Effects of PD169316, a p38 MAPK Inhibitor, on a Guinea Pig Model of Atopic Dermatitis

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ABSTRACT

Atopic dermatitis is the most common chronic inflammatory skin disease among young children, with an increasing prevalence especially in the west. As in Type-1 immediate hypersensitivity reactions, exposure to allergen results in IgE cross-linking, initiating a tyrosine kinase signaling cascade in mast cells that consequently results activation of intracellular signalling cascades including the mitogen-activated protein kinase (MAPK) signal transduction pathway, including the isoform p38, which leads to the accumulation of T cells and eosinophils. Current therapy of antihistamines and steroids for atopic dermatitis are not very effective and may exhibit some side effects. Therapeutics aimed at reducing the production of inflammatory mediators is suggested to provide more effective treatment for inflammatory diseases. The present study examined the effects of p38 inhibitor PD169316 in vivo model of atopic dermatitis in inflammatory response to allergen. The effect on the model is assessed by measuring the development of erythema using tristimulus tricolorimetry and doing histological examination 24 hours after challenge. The results showed that PD 169316 had a significant suppressive effect on the chronic phase of atopic dermatitis but not acute phase.

INTRODUCTION

Atopic dermatitis is an common inflammatory skin disease, characterized by an itchy, erythematous, poorly demarcated skin eruption, which has a tendency to develop in skin creases. Typical of Type-1 immediate hypersensitivity reaction, exposure to allergen results in cross-linking of allergen-specific Immunoglobulin E (IgE) bound on high-affinity Immunoglobulin E receptor (FcεRI) on the mast cell membrane, initiating a tyrosine kinase signalling cascade in mast cells and basophils (Reischl et al., 1999) that consequently results in the activation of multiple intracellular mitogen-activated protein kinase (MAPK) signal transduction pathway. Stimulation of leukocytes by proinflammatory cytokines is known to result in the activation of the MAPK isoform p38 (Herlaar and Zarin, 1999).

Onset of lesions in atopic dermatitis occurs in two phases, acute and chronic. Studies showed that initiation of acute skin inflammation is associated with IL-4 expression whereas maintenance of chronic inflammation is associated with IL-5 expression and eosinophil infiltration, characteristic of Th2-type profile of cytokine expression (Hamid et al., 1994). This imbalance of Th1 and Th2 cells may provide a possible explanation that the immune system of a newborn infant is skewed towards Th2–mediation due to lack of factors that enhance Th1–mediated responses (Romagnani, 2000).
The recognition of chronic, allergic-driven inflammation as the basis for the pathology of atopic diseases has led the suggestion that therapeutics aimed at reducing the production of inflammatory mediators would provide more effective treatment for inflammatory diseases (Walker and Zuany, 2001). Current treatments like anti-histamines and corticosteroids treat only the symptoms and may have side effects (Leung and Bieber, 2003). The rise in both severity and prevalence of atopic dermatitis has a great clinical and economic impact in industrialized countries and thus, there is a high interest in finding new effective treatments for this predisposition. Earlier studies with specific p38 inhibitors have demonstrated potent inhibitory effects on cytokine production both in vivo and in vitro, including anti-inflammatory activity in a variety of animal models (Herlaar and Zarin, 1999). Here, we would like to investigate the effect of PD 169316, a p38 MAPK inhibitor, on a guinea pig model of atopic dermatitis. The inhibition of p38 MAPK will be assessed by quantifying allergen-induced eosinophil infiltration, adapting a cell–counting protocol developed by Sjögren (1995). The experiment will be divided into two sections. The first is the study of ovalbumin (OA)-induced erythema on the skin, using tristimulus colorimetric analysis (Chan & Po, 1993; Takiwaki and Serup, 1994), of sensitized guineapigs challenged locally with OA. The second is the investigation of OA-induced eosinophil infiltration twenty-four hours following the challenge.

MATERIALS AND METHODS

OA-induced erythema

**Animals** Male and Female Hartley guinea pigs of 250-300 g body weight were purchased and kept under standard conditions in the animal house of the Department of Pharmacology.

**Sensitization** Active sensitization for the allergic reaction was performed 18 days prior to testing and booster injections 14 days after sensitization. 10 mg OA together with 100 mg of adjuvant Al(OH)₃, was dissolved in 1 ml 0.9% NaCl solution and injected i.p. (Sjögren (1995)).

**Testing** The skins of the flanks were shaved with an electric razor, and abraded with a normal household shaver, one day prior to testing. On day 18, the animal was anaesthetized using Pentobarbitone (37 mg/kg) and four to six 5 mm areas on the flanks were marked out for testing. A baseline reading of the test areas was taken using Minolta® Chroma Meter CR-200 and designated as T = 0 min. OA was dissolved in saline to 0.3 mg/ml, 1.0 mg/ml, 3.0 mg/ml and 10 mg/ml. 10 µl of each OA concentration was injected i.d. into the marked-out areas. Readings were taken from each of the injected areas at 2-minute intervals for the first 10 minutes (T = 2, 4, 6, 8, 10 min), followed by 4-minute intervals for the next 56 minutes (T = 14, 18, ..., 62, 66 min. All further readings were deducted from the baseline readings, representing an increase in or decrease in the color of the erythema as reflected by the values of L*a*b* colour scheme. This set of experiment was first carried out to find the optimal concentration that will significantly induce erythema on the skin that will be used against the drug.

**Drugs** p38 MAP kinase inhibitor PD169316 [4-(4-Fluorophenyl)-2-(4-nitrophenyl)-5-(4-pyridyl)-1H-imidazole] was dissolved in 6:4 (v/v) ethanol/propylene glycol vehicle into concentrations of 0.3 mg/ml, 1.0 mg/ml and 3.0 mg/ml. All aliquots were stored at -20°C. On day 18, 10 µl of each concentration of the drug was administered topically 45 minutes before OA i.d. challenge at different localizations. In each experiment, one area was chosen to act as a control, with only the vehicle being applied. After the period, the drug treatments were cleaned off and baseline reading was taken before 10 µl of the optimal concentration of OA was injected on each area.
OA-induced eosinophil infiltration

Histological examination 24 hours after the i.d. challenge, the guinea pigs were sacrificed using CO2. A 10 mm by 10 mm biopsy of each test area was taken, fixed in 10 % neutral formalin and embedded in paraffin wax. 7 µm sections were cut using a rotary microtome and stained with Lunar stain before examination under a light microscope.

Counting of the dermal inflammatory cell infiltrate  Under magnification of x600, 6 arbitrarily chosen fields from the hypodermis were counted and totaled up.

Statistical analysis The results of colorimetric analysis were statistically analyzed by one-way ANOVA with Dunnett post-hoc analysis. The results of the cell counting were statistically analyzed using the same analysis and also Student’s independent paired T-test.

RESULTS

OA-induced erythema

The 66-min time courses of OA-induced erythema without and with drug application were measured using tristimulus tricolorimetry and computed as a percentage increase or decrease with respect to the control AUC. OA concentration of 3.0 mg/ml was found to be optimal for the onset of OA-induced erythema. The drug study showed that there was a significant decrease in darkness compared with control (vehicle) only for drug concentration 1.0 mg/ml (Dunnett, p < 0.5) but no significant effect on redness of the skin for any concentration of the drug.

OA-induced eosinophil infiltration

The result of histological examination of biopsy of OA-challenged skin 24 hours later showed almost zero eosinophil infiltration in saline treatment and a significant increase in infiltration for 3.0 mg/ml OA challenge (Dunnett, p<0.05). Topical application of the vehicle without drug did not reduce the infiltration compared to 3.0 mg/ml OA-induced infiltration but topical application of 3.0 mg/ml of PD 169316 in the vehicle significantly reduced eosinophil infiltration (p=0.001).
Fig. 2: (a) Number of OA-induced eosinophil infiltration in hypodermis layer in response to OA challenge. (*Dunnett, p<0.05). (b) Percentage of OA-induced eosinophil infiltration in hypodermis layer in response to OA challenge after topical application of vehicle and drug. (*Students’ independent paired T-test, p<0.01 (p=0.001046)).

CONCLUSION

Our results in the investigation on PD 169316 showed that the drug had a significant suppressive effect on the OA-induced chronic allergic reaction in actively sensitized guinea pigs but not on the immediate of OA-induced allergy.

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REFERENCES


