Journal of Dermatological Science (2006) 43, 117—126

ELSEVIER

Journal of Dermatological SCIENCE

www.intl.elsevierhealth.com/journals/jods

Pimecrolimus and tacrolimus differ in their inhibition of lymphocyte activation during the sensitization phase of contact hypersensitivity

A. Bavandi, H. Fahrngruber, H. Aschauer, B. Hartmann, J.G. Meingassner*, F.S. Kalthoff

Novartis Institutes for BioMedical Research, Brunnerstrasse 59, A 1235 Vienna, Austria

Received 10 December 2005; received in revised form 31 March 2006; accepted 3 April 2006

KEYWORDS
Pimecrolimus;
Tacrolimus;
Lymphocyte activation;
Lymph node hyperplasia;
Sensitization phase;
Contact hypersensitivity;
Drug levels

Summary

Background: As reported previously, oral administration of the calcineurin inhibitors (CNI) pimecrolimus and tacrolimus resulted in equipotent inhibition of the elicitation phase of contact hypersensitivity (CHS) in mice. The sensitization phase was inhibited by tacrolimus but was unaffected by pimecrolimus, even at higher doses.
Objective: The kinetics of lymph node hyperplasia and up-regulation of T and B cell activation antigens were analyzed to obtain a better understanding of the divergent CNI profile in CHS.
Methods: Lymph node (LN) cells of CNI-untreated and treated mice were examined with flow cytometry at various time points after sensitization with oxazolone. LN hyperplasia and drug levels were also determined.
Results: Sensitization induced a higher portion of LN cells expressing the activation antigens CD25, CD69 and CD134 and an increase in activated B cells ($B220^+/CD40^+$) compared to naïve mice. Up-regulation of these markers was completely or profoundly blocked with tacrolimus, whereas pimecrolimus at the three-fold higher dose caused significantly less inhibition. Tacrolimus also completely blocked the sensitization-associated increase of $CD11c^+$ antigen presenting cells (APC) in LN, whereas pimecrolimus showed significantly less inhibition. In contrast to tacrolimus, LN weight and cellularity were not affected by pimecrolimus at any time point after sensitization. Concentration of tacrolimus in blood and in the draining LN substantially exceeded that of pimecrolimus by factors 6.7—14 and 5.6—5.8, respectively, at the same dose levels.

* Corresponding author. Tel.: +43 1 86 634 273; fax: +43 1 96 634 354.
E-mail address: Josef.meingassner@novartis.com (J.G. Meingassner).

doi:10.1016/j.jdermsci.2006.04.001

Conclusion: In contrast to tacrolimus, systemic treatment of mice with pimecrolimus only weakly interferes with lymphocyte activation and does not affect hyperplasia of the draining lymph nodes during sensitization.

1. Introduction

Cutaneous contact hypersensitivity (CHS) reactions can be experimentally induced in mice by epicutaneous applications of potent haptens such as oxazolone. It has been shown that the sensitization to contact allergens depends on the ability of skin-resident antigen-presenting cells, most likely the Langerhans cells (LC), to internalize the hapten-modified proteins, and to migrate to the regional lymph nodes as mature LC, where they present the antigenic materials to naïve T lymphocytes. Successful activation of naïve T cells in the context of co-stimulation causes them to migrate into the B cell rich areas, where they activate antigen-specific B cells via cytokines and the presentation of ligands such as CD154, which binds to the B cell activation molecule CD40. Some of the activated T cells may leave the lymph node and enter the peripheral blood. Due to elevated levels of adhesion receptors, such as the skin-homing selectin CLA and the ICAM-1 counter-ligand LFA-1, the activated T cells have the ability to enter into the skin environment as soon as a subsequent exposure to the contact allergen leads to the local activation of epidermal and endothelial cells expressing inflammatory mediators and appropriate adhesion molecules, respectively [1, review]. Taken together, CHS develops in two phases, the sensitization phase associated with maturation of antigen-loaded antigen presenting cells (APC) and activation of specific T and B cells, and the elicitation phase, characterized by inflammatory cell infiltration at the site of antigenic re-challenge, becoming clinically manifest as allergic contact dermatitis.

Previously, we have shown that the two phases of CHS were differentially affected in mice by the oral administration of the calcineurin inhibitors (CNI), pimecrolimus, tacrolimus and cyclosporine A [2]. All three CNIs were capable of inhibiting the elicitation of CHS, with both pimecrolimus and tacrolimus showing equivalent potency and efficacy and exceeding that of cyclosporine A. The sensitization phase of CHS, however, was inhibited by tacrolimus and — to a lesser extent — by cyclosporine A, but was unaffected by pimecrolimus, even at a four-fold higher dose than tacrolimus. These results were surprising in the light of other data showing that pimecrolimus inhibited primary and secondary T cell activation with a 10-fold higher potency as compared to cyclosporine A in vitro [3]. Furthermore, tacrolimus and pimecrolimus inhibited the induction of cytokine transcripts after activation of the human T cell leukemia cell line Jurkat with similar potency [4]. The different activity profile in the murine cutaneous CHS model might thus be caused by specific, currently unidentified, pharmacodynamic/pharmacokinetic characteristics of the compounds.

The present study was conducted to explore the effect of pimecrolimus and tacrolimus on sensitization in more detail. As shown previously, lymphocyte priming by APC subsequent to the first sensitization with a contact allergen such as oxazolone results in the up-regulation of lymphocyte activation antigens and cytokine genes in the draining lymph nodes [5—7]. This process is accompanied by lymphocyte proliferation, reflected by increases in cellularity and weight of the draining lymph nodes [2,5]. We have followed the kinetics of lymph node hyperplasia and the T and B cell fractions by flow cytometric analysis at different time points after oxazolone sensitization. Particularly, the expression of the activation markers CD25, CD69 and CD134 on T cells and CD40 on B cells was monitored since they are known to be affected by antigen-mediated stimulation [7,8]. In addition, satellite groups of similarly treated animals were evaluated to assess the tissue distribution of the two drugs administered using the same schedule and doses as reported previously [2].

2. Materials and methods

2.1. Test articles, laboratory animals, induction of CHS and treatment

Pimecrolimus was used as a 20% solid solution (Novartis Pharma AG); tacrolimus was used as marketed product (Prograf® 5 mg capsules) and prepared in water for adjustment of the oral dose. Animal strain, housing conditions, the induction procedure of CHS and the treatment schedules were as described previously [2]. In brief, female BALB/c mice were sensitized on the shaved abdomen with 50 μl of oxazolone (2% in acetone) on day 1. Oral pimecrolimus was administered at 30 mg/kg (only for determination of tissue concentrations) and 90 mg/kg, and