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Letter to the Editor

In vivo study of targeted nanomedicine delivery into Langerhans cells by multiphoton laser scanning microscopy

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Abstract: Epidermal Langerhans cells (LCs) function as professional antigen-presenting cells of the skin. We investigated the LC-targeting properties of a special mannose–moiety-coated pathogen-like synthetic nanomedicine DermaVir (DV), which is capable to express antigens to induce immune responses and kill HIV-infected cells. Our aim was to use multiphoton laser microscopy (MLM) *in vivo* in order to visualize the uptake of Alexa-labelled DV (AF546-DV) by LCs. Knock-in mice expressing enhanced green fluorescent protein (eGFP) under the control of the langerin gene (CD207) were used to visualize LCs. After 1 h, AF546-DV penetrated the epidermis and entered the eGFP-LCs. The AF546-DV signal was equally distributed inside the LCs. After 9 h, we observed AF546-DV signal accumulation that occurred mainly at the cell body. We demonstrated in live animals that LCs picked up and accumulated the nanoparticles in the cell body.

Key words: eGFP-Langerin knock-in mice – *in vivo* – Langerhans cells – multiphoton laser microscopy – nanomedicine formulation

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Background

Near-infrared femtosecond laser-based fluorescence microscopy has rapidly evolved and has become capable to monitor fine morphological changes in Langerhans cells (LCs) (1,2). To track interactions between LCs and DermaVir *in vivo*, knock-in mice expressing enhanced green fluorescent protein (eGFP) under the control of the langerin (CD207) gene were used (3). DermaVir is an HIV therapeutic vaccine candidate to boost HIV-specific T-cell responses (4).

Questions addressed

1. After administering DV with the DermaPrep medical device – that interrupts *stratum corneum* – LCs take up DV via receptor-mediated endocytosis (4). Is DV able to penetrate through the intact skin of a murine ear?
2. DV-loaded LCs migrate into the lymph nodes where they express HIV antigens and induce cytotoxic T-lymphocytes (CTLs) (5,6). Does the intracellular distribution of DV nanoparticles change following their uptake by LCs? Do LCs undergo morphologic changes as a sign of their activation?

Experimental design

AF546-DV was prepared by covalently binding Alexa-546 succinimidyl ester (Life Technologies, Carlsbad, CA, USA) to the amine moieties of polyethyleneimine-mannose (PEIm). DermaVir nanoparticles were prepared using the labelled PEIm and plasmid DNA in triethanolamine buffer (pH 7.6) containing mannitol, as described previously (7). Studies using Langerin-EGFP-DTR-knock-in mice (3) and BALB/c mice were approved by the Semmelweis University Animal Care and Use Committee. The anesthetized animals were laid on a 37°C heated microscope table to keep their body temperature constant. In our experiments, the ears of the AF546-DV-treated animals were examined (after 1, 9 and 24 h of the treatment). The *stratum corneum* interruption was performed with DermaPrep medical device using the exfoliating sponge and stripping tapes to remove residual cell matter from the skin surface. As laser light source, a broadly tunable, femtosecond pulse Ti-sapphire laser (FemtoRose 100TUN NoTouch, R&D Ultrafast Lasers Ltd, Budapest, Hungary) was used that generated approximately 190 fs pulses at a repetition rate of approximately 76 MHz. The laser's central wavelength was set to approximately 890 nm, which assured high two-photon excitation efficiency for both the AF546-DV and eGFP-labelled LCs as well as low background autofluorescence signal originating from the intrinsic autofluorescence of inherent endogenous chromophores of the skin like keratin, NAD(P)H and melanin (8).

Results

AF546-DV penetration into the deep layers of mouse ear skin

We examined the potential of AF546-DV to penetrate through the intact mouse skin barrier, when the natural surveillance activity of LCs focuses on AF546-DV exclusively. Anesthetized Langerin-EGFP-DTR-knock-in mice were placed on the microscope stage, and series of *Z*-stack images were recorded at the place of the treatment at three different time points. The compiled *Z*-stack images are presented in Fig. 1 as composite orthogonal cuts. After 1 h of treatment, AF546-DV penetrated into the epidermis reaching an average depth of approximately 10 μm from *stratum corneum*. After 9 and 24 h, diffuse distribution of AF546-DV was observed in a 5–30 μm depth range (Fig. 1).