

In vivo Investigation of The Skin Redox Status Using EPR Imaging

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Abstract

Electron paramagnetic resonance imaging (EPRI) instrumentation at S-band (2.2 GHz) has been developed to enable *in vivo* mapping of the redox status of the skin of rats. ¹⁵N-PDT (4-oxo-2, 2, 6, 6-tetramethyl piperidine-d₁₆-1-¹⁵N-oxyl) used as redox probe. Superoxide dismutase (SOD)-mimetic compound (dichloro (4R,9R,14R,19R)-3,10,13,20,26-pentaazatetracyclo [20.3.1.0^{4,9}.0^{14,19}] hexacos-1(26),22(23),24-triene) was used to induce alterations in the redox status by mediating the oxidative stress and antioxidant potential of the skin constituents. Skin metabolic properties interpreted as the reduction rate constants were obtained. From the EPRI of the SOD-mimetic treated skin, it was observed that the nitroxide in the deeper layer was diminished faster.

Introduction

Various skin diseases are related to the exterior oxidative stress as well as the interior imbalanced production of oxidants such as superoxide anions (O₂^{•-}). SOD enzymes and mimetic compounds can decrease the level of O₂^{•-} and maintain a normal cellular superoxide radical concentration that is critical to the cell viability. Electron paramagnetic resonance imaging (EPRI) has been developed for measuring the kinetics and spatial distribution of free radicals in biological systems (1,2). Nitroxide free radicals have been used as spin probes. A non-charged SOD-mimetic compound was introduced to the skin prior to the application of nitroxide radicals. It was observed that with the topical application of SOD-mimetic, the overall reduction rate of nitroxide radicals was 2.5 fold faster and EPRI showed relatively faster depletion of nitroxide in the deeper dermis and hypodermis area.

Materials and Methods

The nitroxide used was ¹⁵N-PDT with a concentration of 30 mM. The SOD-mimetic was M40403 (MetaPhore Pharmaceuticals, Inc.) with a concentration of 5 mM. Sprague rats were anesthetized with 50 mg/kg pentobarbital i.p. Two circular spots upon the left and right lateral aspect of the legs were sheared (Fig. 1). The right side was pretreated with 50 μ l of 5 mM SOD-mimetic for 30 min. As control, 50 μ l of water was applied to the left side. When the skin spots were completely dried, 3.5 μ l of 30 mM ¹⁵N-PDT solution was applied to the marked skin spots. After 5 minutes, *in vivo* EPR spectroscopy and imaging measurements were performed on a custom-built S-band spectrometer with a surface loop-gap resonator. The parameters were as follows: frequency 2.2 GHz; scan width for the whole spectrum 60 G, scan width for the imaging profile 16 G; scan time 15 sec; gradient magnetic field 50 G/cm.

Results

EPR spectroscopy The penetration of nitroxide into the skin could be approximated as a first order diffusion process with a rate constant k_p . Due to the flow restriction of the sample holder, the main clearance of nitroxide radicals in the skin was the bioreduction by the skin reducing equivalents in the keratinocyte and fibroblast cells with a rate constant k_r (3). The time courses of the decay process of the ¹⁵N-PDT signal intensity were recorded and simulation was performed at both treated and untreated sites. More than 20 rats were studied and it was found that the skin properties were quite different from each other. So we used the relative penetration and reduction rate constants defined as the ratio of the rate constants of the treated skin to that of the untreated skin. Fig.2 shows the statistical studies of the relative penetration and reduction rate constants of 10 rats as $k_p(\text{SOD}/\text{control}) = 1.9 \pm 0.2$ (p value 0.004, $\pm$ SD) and $k_r(\text{SOD}/\text{control}) = 2.6 \pm 0.5$ (p value 0.005, $\pm$ SD). These data indicate that with the pretreatment of SOD-mimetic, the relative reduction rate constant increases 2.6 times. It suggests that the skin redox status is greatly altered by the treatment with SOD-mimetic.

ID spatial imaging In order to depict the spatial distribution of the radicals in the skin and to differentiate the different reduction property of different skin layers, 1D EPRI was performed. The left peak of the EPR sharp component of ¹⁵N-PDT was used for the 1D EPR imaging.

Fig. 3a and 3b show the time dependent 1D spatial profile of the distribution of ¹⁵N-PDT in both control and SOD-mimetic pretreated skin of one animal. There was only one band of nitroxide distribution in the untreated and treated skin. The thickness of this band was around 2 mm. On the epidermis region defined by a thickness of 0.5 mm, the nitroxide distribution was much stronger than that in the deeper region of the skin defined by a skin depth from 0.5 to 2 mm. On the control skin, the distribution of nitroxide stayed longer in the epidermis and deeper into the skin. With the treatment of SOD-mimetic, the distribution of nitroxide decayed rapidly and was cleared out faster in the deeper skin layer. The image data suggested that with the pretreatment of SOD-mimetic, the reducing ability at both the epidermis and dermis layer was increased and an even faster radical reducing potential in the deeper layer was observed.

Summary

The kinetic data showed that permeability and metabolic behavior of the skin were increased considerably on pretreatment with SOD-mimetic. The time dependent spatial distribution of nitroxide radicals was mapped with a spatial resolution of 50 μ m. The nitroxide compound was distributed in a continuous single band and the thickness of this band was about 2 mm. The EPR images also demonstrated that SOD-mimetic changed the distribution of the nitroxide in the skin by increasing the decay on both the epidermis and dermis layers and resulted in faster reduction in the deeper skin layers.

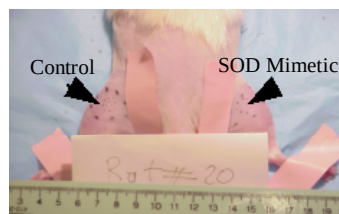


Fig. 1 Photograph of the rat showing two spots on the legs for the application of SOD and nitroxide.

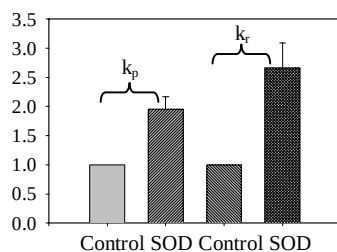


Fig. 2 Relative penetration and reduction rate constants with and without SOD treatment.

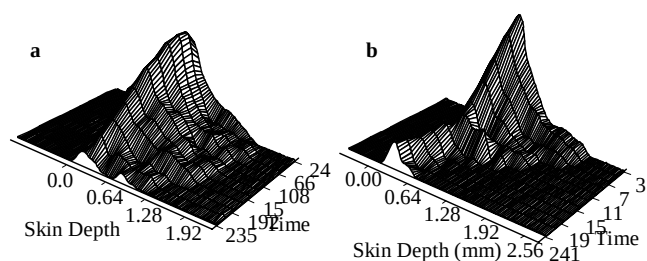


Fig. 3 The time dependent 1-D spatial distribution of ¹⁵N-PDT in the control (a) and SOD treated (b) skin spots.

Reference

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