 1. Chemical structure of β -escin. β -Escin is a mixture of triterpene saponins isolated from horse chestnut seeds that varies with respect to the composition of the sugar moieties linked at C3 and the position indicated by 'R', which most frequently represents angelic or tiglic acid. The arrow indicates the frequent conversion of β -escin into krypto-escin by the migration of an acetyl-group from position C-22 to C-28. Both forms are known to be pharmaceutically active.

ease. However, a contribution of mast cells to the allergic reaction was not discernible in this model, and therefore it was not well suited for our interest in mast cell-mediated type I hypersensitivity. A porcine type I allergy model has not been reported so far. Therefore, we initiated the development of a porcine model that is representative of the acute response of a type I hypersensitivity reaction, such as a skin prick test. Skin prick tests are a standard means in human medicine to determine hypersensitivities to type I allergens. Exposure to small amounts of allergens on the site of the prick causes the activation of local mast cells and the release of inflammatory mediators. This leads to the extravasation of fluids, visible as wheal formation or swelling, and erythema of the surrounding skin tissue. The extent of swelling and erythema can be clinically scored and is proportional to the extent of the allergic reaction. This test is therefore useful not only to assess the allergy status of patients but also to test the efficacy of treatments that may suppress the allergic response.

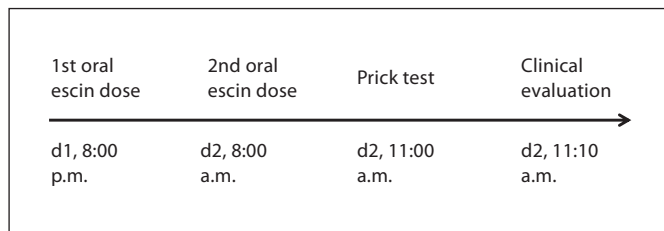


Fig. 2. Experimental protocol. Time line of the experimental protocol of the dose-response study. d1 = Day 1; d2 = day 2.

In this report, we describe the topical application of the mast cell degranulating substances histamine and compound 48/80, the former being used as a gold standard positive control in routine prick tests, as a novel model for mast cell-mediated hypersensitivity and the application of this model to demonstrate the anti-allergic efficacy of oral escin treatment.

Methods

Animals and Prick Test

All experimental procedures were discussed and approved by the institutional and national animal ethics committees and performed with Austrian experimental animal license numbers GZ 68.205/0015-II/10b/2010 and GZ 68.205/0119-II/10b/2010. Young pigs of the Large White breed with a mean body mass of 20 kg were used for the experiments. An equal gender distribution was maintained when animals were assigned to the different treatment groups. For the prick test, 25 μ l of histamine (histamine-HCl, 10 mg/ml; ALK-Abelló), compound 48/80 (10 mg/ml; Sigma), or negative control solution (0.9% NaCl solution) were applied on the shaved back, on an area starting caudal to the interscapular region and paramedian at a distance of 2 cm to the spine. At the application site of these solutions, the skin was pricked with a conventional prick lancet (ALK Lancet; ALK-Abelló). Two minutes later the residual solutions were wiped off. Ten and twenty minutes after pricking, redness and swelling, which is defined as an elevation above the normal surrounding skin level, were analyzed by an experienced clinician in a semi-quantitative fashion (scores 0–3, with 0 = no changes, 1 = mild, 2 = moderate, and 3 = severe symptoms for redness and swelling each).

For a dose-response study with oral escin treatment, the contents of commercially available escin capsules were filled into new capsules at doses of 20 or 40 mg (Pharmacy of the University of Veterinary Medicine Vienna). Capsules were orally administered at two time points: 15 min and 3 h before performing the prick test. Three pigs per dose group were treated with a bolus of 20, 40, or 100 mg escin, corresponding to 1, 2, or 5 mg/kg body mass. The basic dose of 1 mg/kg was chosen according to the dose used in an already licensed drug for treatment of venous diseases in humans. The other two doses were intended to show a dose-depen-

dent response in this model. After the final clinical assessment of the prick sites, blood samples were taken at the jugular vein and serum was prepared for escin quantification. Serum samples were kept frozen at -80°C until analysis. Figure 2 gives the time line for the experimental protocol of the dose-response study.

Quantification of Escin in Pig Serum by High-Pressure Liquid Chromatography-Mass Spectrometry

The concentration of escin and krypto-escin (a mixture of free escin and Na-salt in one peak) in pig serum was determined by high-pressure liquid chromatography (HPLC; 1100 HPLC series; Agilent Technologies) followed by mass spectrometry [MS; API 4000 Qtrap[®] with multiple reaction monitoring (MRM); MDS Sciex]. The methodology for escin determination in serum was developed according to previous publications [31, 32]. Serum samples were prepared for chromatography via solid phase extraction using Strata-X columns (30 mg/ml; Phenomenex). Briefly, 200 μ l of serum were spiked with 1 ng betamethasone (Vetranal[®]; Fluka Analytical) as an internal standard, diluted with 200 μ l water, and loaded onto the column at flow rates up to 1–2 ml/min. The column was washed with 1 ml of 50 mM Na-phosphate pH 3 buffer in 10% methanol, and the sample was eluted with 80% methanol. Fractions were dried and redissolved in a 3:2 mix of eluents A and B [eluent A: 5 mM ammonium formate adjusted to pH 3.5 with formic acid in water; eluent B: 5 mM ammonium formate in MeCN/H₂O (98/2) adjusted with formic acid to pH 3.5].

Samples were chromatographed on a Synergy Hydro RP C18[®] column (3 μ m, 2 $\times$ 150 mm; Phenomenex) equipped with a C8 wide-bore precolumn (4 $\times$ 3 mm; Phenomenex). The analytes were eluted with the following gradient: 45% eluent B for 2 min, then 45–98% eluent B within 6 min at a flow of 0.4 ml/min at 25°C. Analytes were detected by positive ion electrospray ionization LC MS/MS analysis with a spray voltage of +4.5 kV and a declustering potential of 136 and 151 kV for escin and escin-Na. The Turbo-spray source injectors were heated to 350°C to allow connection to the HPLC system without mobile phase splitting. The values of the curtain gas and nebulizer gas were 20 and 50 psi, respectively. The dwell time for MRM was 150 ms. MS/MS was performed using nitrogen as the collision gas (collision-activated dissociation gas setting 6) at a collision cell exhibit a potential of 8–10 V. The optimal collision energies for escin and escin-Na were 44 and 42 V, respectively. The optimal MRM transitions monitored for escin were m/z 1131.4/505.3 and 1131.4/435.5 and for escin-Na they were 1153/523.2 and 1153/281.1, respectively. Results were based on the average of all MRMs of escin and escin-Na. For an accurate sample comparison, quantification of escin was normalized to the internal standard using the peak area ratios of the analyte and the internal standard.

Western Blot Analysis of GC Receptor- α Phosphorylation

Murine CFTL-12 mast cells were a kind gift from Dr. Melissa Brown. Cells were cultured as previously described [33]. After incubation with 1 μ M escin (Nobilus Ent) or dexamethasone (Sigma-Aldrich) for 15 min, 2 h, and 5 h at 37°C, 5×10^6 cells per sample were pelleted. Whole cell extracts were prepared by resuspending pellets in 300 μ l Laemmli buffer (Sigma-Aldrich) and forcing the suspension through a 26G1/2 needle. Protein extracts (30 μ l) were separated on 10% Tris-glycin gels (Invitrogen) and

transferred to PVDF membranes (Invitrogen). Membranes were stained with Ponceau S solution (Sigma-Aldrich) and probed with antibodies against GC receptor (GR) phosphorylated on serine 211 (Cell Signaling) or GR independent of the phosphorylation status (Santa Cruz) overnight at 4°C in a dilution of 1:1,000 or 1:500, respectively. On the next day, membranes were washed and incubated with an HRP-conjugated goat-anti-rabbit IgG antibody (Biorad) in a dilution of 1:5,000 for 1 h at room temperature. Blots were washed again and developed using Super Signal West Pico® (Thermo Scientific). Signals were visualized on Amersham Hyperfilm ECL® (GE Healthcare).

Results

Establishment of a Porcine Model of Type I Hypersensitivity Reactions in Skin

In the absence of known antigens that may trigger a cutaneous allergic response in pigs without leading to additional signs of irritative dermatitis, we tested histamine and the known mast cell degranulating agent compound 48/80 [34] as surrogates for potential allergens to set up a porcine skin prick test model. The response was observed for 20 min and semiquantitatively assessed by clinical scores for redness and swelling at 10 min (fig. 3) and 20 min (data not shown) after pricking. As expected, the negative control did not cause any symptoms within the observation period. In contrast, histamine clearly provoked erythema and swelling at the prick site (scores of 0.8 ± 0.3 for erythema and 1.7 ± 0.6 for swelling after 10 min, respectively). The response was even more pronounced for compound 48/80. Erythema scored higher than swelling in this case (scores of 2.8 ± 0.3 and 2.2 ± 0.3 after 10 min, respectively) and both symptoms exceeded the scores for the histamine-treated sites. As no further increase in symptoms was observed 20 min after pricking, the read-out of the reaction 10 min after pricking was defined as the time point for the assessment of test substances.

Oral Escin Treatment Dose-Dependently Inhibits Allergic Skin Responses in the Prick Test

We used the newly established prick test model to assess the anti-allergic efficacy of escin in this system. In contrast to previous experiments in the mouse, where escin was applied intraperitoneally, oral application was tested. Figure 2 gives an overview of the experimental protocol. Treatment sites were clinically scored and photo-documented, an example of which is presented in figure 4 for one animal before (day 1, untreated) and after receiving 5 mg/kg escin (day 2). While the application site of the negative control was barely visible on both days,

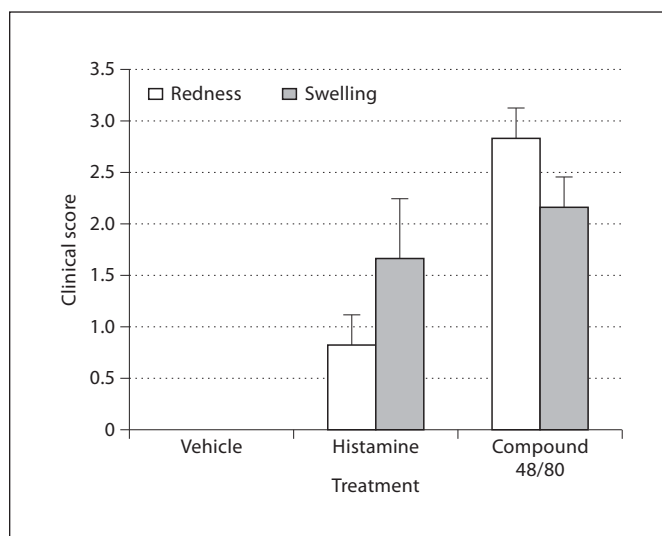


Fig. 3. Establishment of a porcine prick test model for allergic cutaneous reactions. The skin of pigs was pricked in the presence of 0.9% NaCl as a negative control, histamine as a typical positive control for clinical prick tests in human patients (10 mg/ml), or the mast cell-degranulating compound 48/80 (10 mg/ml). Semiquantitative clinical assessment of each test site was performed 10 min after pricking, using scores of 0–3 for redness and swelling. The graph shows the mean scores and standard deviations of 3 animals.

compound 48/80 caused marked symptoms of redness and swelling at baseline on day 1 as observed during the establishment of the assay. In contrast, treatment with 5 mg/kg escin strongly impaired the allergic skin response on day 2. Both redness and swelling were remarkably reduced. Figure 5a summarizes the scoring results for all dose groups. In comparison to untreated animals, 1, 2, and 5 mg/kg escin showed a dose-dependent reduction of symptoms and were correlated with increased escin serum titers (fig. 5b).

Escin Treatment of Mast Cells in vitro Stimulates Phosphorylation of the GR-α

To investigate the mode of action of escin and involved intracellular pathways, we studied potential similarities between the GC dexamethasone and escin. Therefore, we examined the effects of escin on GR phosphorylation. Engagement of the GR was visualized by its phosphorylation on serine 211 upon treatment of the murine mast cell line CFTL-12 (fig. 6). Western blots showed comparable phosphorylation kinetics of the GR for dexamethasone and escin treatment. However, the degree of phosphorylation was markedly higher in cells treated with dexamethasone

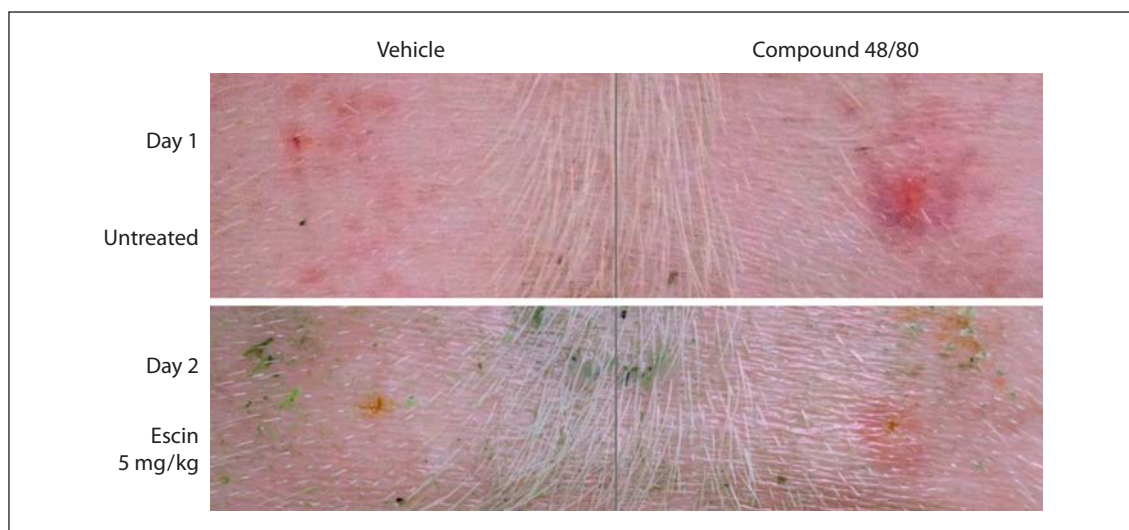


Fig. 4. Reduced redness and swelling at prick test sites after oral treatment with escin. On day 1, prick tests were performed on the skin of untreated pigs with compound 48/80 or the negative control, as indicated. Pigs were then orally treated with 1, 2, or 5 mg/kg escin in the evening and in the morning of the next day.

Three hours after the second treatment, the prick test was repeated. The pictures show the test sites for the negative control and compound 48/80 in one representative animal before and after treatment with 5 mg/kg escin. Scores are summarized in figure 5.

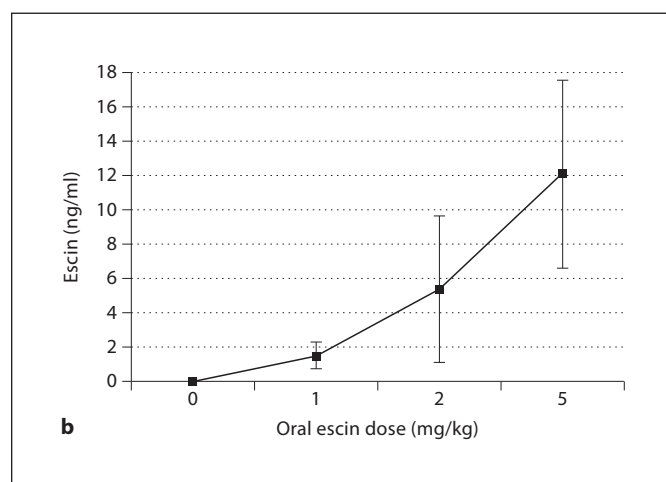
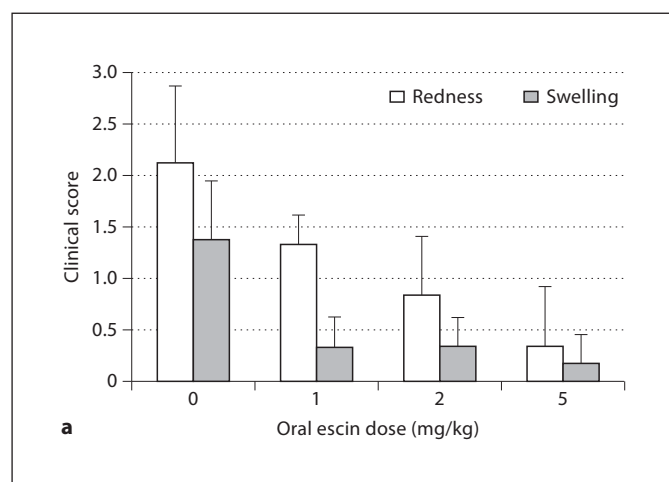


Fig. 5. Dose-dependent efficacy of oral treatment with escin in mast cell-mediated allergic skin reactions. **a** The skin was pricked in the presence of the negative control solution or the mast cell-stimulating compound 48/80. Ten minutes after pricking, the clinical symptoms redness and swelling were scored for each spot. The graph presents results for sites that were stimulated with compound 48/80, summarizing the mean scores and standard deviations of 12 untreated animals and 3 animals per treatment dose. **b** Escin concentration in the serum of pigs orally treated with increasing doses of escin. Blood samples from pigs presented in **a** were taken at the termination of the experiment, and the concentration of β -escin and krypto-escin was determined in serum by HPLC-MS. The graph presents the means and standard deviations of the total serum escin of 3 animals per dose group.

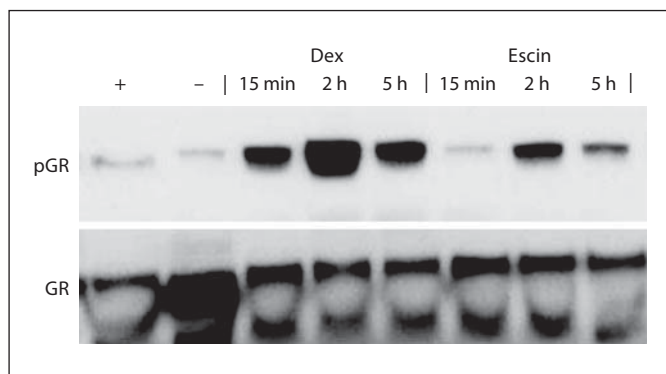


Fig. 6. Escin stimulates phosphorylation of the GR at a site involved in activation. CFTL-12 murine mast cells were treated with 1 μ M escin or dexamethasone (Dex) for the indicated periods of time. Whole cell extracts were then analyzed by Western blots using antibodies against GR phosphorylated at position serine 211 (pGR) and a phosphorylation-independent GR antibody as a control to correlate amounts of total GR protein in each sample. Unstimulated cells served as a negative control (-). The image of the untreated control was taken from the same blot at the same exposure but was appositioned next to the Dex samples for better comparison. The blot is representative of 3 independent experiments.

compared to equimolar amounts of escin, suggesting overlapping but nonetheless different signaling mechanisms of these two substances.

Discussion

In this report we describe the establishment of a simple but effective model of cutaneous type I allergy in a large animal. This model does rely directly on the pathophysiological cascades induced by mast cell degranulation and therefore there was no need to sensitize animals to provoke an increase in allergen-specific IgE antibody titers. As a consequence, this model is limited to the pre-clinical evaluation of mast cell-stabilizing substances. In a second step, we used this model to demonstrate the therapeutic potential of escin as a novel anti-allergic substance. Animal models for the study of allergic reactions or the action of potential treatments are mostly based on rodents. While these models have greatly enhanced our scientific knowledge, they have also been criticized because of species differences and sometimes insufficient predictability of treatment effects in humans. Based on high anatomical and metabolic similarities between pigs and humans, this species is particularly interesting for biomedical research [35]. We

took advantage of the immediate allergic response known from skin prick tests in the clinic and performed this test in pigs, using the mast cell-activating compound 48/80 in place of a specific antigen and histamine as a positive control substance.

Compound 48/80 is a polymer produced by the condensation of N-methyl-p-methoxyphenethylamine with formaldehyde, which is known to induce mast cell degranulation and histamine release [34]. We found that the application of compound 48/80 in our porcine model proved to be even more effective in inducing acute allergic dermatitis, with prominent redness and swelling, than comparable amounts of the gold standard histamine. Wheal formation after allergen exposure is caused by a combination of low-level endogenous histamine and additional stimuli. A much higher concentration of externally applied histamine is required to provoke the same allergic reaction [36]. In the absence of known specific allergens in the pig, a direct comparison of allergen, histamine, and compound 48/80 is not feasible. However, it is tempting to speculate that the mast cell degranulating compound 48/80 may yield a more similar response to allergens than histamine as a single agent. Even if histamine is an established positive control in clinical settings, stimulation with compound 48/80 may be more representative of a natural allergic response, which makes it an attractive tool to study anti-allergic compounds, particularly if their mode of action is different from anti-histamines. Unfortunately, histopathological examinations of cutaneous mast cell numbers and mast cell morphology in skin biopsies did not resolve the difference between the treatments due to a high sample variability (data not shown).

While human wheal and flare reactions in response to prick tests were well reflected in terms of swelling and redness in pigs, surprisingly, pigs did not develop pruritic signs following epidermal histamine and compound 48/80 contact. Lack of pruritus during an allergic skin response appears to be a novel, specific characteristic of this species, which has not been described before. On the other hand, pruritus is not completely missing in the porcine species as it is a prominent symptom when suffering from mange (*Sarcoptes suis*). However, this unexpected finding did not influence the focus of our model.

In our previous studies, we successfully used escin to inhibit early and late phases of type I hypersensitivity reactions in a passive cutaneous anaphylaxis model and in allergic airway inflammation in mice, respectively [11]. Escin was applied intraperitoneally in these murine allergy models. However, for further development of the

drug we aimed to test alternative treatment routes as well as alternative model species. Our new allergy model now provided the opportunity to test the anti-allergic effect of escin after oral application in pigs. As expected, the comparison of symptom scores before and after escin treatment clearly confirmed the anti-allergic properties of escin. Moreover, a dose-response relationship was observed for the effect of escin in the pig model, which substantiated the results of murine models. It also strengthened the value of the new model as it obviously allows differentiation of the extent of the response depending on different doses of treatment. Preliminary data also suggest a comparable *in vivo* effectiveness of topical escin and cortisone treatment in this model, thus supporting the possible clinical relevance of these findings.

It is not yet clear whether the benefit of escin for the treatment of allergy is based on the same or a similar mode of action as in traditional indications, such as CVI or postoperative edemas. Consequently, it was not self-evident that the oral treatment route used for CVI would also yield a therapeutic effect of escin in allergy. Thus, the successful reduction of the allergic skin response after oral escin therapy in pigs is of major scientific significance. In addition, the development of oral escin preparations as anti-allergic medication in human medicine is supported by the rare occurrence of adverse reactions to oral escin treatment in previous clinical trials [37, 38].

In the attempt to provide alternative treatments to current drugs, it is of interest whether escin targets similar or different effector molecules or pathways compared to traditional drugs, such as corticosteroids. Recent literature described a protective effect of escin in murine models of acute injury of the lung or the liver, which was accompanied by the upregulation of GR protein levels in lung tissue 8 h after treatment with escin [39, 40]. Moreover, a synergistic anti-inflammatory effect was indicated for escin in combination with GCs in models of paw edema and pleuritis, and for the inhibition of macrophage activation *in vitro* [41]. The synergy of escin with GCs might be explained by an upregulation of their target, the GR. However, our own studies of mast cell activation in the mast cell line CFTL-12 did not show an upregulation of GR protein expression in a time course of escin treatment (fig. 5, additional data not shown), suggesting that upregulation by escin may be cell type-dependent or secondary to other biological effects of escin *in vivo*. Interestingly, we found another sign of involvement of the GR pathway by escin. The activation of the GR pathway by GCs is marked by the phosphorylation of Ser211 of GR [42]. Side-by-side

treatment of CFTL-12 mast cells with escin or dexamethasone revealed that exposure to either compound leads to phosphorylation of the GR at this site with the same temporal pattern. The extent of phosphorylation was, however, much lower for escin, which suggests that escin does not simply mimic a GC signal. It remains to be clarified whether escin uses additional components of the GR pathway and whether or how it affects the transcriptional activity of cells that were treated with escin in comparison to dexamethasone. Beneficial aspects of GC treatment are commonly attributed to transrepression whereas transactivation is considered responsible for undesired side effects, but studies with so-called 'dissociated GR ligands' indicate that this distinction is not clear cut [43]. Further investigation of the effects of escin on the transcriptional modulation of GR target genes will therefore be important to provide more insight into the contribution of escin to this pathway. Moreover, cases of patients that are resistant to GC treatment or become GC insensitive in the course of therapy necessitate alternative treatment options [44–46]. The elucidation of a different or overlapping mode of action of escin with GCs for the modulation of allergic responses and inflammation will therefore be exciting for the further development of escin against allergic conditions.

Conclusions

A novel cutaneous allergy model based on skin prick tests was established in pigs. This model of type I hypersensitivity was successfully used to demonstrate the oral efficacy of our model substance escin and will provide a valuable tool for future preclinical studies of anti-allergic compounds. Our previous studies of murine models of allergy and the current report show that escin may enhance or even replace current allergy treatments, thus offering new treatment alternatives particularly for patients who are unresponsive to standard therapies. The results of this study should expedite further studies for the clinical use of escin in allergic conditions due to its high tolerability, good anti-allergic effectiveness, and oral availability.

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Disclosure Statement

B.R., A.G., E.P.-G., and P.D. are employed by Marinomed Biotechnologie GmbH. M.F. was a Marinomed employee at the time of the in vitro experiments. H.U. is co-founder of Marinomed.

A.G. and E.P.-G. are inventors on patent No. WO 2008015007 held by Marinomed Biotechnologie GmbH that relates to the content of the manuscript. Marinomed Biotechnologie GmbH finances the processing charge of this paper. W.S. declares that he has no competing interest.

References

- ▶ 1 Devereux G: The increase in the prevalence of asthma and allergy: food for thought. *Nat Rev Immunol* 2006;6:869–874.
- ▶ 2 Platts-Mills TA, Woodfolk JA: Allergens and their role in the allergic immune response. *Immunol Rev* 2011;242:51–68.
- ▶ 3 Baumruker T, Prieschl E: Mast cells and their activation – from the molecular mechanism to clinical relevance. *Mod Asp Immunobiol* 2001;1:259–262.
- ▶ 4 Bloemen K, Verstraelen S, Van Den HR, Witters H, Nelissen I, Schoeters G: The allergic cascade: review of the most important molecules in the asthmatic lung. *Immunol Lett* 2007;113:6–18.
- ▶ 5 Galli SJ, Tsai M, Piliponsky AM: The development of allergic inflammation *Nature* 2008;454:445–454.
- ▶ 6 Marshall JS: Mast-cell responses to pathogens. *Nat Rev Immunol* 2004;4:787–799.
- ▶ 7 Schacke H, Docke WD, Asadullah K: Mechanisms involved in the side effects of glucocorticoids. *Pharmacol Ther* 2002;96:23–43.
- ▶ 8 Baroody FM, Naclerio RM: Antiallergic effects of H1-receptor antagonists. *Allergy* 2000;55(suppl 64):17–27.
- ▶ 9 Rizzo MC, Sole D: Inhaled corticosteroids in the treatment of respiratory allergy: safety vs. efficacy. *J Pediatr (Rio J)* 2006;82:S198–S205.
- ▶ 10 Adcock IM, Barnes PJ: Molecular mechanisms of corticosteroid resistance. *Chest* 2008;134:394–401.
- ▶ 11 Lindner I, Meier C, Url A, Unger H, Grassauer A, Prieschl-Grassauer E, et al: Beta-escin has potent anti-allergic efficacy and reduces allergic airway inflammation. *BMC Immunol* 2010;11:24.
- ▶ 12 Guillaume M, Padioulet F: Veinotonic effect, vascular protection, antiinflammatory and free radical scavenging properties of horse chestnut extract. *Arzneimittelforschung* 1994;44:25–35.
- ▶ 13 Sirtori CR: Aescin: pharmacology, pharmacokinetics and therapeutic profile. *Pharmacol Res* 2001;44:183–193.
- ▶ 14 Matsuda H, Li Y, Murakami T, Ninomiya K, Araki N, Yoshikawa M, et al: Antiinflammatory effects of escins Ia, Ib, IIa, and IIb from horse chestnut, the seeds of *Aesculus hippocastanum* L. *Bioorg Med Chem Lett* 1997;7:1611–1616.
- ▶ 15 Matsuda H, Li Y, Murakami T, Ninomiya K, Yamahara J, Yoshikawa M: Effects of escins Ia, Ib, IIa, and IIb from horse chestnut, the seeds of *Aesculus hippocastanum* L., on acute inflammation in animals. *Biol Pharm Bull* 1997;20:1092–1095.
- ▶ 16 Wang T, Fu F, Zhang L, Han B, Zhu M, Zhang X: Effects of escin on acute inflammation and the immune system in mice. *Pharmacol Rep* 2009;61:697–704.
- ▶ 17 Wei F, Ma LY, Jin WT, Ma SC, Han GZ, Khan IA, Lin RC: Antiinflammatory triterpenoid saponins from the seeds of *Aesculus chinensis*. *Chem Pharm Bull* 2004;52:1246–1248.
- ▶ 18 Xie Q, Zong X, Ge B, Wang S, Ji J, Ye Y, et al: Pilot postoperative ileus study of escin in cancer patients after colorectal surgery. *World J Surg* 2009;33:348–354.
- ▶ 19 Carrasco OF, Vidrio H: Endothelium protectant and contractile effects of the antivaricose principle escin in rat aorta. *Vascul Pharmacol* 2007;47:68–73.
- ▶ 20 Fu F, Hou Y, Jiang W, Wang R, Liu K: Escin: inhibiting inflammation and promoting gastrointestinal transit to attenuate formation of postoperative adhesions. *World J Surg* 2005;29:1614–1620.
- ▶ 21 Ottillinger B, Greeske K: Rational therapy of chronic venous insufficiency – chances and limits of the therapeutic use of horse-chestnut seeds extract. *BMC Cardiovasc Disord* 2001;1:5.
- ▶ 22 Wetzel D, Menke W, Dieter R, Smasal V, Giannetti B, Bulitta M: Escin/diethylammonium salicylate/heparin combination gels for the topical treatment of acute impact injuries: a randomised, double blind, placebo controlled, multicentre study. *Br J Sports Med* 2002;36:183–188.
- ▶ 23 Tan SM, Li F, Rajendran P, Kumar AP, Hui KM, Sethi G: Identification of beta-escin as a novel inhibitor of signal transducer and activator of transcription 3/Janus-activated kinase 2 signaling pathway that suppresses proliferation and induces apoptosis in human hepatocellular carcinoma cells. *J Pharmacol Exp Ther* 2010;334:285–293.
- ▶ 24 Harikumar KB, Sung B, Pandey MK, Guha S, Krishnan S, Aggarwal BB: Escin, a pentacyclic triterpene, chemosensitizes human tumor cells through inhibition of NF-kappaB signaling pathway. *Mol Pharmacol* 2010;77:818–827.
- ▶ 25 Ming ZJ, Hu Y, Qiu YH, Cao L, Zhang XG: Synergistic effects of beta-aescin and 5-fluorouracil in human hepatocellular carcinoma SMMC-7721 cells. *Phytomedicine* 2010;17:575–580.
- ▶ 26 Zhang Z, Gao J, Cai X, Zhao Y, Wang Y, Lu W, et al: Escin sodium induces apoptosis of human acute leukemia Jurkat T cells. *Phytother Res* 2011;25:1747–1755.
- ▶ 27 Patlolla JM, Raju J, Swamy MV, Rao CV: Beta-escin inhibits colonic aberrant crypt foci formation in rats and regulates the cell cycle growth by inducing p21(waf1/cip1) in colon cancer cells. *Mol Cancer Ther* 2006;5:1459–1466.
- ▶ 28 Wang YW, Wang SJ, Zhou YN, Pan SH, Sun B: Escin augments the efficacy of gemcitabine through down-regulation of nuclear factor-kappaB and nuclear factor-kappaB-regulated gene products in pancreatic cancer both in vitro and in vivo. *J Cancer Res Clin Oncol* 2012;138:785–797.
- ▶ 29 Meyer W, Schwarz R, Neurand K: The skin of domestic mammals as a model for the human skin, with special reference to the domestic pig. *Curr Probl Dermatol* 1978;7:39–52.
- ▶ 30 Vana G, Meingassner JG: Morphologic and immunohistochemical features of experimentally induced allergic contact dermatitis in Gottingen minipigs. *Vet Pathol* 2000;37:565–580.
- ▶ 31 Liu L, Wu X, Wu D, Wang Y, Li P, Sun Y, et al: A liquid chromatography-tandem mass spectrometry method for the simultaneous quantification of escin Ia and escin Ib in human plasma: application to a pharmacokinetic study after intravenous administration. *Biomed Chromatogr* 2010;24:1309–1315.
- ▶ 32 Li B, Abliz Z, Fu G, Tang M, Yu S: Characteristic fragmentation behavior of some glucuronide-type triterpenoid saponins using electrospray ionization tandem mass spectrometry. *Rapid Commun Mass Spectrom* 2005;19:381–390.
- ▶ 33 Henkel G, Weiss DL, McCoy R, Deloughery T, Tara D, Brown MA: A DNase I-hypersensitive site in the second intron of the murine IL-4 gene defines a mast cell-specific enhancer. *J Immunol* 1992;149:3239–3246.
- ▶ 34 Lagunoff D, Martin TW, Read G: Agents that release histamine from mast cells. *Annu Rev Pharmacol Toxicol* 1983;23:331–351.

- ▶ 35 Vodicka P, Smetana Jr K, Dvorankova B, Emerick T, Xu YZ, Ourednik J, et al: The miniature pig as an animal model in biomedical research. *Ann NY Acad Sci* 2005; 1049:161–171.
- ▶ 36 Devillier P, Bousquet J: Inhibition of the histamine-induced weal and flare response: a valid surrogate measure for antihistamine clinical efficacy? *Clin Exp Allergy* 2007;37: 400–414.
- ▶ 37 Greeske K, Pohlmann BK: Horse chestnut seed extract – an effective therapy principle in general practice: drug therapy of chronic venous insufficiency. *Fortschr Med* 1996; 114:196–200.
- ▶ 38 Oschmann R, Biber A, Lang F, Stumpf H, Kunz K: Pharmacokinetics of beta-escin after administration of various *Aesculus* extract containing formulations. *Pharmazie* 1996; 51:577–581.
- ▶ 39 Jiang N, Xin W, Wang T, Zhang L, Fan H, Du Y, et al: Protective effect of aescin from the seeds of *Aesculus hippocastanum* on liver injury induced by endotoxin in mice. *Phytomedicine* 2011;18:1276–1284.
- ▶ 40 Xin W, Zhang L, Fan H, Jiang N, Wang T, Fu F: Escin attenuates acute lung injury induced by endotoxin in mice. *Eur J Pharm Sci* 2011; 42:73–80.
- ▶ 41 Xin W, Zhang L, Sun F, Jiang N, Fan H, Wang T, et al: Escin exerts synergistic anti-inflammatory effects with low doses of glucocorticoids in vivo and in vitro. *Phytomedicine* 2011;18:272–277.
- ▶ 42 Wang Z, Frederick J, Garabedian MJ: Deciphering the phosphorylation ‘code’ of the glucocorticoid receptor in vivo. *J Biol Chem* 2002;277:26573–26580.
- ▶ 43 De Bosscher K, Beck IM, Haegeman G: Classic glucocorticoids versus non-steroidal glucocorticoid receptor modulators: survival of the fittest regulator of the immune system? *Brain Behav Immun* 2010;24:1035–1042.
- ▶ 44 Adcock IM, Chou PC, Durham A, Ford P: Overcoming steroid unresponsiveness in airways disease. *Biochem Soc Trans* 2009;37: 824–829.
- ▶ 45 Gross KL, Lu NZ, Cidlowski JA: Molecular mechanisms regulating glucocorticoid sensitivity and resistance. *Mol Cell Endocrinol* 2009;300:7–16.
- ▶ 46 Barnes PJ: Mechanisms and resistance in glucocorticoid control of inflammation. *J Steroid Biochem Mol Biol* 2010;120:76–85.