Protoporphyrin IX distribution after intra-articular and systemic application of 5-aminolevulinic acid in healthy and arthritic joints

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ABSTRACT

Arthroscopic synovectomy, which is limited today to the large joints, is an important early treatment of rheumatoid arthritis (RA). Photodynamic therapy (PDT) is potentially to be a less invasive method of removing the synovial membrane. Therefore, in a rabbit model of RA, the accumulation of the photosensitizer Protoporphyrin IX (PPIX) after intra-articular and systemic application of ALA into arthritic rabbit knee joints was studied in skin, patella, synovial tissue, and meniscus by fluorescence microscopy. PPIX fluorescence was measured in biopsies taken at different times after application of neutral and acid ALA solutions. Significant PPIX fluorescence was observed in the synovial membrane and skin 2 and 4 hours after application. Using intra-articular application, ALA solutions prepared with pH 5.5 were at least as efficient as neutral solutions in sensitizing the synovial membrane. Skin also showed PPIX within 4 hours after application. After 24 hours, a marginal PPIX fluorescence was detected in these tissues. On the other hand, in cartilage and meniscus significant PPIX accumulation was still observed 24 hours after ALA injection. Systemic application of ALA also showed a good accumulation of PPIX. Further experiments are needed to show whether accumulation of the photosensitizer and tissue selectivity are sufficient for a successful treatment of rheumatoid synovitis.

Key Words: photodynamic therapy, ALA, Protoporphyrin IX, rheumatoid arthritis, rabbit

1. INTRODUCTION

In the western industrialized countries 1-3% of the population suffer from rheumatoid arthritis (RA). The progression of this systemic disease leads in most cases to a destruction of articular structures by the inflammatory process and often results in disability. Since the etiology of RA is still not exactly known no causal therapy exists today. Removal of the synovial membrane (synovectomy) has proved a successful local treatment, especially if performed early, but open surgery is costly and invasive and the arthroscopic synovectomy is restricted to the larger joints. Photodynamic therapy (PDT), however, could be used to destroy and remove the synovial membrane with minimal injury. This was demonstrated with the photosensitizers Photosan 3 and benzoporphyrin derivative (BPD) in a rabbit model of RA [1, 2].

5-aminolevulinic acid (ALA) induced Protoporphyrin IX (PPIX) is a second generation photosensitizer with properties which make it interesting for the PDT of RA. ALA can be applied locally or systemically, irradiation is possible only a few hours after application, and due to the rapid clearing of PPIX within 24 hours skin phototoxicity is only a minor problem. The rapid photobleaching of PPIX has the advantage that

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the dosimetry can become independent of the light dose [3, 4]. This study addresses three important questions:

1. Does the synovial membrane accumulate PPIX?
2. Does other tissue become sensitized in such a way that side effects have to be expected?
3. Which is the best method of applying ALA?

Experiments were performed in a rabbit model of RA, in which, by an immunological reaction, inflammatory symptoms of the RA were induced in the rabbit's knee joints. Histologically all important symptoms of the proliferative phase of RA can be found in this model [5]. PPIX distribution in different tissues was studied 2, 4, and 24 hours after intra-articular and systemic application by fluorescence microscopy.

2. MATERIAL AND METHODS

In order to induce symptoms of RA a total of 18 New Zealand white rabbits weighing between 2.7 and 4.5 kg were sensitized by a 2 ml intradermal injection of 4 % heat-inactivated human IgG (Baxter Gammagard) in Freund's adjunvanz. Animals showing a positive immunological reaction were injected four times intra-articular with heat-inactivated IgG in their right knee joint.

For the photodynamic therapy one group of animals was sensitized by an intravenous injection of 250 mg/kg body weight ALA (100 mg/ml in phosphate-buffered saline at pH 6). Two other groups were sensitized by an intra-articular injection of 3 ml ALA (30 mg/ml in phosphate-buffered saline) with a pH of 5.5 and 7 respectively. The animals were sacrificed 2, 4, and 24 hours after ALA-application and samples of synovial membrane, cartilage, meniscus, and skin near the joint were removed and stored immediately in liquid nitrogen. The distribution and relative intensity of the PPIX fluorescence were measured with a fluorescence microscope (Leitz Aristoplan), coupled to a sensitive cooled slow-scan CCD-camera (Princeton Instruments). The 10 and 20 μm thick cryo-sections of the samples were excited with a HBO lamp between 400 and 450 nm. Fluorescence was detected between 500 and 550 nm (green image) and 600 and 800 nm (red image). By subtraction of the green image from the red image tissue fluorescence in the red spectral range could be removed from the fluorescence images. Measurements of the intensity of PPIX fluorescence were carried out by acquiring spatial resolved fluorescence spectra of the tissue samples. A spectrograph was mounted between the fluorescence microscope and the CCD-camera allowing the emission spectra of a whole line to be to measured simultaneously. In order to compensate for variations in the sample thickness and the excitation, the intensity of the PPIX fluorescence was normalized by the green autofluorescence of the tissue. By measuring complete spectra instead of taking fluorescence images at a few fixed wavelengths it is possible to subtract accurately the fluorescence background of the tissue and the optics. The normalized PPIX-fluorescence I_{PPIX} was calculated from the fluorescence intensity I_N at four wavelengths:

$$I_N = \frac{I(635\,nm) - I(616\,nm)}{I(525\,nm) - I(770\,nm)}$$

Multiplication of I_N by the mean tissue autofluorescence at 525 nm allows comparison of the absolute fluorescence intensity I_{PPIX} in the different tissues.

3. RESULTS

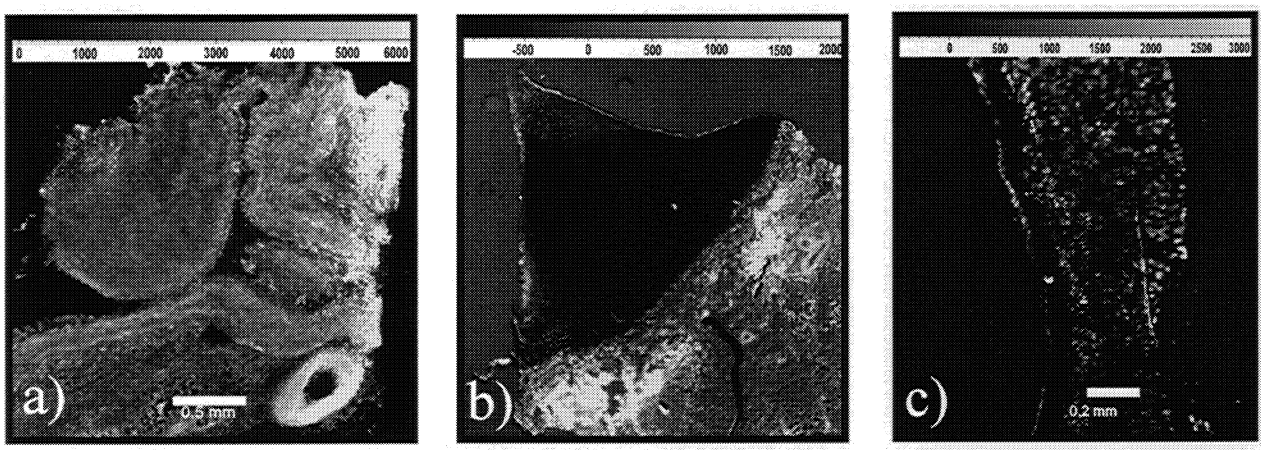

Fig. 1. Fluorescence images of cryo-section from arthritic rabbit joints after systemic application of ALA:
a) synovial membrane (4 hours application),
b) meniscus (2 hours application), and
c) cartilage (24 hours application).

All animals developed the symptoms of a heavy synovitis with swelling, thickening of the endothelial layer and a massive infiltration of leukocytes. Histological examination of the articular tissue after intra-articular application of ALA revealed no adverse effects of the ALA solution. Even the acid solution with pH 5.5 was well tolerated.

PPIX-fluorescence was observed in all tissue samples. The synovial membrane showed PPIX in the endothelial layers, the infiltrating leukocytes and the tunica media of the supporting blood vessels (Fig. 1a). In the skin PPIX was accumulated in the epidermis, glands, and hair follicles after both systemic and intra-articular application (fluorescence image not shown). Since the skin samples were taken in the direct vicinity of the sensitized joint, ALA may well have been diffused from the joint to the skin. In meniscus and cartilage a spotty fluorescence pattern was observed which could be attributed to the chondrocytes, whereas the extracellular matrix was free of PPIX-fluorescence (Fig. 1b and 1c).

Measuring spatial-resolved emission spectra, PPIX accumulation at different times was calculated. In skin and synovial membrane the PPIX appears rapidly within 2 hours (Fig. 2). After 24 hours the photosensitizer has nearly completely cleared from the tissue. These data are consistent with the reported pharmacokinetics of PPIX in rabbits [6]. In the bradytrophic tissue of cartilage and meniscus the PPIX-fluorescence further increases 2 between 4 hours, and after 24 hours a significant amount of PPIX was observed.

The absolute fluorescence intensity was at a maximum in the skin. Synovial membrane and meniscus had comparable fluorescence intensities, whereas cartilage fluorescence was much weaker. Differences in application had no drastic effect on the sensitization of the synovial membrane. However a higher PPIX-

fluorescence was observed after systemic or intra-articular application with pH 5.5 compared to the neutral solution.

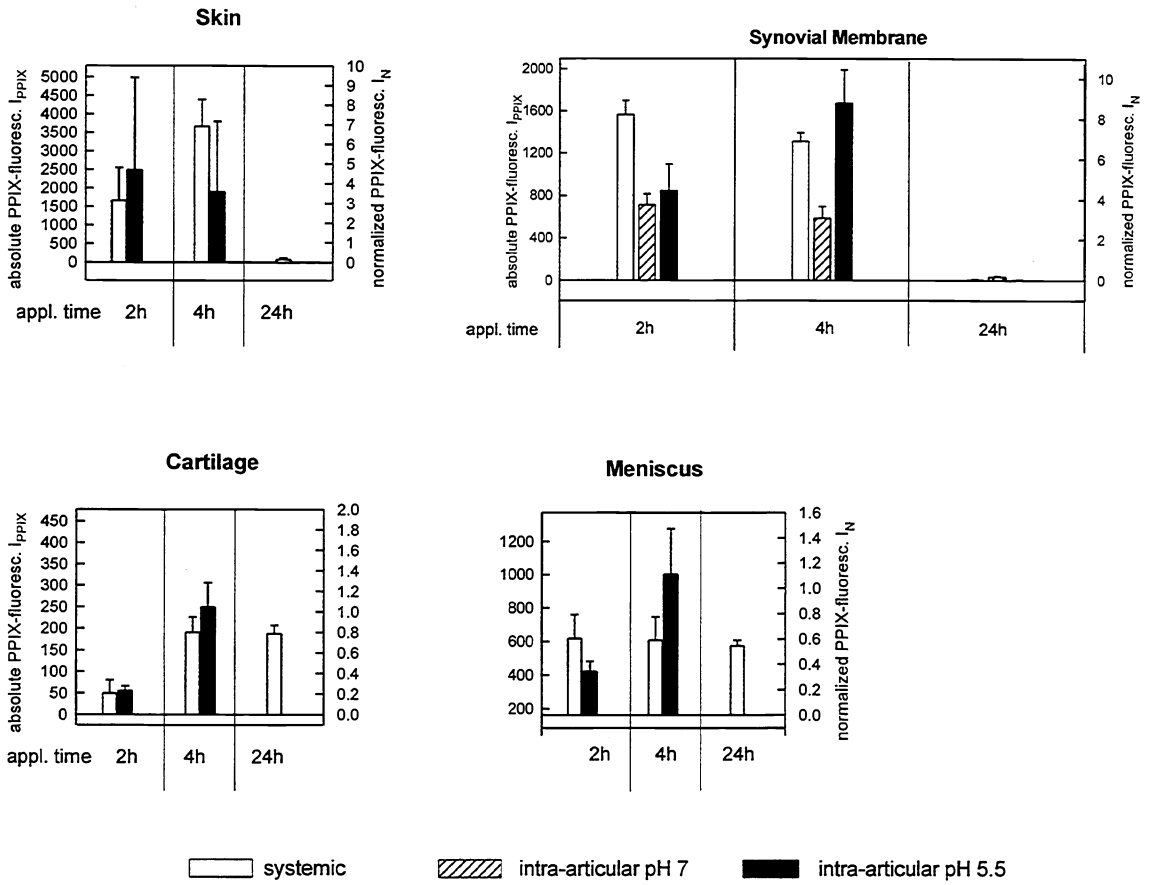

Fig. 2. Fluorescence intensity of PPIX in cryo-sections of skin, synovial membrane, cartilage, and meniscus after different times and methods of ALA application.

4. DISCUSSION

Both the systemic and the local application of ALA induced a significant accumulation of PPIX in synovial membrane, cartilage, meniscus and skin. Since the intensity of PPIX fluorescence was comparable with the fluorescence of human skin samples sensitized for the PDT of basal cell cancer [7], it is expected that the induced sensitization will be high enough to cause a photodynamic effect under irradiation.

Side effects can be expected by the PPIX accumulation in skin, cartilage and meniscus. Photosensitization of the skin near the joint to be treated cannot be avoided by intra-articular application. Since a significant amount of light may reach the surface during irradiation, skin necrosis may be possible.

The delayed appearance of PPIX in the chondrocytes of cartilage and meniscus, which can be explained by a slow diffusion process of PPIX into the collagen matrix, allows side effects to these tissues to be reduced by a short application time of 2 hours or less.

Systemic and local application of ALA is possible since the efficacy of the three different ways of ALA application did not vary very much. An acid ALA solution is preferred for intra-articular application since a neutral solution is not stable at the high concentrations used [8]. In a next step the effect of irradiation after ALA application on the different articular structures will be studied, in order to demonstrate the feasibility of photodynamic destruction of the synovial membrane with this sensitizer.

5. REFERENCES

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