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The influence of reactive oxygen species and NO on oxidative metabolism and dielectric properties of living tissue

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ABSTRACT

The aim of this work was a comprehensive assessment of the action of reactive oxygen species and nitric oxide on the scar tissue fragment ex-vivo. The study was performed using fragments of scar tissue (n=10) removed intraoperatively in patients with Dupuytren's contracture and preserved in an isotonic solution of sodium chloride until the beginning of the experiment (for saving of living state of cells in specimens). Each fragment was divided into 3 equal parts. No manipulations were performed to the first part, the second one was treated with singlet oxygen, the third piece was processed with nitrogen oxide (20 ppm). The duration of the tissue treatment period was 5 minutes. Upon completion of the experiment in all samples, the methods of near-field resonance microwave sensing evaluated the dielectric properties of tissue using a software package developed at the Institute of Applied Physics of the Russian Academy of Sciences. Further, each portion of the tissue was homogenized using the certified apparatus "UltraTurrax" according to the standard procedure. The parameters of oxidative metabolism (intensity of free radical oxidation and total antioxidant activity) were studied in the homogenates by Fe - induced biochemiluminescence. It was found that the treatment of scar tissue fragments by gas flow from singlet oxygen and nitric oxide generators leads to a change in the dielectric properties of the tissue and the intensity of free radical processes in it, and the nature of the response is specific to the influencing factor (NO or singlet oxygen). It is shown that the singlet oxygen effect is a moderate increase in the dielectric permeability of the tissue and a balanced stimulating effect on the pro - and antioxidant systems. The NO effect at a concentration of 20 ppm is associated with a marked increase in dielectric permittivity and conductivity, as well as a significant increase in the antioxidant potential of the tissue.

Keywords: *Reactive oxygen species, nitric oxide, oxidative metabolism, tissue dielectric properties, microwave probing*

1. Introduction

Modern physiotherapy has a wide range of diverse therapeutic application. At the same time, their type is constantly expanding. Thus, since some decades ago, ozone therapy, has found application in various fields of practical medicine [1]. Our previous works and also from other teams have shown that not only ozone, but also other exogenous forms of oxygen and nitrogen can have positive effects on living systems [2-7]. In particular, positiveshifts for low concentrations of nitrogen monoxide (NO; 20-100 ppm) [8] and gas flow from the singlet oxygen generator were demonstrated for blood samples [3, 9, 10]. It was found that at certain levels these aforementioned applications increase the antioxidant potential of blood plasma and moderately stimulate the activity of one of the main antioxidant enzymes – superoxide dismutase [9, 10]. Also, the beneficial effect of singlet oxygen on the parameters of energy metabolism was revealed [8], however, all these results related to the treatment of blood in vitro. It should be noted that there are no data on the nature of modification of tissue parameters in the literature.

In this regard, the aim of the work was a comparative assessment of the action of reactive oxygen species and nitric oxide on a fragments of scar tissue.

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2. Material and Methods

For the experiment, a special installation was assembled (fig. 1), which allows to create a gas environment around a fragment of tissue under a glass dome, into which a gas stream was pumped from a singlet oxygen generator or NO-generator. The duration of the tissue treatment period was 5 minutes for all factors. The NO concentration in the gas stream was 20 ppm, singlet oxygen was created in the mode of 100% of the power of the generator.

The study was performed using fragments of scar tissue (n=10) removed intraoperatively in patients with Dupuytren's contracture and preserved in an isotonic solution of sodium chloride until the beginning of the experiment (for saving of living state of cells in specimens). Each fragment was divided into 3 equal parts, the first was intact (did not carry out any manipulations with it), the second one was treated with singlet oxygen, the third one was treated with nitrogen oxide.

Upon completion of the experiment in all samples, we evaluated the dielectric properties of the tissue with near-field resonance microwave sensing method using a software package developed at the Institute of Applied Physics of the Russian Academy of Sciences (Nizhny Novgorod) [11-13]. This complex allows to calculate dielectric permittivity (ε) and conductivity (σ) of the biomaterial. The depth of sounding of the biological sample is 5 mm.

Further, each portion of the tissue was homogenized using certified "UltraTurrax” apparatus according to the standard procedure is not saturated the tissue specimen by side substances. The parameters of oxidative metabolism were studied in the obtained homogenates by Fe-induced biochemiluminescence: the intensity of free radical oxidation processes (by the level of the maximum flash, I max) and the total antioxidant activity (as the value of the inverse light sum of biochemiluminescence in 30 seconds, 1/S). Biochemiluminescence analysis was performed with certified biochemiluminometer “BHL-06” (NizhnyNovgorod, Russia). It automatically registers chemiluminescence, which can be spontaneous or induced by different substances, such as Fe, luminol etc. [2, 4, 15, 16].

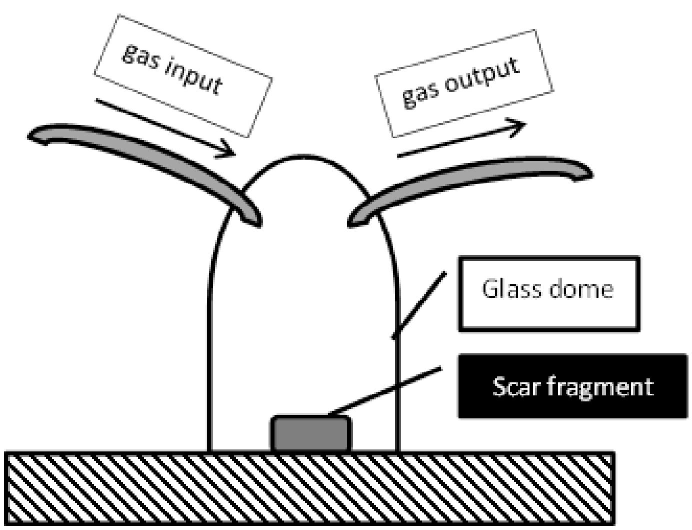

Figure 1 | Installation scheme for the experiment.

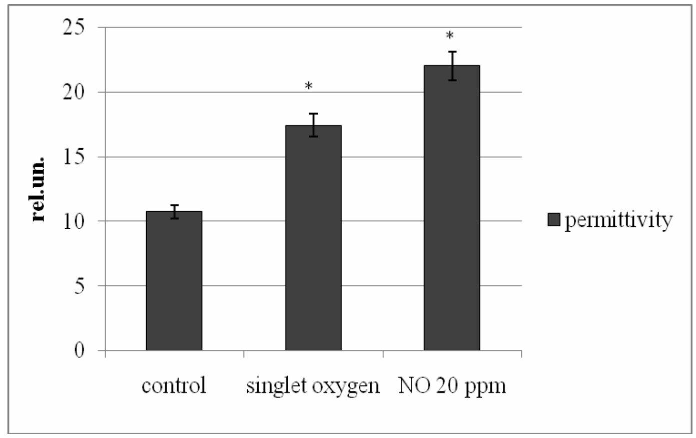

Figure 2 | The level of dielectric permittivity of intact tissues and specimens processed with singlet oxygen and NO («*» - p<0,05).

Statistical processing of the results was performed using Statistica 6.1 for Windows. The normality of the distribution of parameter values was evaluated using the Shapiro-Wilk criterion. Taking into account the nature of the distribution of the trait, the Kruskal-Wallace H-criterion was used to assess the statistical significance of the differences. The critical level of significance in testing statistical hypotheses in this study was taken to be 0.05.

3. Results

It was found that the treatment of a fragment of scar tissue by radical sources for 5 min leads to significant shifts in their dielectric properties and the state of free radical processes. Thus, both factors under consideration provide an increase in the dielectric permeability of the tissue, but the severity of this permeability depends directly on the nature of the impact (Fig. 2). In particular, the treatment of the biomaterial with a gas stream initially containing singlet oxygen leads to an increase in the parameter value by 1.63 times ($p<0.05$ relative to the intact fragment), while the effect of NO induces a more significant increase in the dielectric constant (2 times $p<0.01$ compared to the untreated piece of tissue).

The variability of the response was also found for conductivity (Fig. 3). At the same time, the effect of singlet oxygen did not contribute to the formation of shifts in this criterion, while the treatment of no biotissue at a concentration of 20 ppm led to an increase in the value of the indicator by 1.57 times ($p<0.01$ relative to the control).

Changes in the dielectric characteristics of the samples were accompanied by shifts in free-radical processes in them.

Thus, the activity of the studied factors was opposite (Fig. 4). Treatment of tissue fragments with singlet oxygen provided moderate stimulation of radical reactions is estimated by dynamics of level of the maximum flash (in

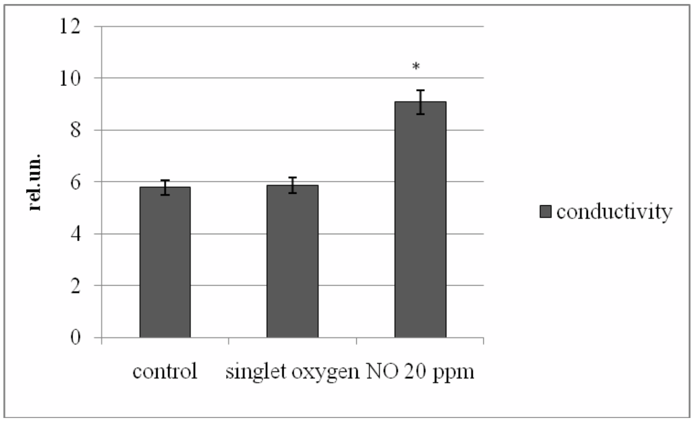

Figure 3 | The level of conductivity of intact tissues and specimens processed with singlet oxygen and NO («*» - p<0,05).

1.21 times; p<0.001 compared to the control sample, with which no manipulation was performed). On the contrary, when using a low concentration of nitrogen monoxide (20 ppm), a decrease in the studied parameter by 12.4% (p<0.05 relative to the intact tissue fragment) was found.

According to the effect on the total antioxidant activity of biomaterial, both estimated factors show a tendency to increase the value of the parameter (Fig. 5).

It was found that nitric oxide to a greater extent increases the antioxidant potential of the biological sample is measured by value of the inverse light sum of biochemiluminescence in 30 seconds (by 28.9%; p<0.05 compared with intact tissue fragment). Coupled with a decrease in the intensity of free radical processes, this indicates a pronounced antioxidant effect of nitric oxide. The impact of singlet oxygen also leads to increase of antioxidant activity of homogenates of the tissue, but these changes are expressed to a lesser extent (+15,7%; p<0.05 relative to the fragment of the biomaterial, which has not carried out impacts). In this case, it is possible to assume the balance of the factor influence on the state of Pro - and anti-oxidant systems of the biological sample.

4. Discussion

Our experimental studies were aimed at verification of the modulating effect of gaseous sources of reactive oxygen species and nitric oxide on a fragment of biological tissue. Confirmation of this effect is fully consistent with the previously obtained data on human blood samples (in vitro) and animal organism (in vivo) [8-10, 14]. It is important to emphasize that the effect is directly determined by the nature of the factor. At the same time, the features of the applied research methods allow us to conclude that in the treated tissue fragments not only metabolic reactions (primarily shifts in oxidative metabolism as a direct target of the action of reactive oxygen species and NO by dynamics of biochemiluminescence test [1, 15, 16]), but also other processes due to changes in the degree of tissue hydration (in the dynamics of dielectric characteristics of the analyzed objects depending on the content of water and aqueous solutions [17, 18]) are triggered. The given variants of the reaction can be both parallel manifestations of molecular-cellular tissue response to the action of reactive oxygen species and nitrogen monoxide, and dependent processes mediated through ROS-dependent regulatory cascades that determine the permeability of cell membranes for water molecules [15-19].

The obtained data allow to justify the expediency of local therapy carried out and the use of singlet oxygen generators and nitric oxide, but to clarify the nature of the processes involved, it is necessary to decipher the mechanisms for the implementation of the identified effects. This task is expected to be solved in further studies

5. Conclusions:

The experiment made it possible to establish that the treatment of scar tissue fragments by gas flow from singlet oxygen generators and nitrogen monoxide leads to a change in the dielectric properties of the tissue and the intensity of free radical processes in it , and the nature of the response is

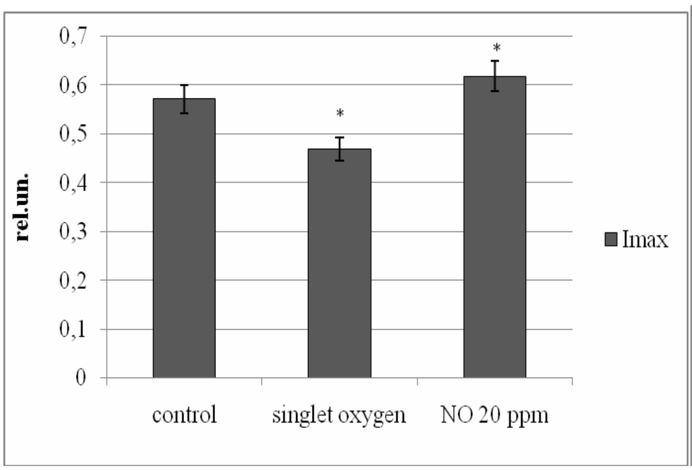

Figure 4 | Intensity of free radical processes of intact tissues and specimens processed with singlet oxygen and NO («*» - p<0,05).

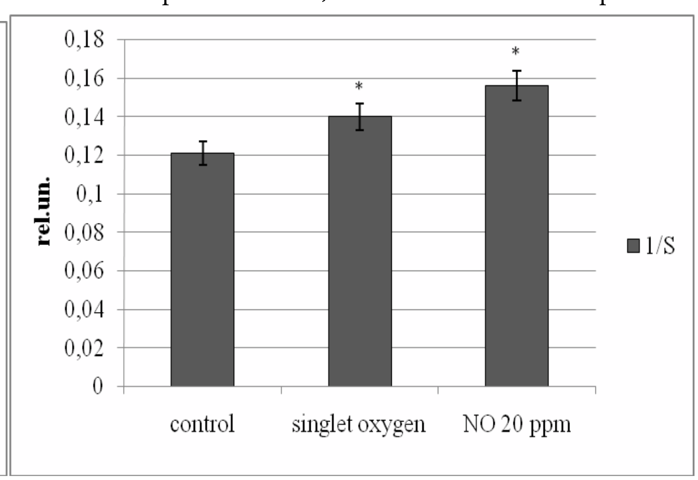

Figure 6 | Total antioxidant activity of intact tissues and specimens processed with singlet oxygen and NO («*» - p<0,05).

specific with respect to the nature of the influencing factor. It is shown that the peculiarity of the singlet oxygen action is a moderate increase in the dielectric permeability of the tissue and a balanced stimulating effect on pro- and antioxidant systems. The effect of NO at a concentration of 20 ppm is associated with a marked increase in dielectric permeability and conductivity, as well as a significant increase in the antioxidant potential of the tissue.

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