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Fluorescence investigation of the detachment of aluminum phthalocyanine molecules from aluminum phthalocyanine nanoparticles in monocytes/macrophages and skin cells and their localization in monocytes/macrophages

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Summary
Background: Nanoparticles made from aluminum phthalocyanine (AlPc) are non-fluorescent in the nanoparticle form. Once AlPc molecules become detached from the particle, fluorescence occurs. Preliminary work showed the benefit of using aluminum phthalocyanine nanoparticles (nAlPc) for the rating of the rejection risk of skin autografts in mice by measuring fluorescence intensities of detached AlPc. Skin autografts showing a high fluorescence intensity were finally rejected suggesting an inflammatory process. In contrast, autografts with normal autofluorescence were accepted. This work was focused on the mechanism of this finding. The aim is detecting inflammatory processes and the potential use of nAlPc for PDT as a new treatment modality.
Methods: The effect of the lipopolysaccharide-stimulated monocyte/macrophage murine cell line J774A.1 on the monomerization of internalized nAlPc was tested. Further, we investigated the influence of J774A.1 cells and the normal skin cell lines L-929 or HaCaT on the dissolution of nAlPc by laser scanning microscopy and flow cytometry. Localization of AlPc molecules after uptake and dissolution of nanoparticles by the cells was surveyed.

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Results: In co-culture models composed of J774A.1 and HaCaT/L-929 cells, the AlPc fluorescence intensity in J774A.1 cells is 1.38/1.89 fold higher, respectively. According to localization measurements in J774A.1 cells it can be assumed that nAlPc is taken up via endocytosis and remains in endosomes and/or lysosomes dissolving there. Detached molecules of AlPc cause rapture of the endosomal and/or lysosomal membrane after irradiation to become quite uniformly distributed in the cytoplasm.
Conclusions: Evidence for monocytes/macrophages being the origin of the measured AlPc fluorescence in rejected skin autografts was confirmed.

Introduction

Nanoparticles or nanoemulsions with or from an appropriate photosensitizer offer a new promising drug delivery system for hydrophobic sensitizers [1,2]. They can be used for fluorescence diagnostics and treatment by photodynamic therapy (PDT) [3]. Nanoparticles made from large-dispersed aluminum phthalocyanine (AlPc) crystals are floating in water. Aluminum phthalocyanine nanoparticles (nAlPc) are therefore suitable for clinical use by the possibility of good transportation in aqueous media and penetration into tissue. They are also suitable for fluorescence diagnostics because AlPc does not fluoresce in the nanoparticle form but in the monomeric form it does [3,4]. More precisely, there is already a fluorescence occurring when AlPc molecules are arranged vertically attached to the surface of the nanoparticles during the dissolution process.

The application of nAlPc was successfully tested for the rating of the rejection risk of skin autografts by monitoring the fluorescence spectra of AlPc monomers. Skin autografts in mice which showed a high fluorescence intensity due to monomeric AlPc (especially at the border of the autografts) were finally rejected indicating an inflammation. Autografts showing normal tissue autofluorescence, however, were accepted. In comparison, a water-soluble form of AlPc produced a nonselective staining of transplanted and intact skin surface [4].

In this study, the work was focused on the conditions responsible for the monomerization of nAlPc. With the knowledge of the underlying mechanism, it will be possible to detect inflammatory processes leading to skin flap rejection and to use PDT as a treatment modality.

The method of choice to analyze the molecular mechanism is the investigation of the uptake and monomerization of nAlPc by the monocyte/macrophage murine cell line J774A.1 [5] during an inflammatory process. In a study [6] the penetration behavior of polymeric submicron particles was tested by applying them to inflamed skin in mice models. In healthy skin no penetration into deep layers was observed. However, nanoparticles from 50 to 100 nm could penetrate deeply into inflamed skin. The penetration depth of the smaller nanoparticles was increased. Furthermore, intense inflammatory cell infiltration (monocytes, neutrophils and lymphocytes) was observed.

In this paper the effect of stimulating J774A.1 cells with lipopolysaccharides (LPS) on the monomerization of nAlPc in the cells was tested by laser scanning microscopy (LSM 710 NLO, Carl Zeiss MicroImaging GmbH, Jena, Germany, incubator: PeCon GmbH, Erbach, Germany), also colocalization with different cell organelles (cell nucleus, mitochondria, lysosomes) was analyzed. Endocytosis was assumed to be the kind of uptake of nAlPc. Furthermore, we established a co-culture model with J774A.1 cells and the well-established normal skin cell lines L-929 (murine fibroblasts) or HaCaT (human keratinocytes) to check the uptake and monomerization of nAlPc in the different cell types. Co-culture models of normal cells together with cancer cells for analyzing nanoparticle uptake are already used to check the targeting specificity. Costa et al. [7] used MCF-7 cells (human breast adenocarcinoma) co-cultured with hFIB cells (normal human dermal fibroblasts) to analyze the uptake of chitosan-histidine-arginine/pDNA (rhodamine B isothiocyanate-labeled) nanoparticles by confocal laser scanning microscopy and flow cytometry. The nanoparticle uptake by cancer cells was higher than by normal cells for all tested ratios.

Materials and methods

Production of nAlPc

The water colloids of AlPc nanoparticles in a concentration of 1 g/l was received from Natural Sciences Center of A.M. Prokhorov General Physics Institute, Russian Academy of Sciences, Moscow, Russia.

Large-dispersed water-insoluble AlPc powder (FGUP GNC ''NIOPIK'', Moscow, Russia) was used as starting material for producing nanoparticles. The initial size of the large-dispersed AlPc crystals was in the range of 0.1—200 μm. 50 mg of AlPc were introduced into 50 ml distilled water and subjected to ultrasonic dispergation for 50 min using a Bandelin SONOPLUS HD2070 ultrasonic homogenizer with a KE76 attachment (20 kHz, 165 μm amplitude). This resulted in formation of a colloidal solution of AlPc nanoparticles in a concentration of 1 g/l. The hydrodynamic radius of the obtained nanoparticles measured by use of a dynamic light-scattering spectrometer (Photocor Complex, Photocor Instruments Inc., USA) was $\sim 180 \pm 60$ nm. The absorption spectra of nAlPc show the Soret band (π—π^*) in the near-UV region and the Q-bands (n—π^*) in the red region of the spectra (Fig. 1). It is significant that the colloidal solution of the nanoparticles does not fluoresce when it is excited at 633 nm (Fig. 2). A lambda-scan of nAlPc-incubated cells, however, shows emission in the red spectrum (Fig. 3).