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Communication

Cold helium plasma as a modifier of free radical processes in the blood: in vitro study

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Abstract: We studied the influence of various exposures (1, 2, 3, 5, 10 and 15 min.) effects of cold helium plasma on the state of free radical processes in the biological fluid (human blood). It was found that the nature of the response of antioxidant systems of blood to cold plasma treatment is directly determined by exposure, as evidenced by the results of chemiluminescence assessment of the intensity of free radical processes and total antioxidant activity, as well as the dynamics of the concentration of malonic dialdehyde. At the same time, the short-term processing mode of biological fluid allows us to establish the presence of a two-phase influence of the factor on oxidative processes ("the phenomenon of the antioxidant window").

Keywords: cold helium plasma; free radical processes; malonic dialdehyde; blood

1. Introduction

Currently, cold plasma is considered as a new physical therapeutic factor that has a number of positive biological effects [1–5]. On the other hand, the focus of research is mainly on its antibacterial activity [1,6,7], due to the high oxidative potential of this effect [1–3,7–9]. The presence of the latter is

due to the fact that the treatment of surfaces and biological objects with cold plasma contributes to the intensive generation of radicals in them (primarily active forms of oxygen [8–12]). In turn, the resulting stimulation of free radical formation provides the necessary antibacterial effect [1–5]. It is important to note that it requires a sufficiently long exposure to cold plasma [1–3,5,6]. On the contrary, the effect of short-term processing by this factor on biological systems has been studied significantly less. There are only isolated reports of significantly lower exposure to cold plasma (from 35 seconds) to provide an antibacterial effect [13]. At the same time, there are some works that indirectly indicate a special effect on free-radical processes of short-term cold plasma treatment [8–10], as indicated by our previous works [4], but direct evidence of this phenomenon has not been found in the literature. There is also no comparative assessment of modification of radical reactions in biological systems under short-term and long-term exposure to cold plasma.

In this regard, the aim of the work was to compare the effect of different exposures of cold plasma on the state of free radical processes in the biological fluid (human blood).

2. Materials and Method

Our *in vitro* experiment was performed on 15 blood samples of healthy volunteers (3.5 ml each). Earlier in our research [4] and the work of other authors [5,6,11], it was shown that this approach, based on the analysis of the effects of physical effects on blood samples, is convenient and informative. Each sample was divided into 7 equal portions, the first of which was a control one (no manipulations were performed), other samples were treated with cold helium plasma. The duration of exposure for the experimental portions (from second to seventh) was 1, 2, 3, 5, 10 and 15 minutes, respectively. The distance from the end of the "plasma torch" to the surface of the biological fluid was equal to 1.0–1.5 cm. The duration of exposure after cold plasma treatment is 5 minutes.

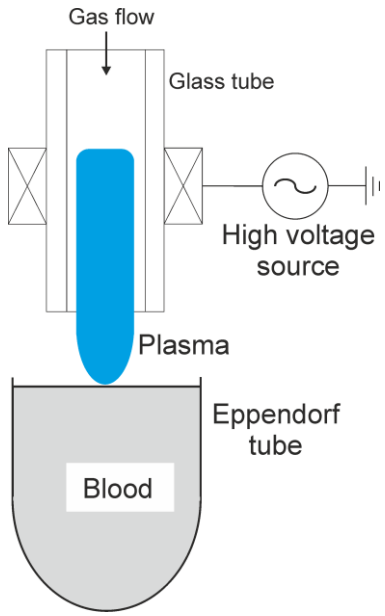

Figure 1. A schematic diagram of the cold plasma device and experiment installation.

Cold plasma was produced using a special device using the principle of microwave-induced ionization of the gas stream and developed at the Institute of Applied Physics of the Russian Academy of Sciences (Nizhny Novgorod) [Figure 1]. As a plasma carrier gas, we used balloon helium of grade A (purity level–99.99%). The temperature of the weakly ionized gas jet was 32 degrees Celsius, and the average power of the source did not exceed 20 W.

The intensity of free radical processes in blood plasma was studied on the apparatus "BHL-06" (Nizhny Novgorod, Russia) by Fe-induced biochemiluminescence [14,15]. For this analysis we created reaction mixture, including: 1) blood plasma; 2) standard phosphate buffer; 3) water solution of iron hydroxide; 4) hydrogen peroxide. Two main parameters were registered. They are the maximum level of flash light (Imax) as index of total intensity of free radical processes and reverse light sum (1/S) as indicator of total antioxidant activity of the biological fluid. The positive control for this method was *tert*-Butyl hydroperoxide (TBHP).

The level of malonic dialdehyde (MDA) in red blood cells was assessed using a test kit (JSC "AGAT", Russia).

The results were processed using the Statistica 6.0 program. The normality of the distribution of parameter values was evaluated using the Shapiro-Wilk criterion. Taking into account the nature of the attribute distribution, the Kraskal-Wallace H-test was used to assess the statistical significance of differences. Data was presented in the format M ± m. The differences were considered significant at a significance level of $p < 0.05$. We calculated the true level of statistical significance of differences in the average values of indicators.

3. Results

The conducted studies allowed us to establish that the level of maximum photo-flash of biochemiluminescence in blood samples progressively increases with increasing duration of treatment with cold helium plasma (Figure 2).

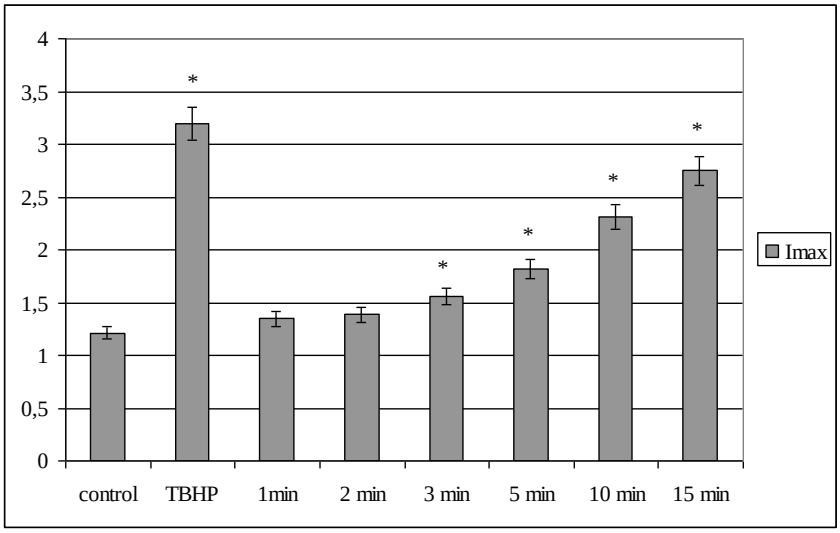

Figure 2. The level of maximal chemiluminescence flash in blood plasma depending on the duration of cold plasma action ("*"- statistical significance of differences relative to the control sample $p < 0.05$; TBHP -*tert*-Butyl hydroperoxide).

At the same time, it is important to emphasize that at low exposures (1–2 min.), the studied parameter shows a tendency to increase (+ 11.0 and + 14.1% relative to the control sample, respectively; $p < 0.1$ only for the second mode).

A rapid increase in the level of maximum flash light is observed only starting from 3-minute processing of the biological medium (+ 49.7%; $p < 0.05$) and reaching a maximum exposure of 15 min. (an increase of 2.27 times compared to the intact sample; $p < 0.01$). Taking into account the fact that the studied parameter registers the number of free radicals in the studied system, we can conclude that from the 3rd minute the concentration of radicals highly increases, and until this time there is a certain "window".

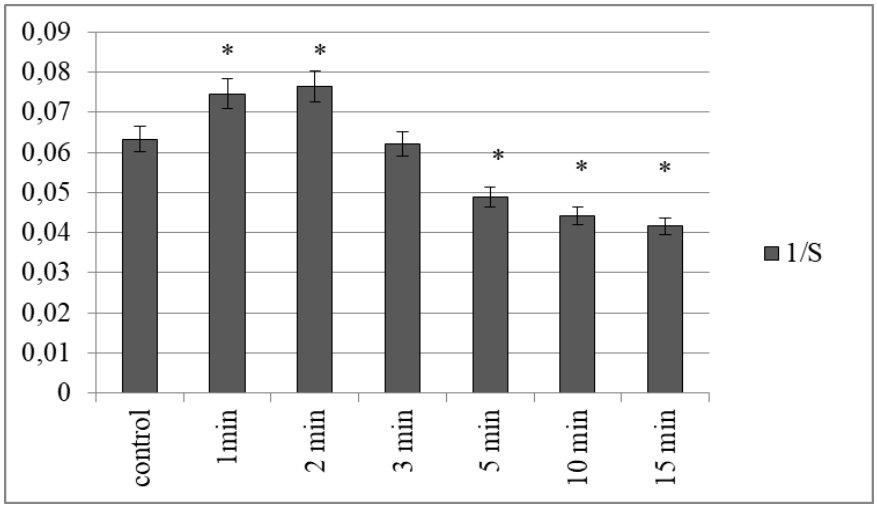

Figure 3. Total antioxidant activity of blood plasma depending on the duration of cold plasma action ("*"- statistical significance of differences relative to the control sample $p < 0.05$).

The presence of such an unusual effect of cold plasma is also evidenced by the dynamics of the overall antioxidant activity of the biological medium (Figure 3). It was found that short-term (1–2 min.) treatment of blood samples with helium cold plasma contributes to an almost identical increase in the value of the indicator (by 18.0 and 20.6% relative to the control; $p < 0.05$ for both cases), while 3-minute exposure demonstrates the level of the parameter that does not differ from the intact sample. This confirms the results of our previous in vivo studies [4]. Longer exposures of the studied factor (5 minutes or longer) reduce the antioxidant reserve of biological fluid (by 21.9–34.2%; $p < 0.05$).

The results of chemiluminescent studies are fully confirmed based on the analysis of the dynamics of the standard indicator of the intensity of free radical processes-the concentration of malonic dialdehyde in blood plasma (Figure 4).

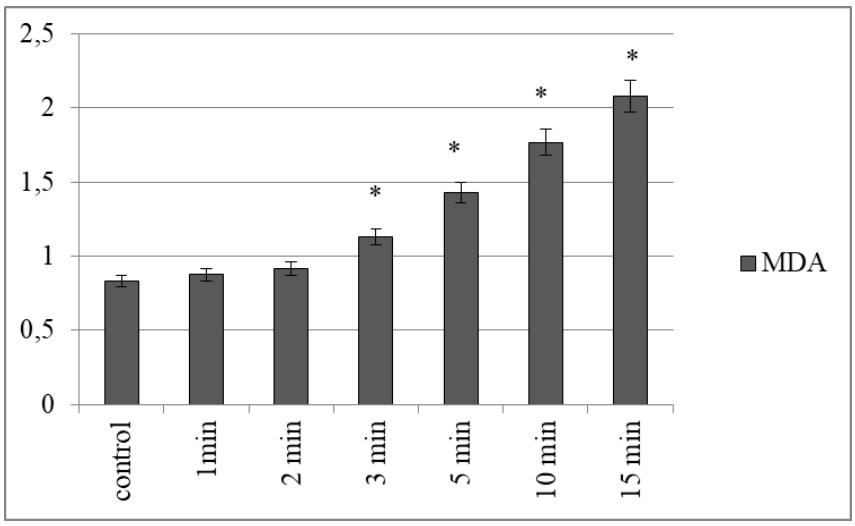

Figure 4. The level of malonic dialdehyde (mmol/l) in blood plasma depending on the duration of cold plasma action ("*"- statistical significance of differences relative to the control sample $p < 0.05$).

The results of chemiluminescent studies are fully confirmed based on the analysis of the dynamics of the standard indicator of the intensity of free radical processes-the concentration of malonic dialdehyde in blood plasma (Figure 4).

It was found that its level increases insignificantly with short exposure to cold plasma (1-and 2-minute treatment), increasing only by 5.2 and 9.7% compared to the control sample ($p > 0.05$). On the contrary, starting from the 3-minute mode (+ 36.0%; $p < 0.05$), an almost linear increase in the parameter value was recorded, up to the maximum exposure (15 min.), at which there was an increase in the concentration of the studied secondary product of peroxidation by 2.50 times ($p < 0.05$).

Additionally, we have shown that cold helium plasma does not affect the morphology of red blood cells in all used exposures, which indirectly indicates that there is no cytotoxic effect of exposure. More research is needed to clarify this issue.

4. Conclusion

Our research allowed us to establish that the nature of the response of antioxidant systems of the blood to treatment with a stream of cold helium plasma is directly determined by exposure, as evidenced by the results of chemiluminescence assessment of the intensity of free radical processes and total antioxidant activity, as well as the dynamics of the concentration of malonic dialdehyde. At the same time, the short-term processing mode of biological fluid allows us to establish the presence of a two-phase influence of the factor on oxidative processes ("phenomenon of the antioxidant window", the limit of which for our exposure conditions was an exposure of 2 min.). This fact confirms the presence of an antioxidant effect in cold plasma, which was previously demonstrated in a few works performed on biological objects [16,17]. We assume that at short-time exposures to cold plasma, the resulting moderate amounts of reactive oxygen species have a bioregulatory effect, as is observed during low-dose ozone therapy [18–21] and nitric oxide [22]. Thus, according to the fluorescence assessment, the concentration of reactive oxygen species during 1-minute treatment

with helium plasma of fibroblast culture does not differ from the control level [9]. Similar data were obtained on isolated cells by other authors for 1–3 minute exposure [10–12]. However, to reveal the mechanism of the phenomenon, additional research is required.

Conflict of interest

The authors declare no conflict of interest.

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