In vivo/ex vivo targeting of Langerhans cells after topical application of the immune response modifier TMX-202: confocal Raman microscopy and histology analysis

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Abstract. The increased ability of TMX-202 (derivative of imiquimod) to penetrate the intact stratum corneum (SC) and the follicular orifices of porcine ear skin was shown ex vivo using confocal Raman microscopy and laser scanning microscopy. Moreover, to assess whether TMX-202 is able to reach the immune cells, Langerhans cells extracted from pretreated human skin were investigated ex vivo using confocal Raman microscopy combined with multivariate statistical methods. Tracking the Raman peak of dimethyl sulfoxide centered at 690 cm⁻¹, the absorption of TMX-202 containing formulation by Langerhans cells was shown. To answer the question whether the TMX-202 active ingredient is able to reach Langerhans cells, the attraction of immune cells to TMX-202 containing formulation treated skin was measured in the in vivo rodent model Mastomys coucha. The results show that TMX-202 active ingredient is able to reach Langerhans cells after penetrating through the intact skin and subsequently attract immune cells. Both the intercellular/transcellular as well as the follicular pathways allow the penetration through the intact barrier of the SC.

Keywords: principal component analysis—linear discriminant analysis method; low concentration; traces; dimethyl sulfoxide; immune cells; Raman spectrum of Langerhans cells; Raman spectra analysis.

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1 Introduction

Toll receptor 7 (TLR7) has emerged as a particularly important and unique target for the development of drugs that modulate the innate immune system as it binds not only naturally occurring single stranded RNA but also synthetic low molecular weight ligands with classical drug-like properties, such as imiquimod (an imidazoquinoline) and purine-like molecules.¹ Acting as an immune response modifier, imiquimod is widely used as a topical agent in dermatology for the treatment of various skin diseases, such as actinic keratosis and nonmelanoma skin cancer,² which in humans are known to be linked to UV exposure, immunosuppression, and papillomavirus infection.³ Imiquimod and its derivatives modulate innate immune responses by binding to TLR7 in dendritic and Langerhans cells, monocytes, and macrophages to induce the synthesis of IFN-α and proinflammatory Th1 cytokines.⁴

Therefore, the topically applied immune response modifier must be able to overcome the cutaneous barrier in order to reach their targets and exert their function. Enhancers of penetration are important components of cosmetic and medical formulations, thus enabling active substances to enter easier through the cutaneous barrier.⁵ Passive penetration of imiquimod through the skin barrier is negligible; therefore, its combination with an enhancing carrier is an important step toward improved clinical efficacy. TMX-202 is a derivative of imiquimod with lesser systemic toxicity, prepared by conjugating to a C-12 phospholipid via a versatile benzoic acid functional group.⁶ This modification should enhance the penetration of the formulation through the skin barrier facilitating its absorption by the living cells of the stratum granulosum and stratum spinosum, including Langerhans cells. Local application of TMX-202 activates the TLR7 receptor in the immune cells, leading to their attraction to the skin.

The different routes, i.e., directly through the stratum corneum (SC) and into the hair follicles, were shown to take a part in the penetration processes.⁷ To test whether the TMX-202 is able to penetrate through the SC barrier and to reach the Langerhans cells, confocal Raman microscopic measurements were performed ex vivo on human skin samples pretreated with a TMX-202 containing formulation, and on Langerhans cells extracted therefrom. The skin samples were obtained from healthy patients undergoing abdominal reduction esthetic surgery. Additionally, the biologic effect of TMX-202, namely the attraction of immune cells, was shown in vivo in the rodent