Microwave study of the effect of cold argon plasma on functional state of rat's skin

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Abstract. The purpose of this study was a comparative study of the dielectric parameters of rat skin when treated with argon and argon cold plasma. The experiment was performed on 40 male Wistar rats divided into 4 equal groups. The first group of animals (n=10) was a control (intact). The rats of the remaining groups (n=10 in each) were treated daily with a pre-epilated area of the skin of the back (area = 1x1 cm). The duration of the course for all experienced groups is 10 procedures. Animals of the second group were treated with a non–ionized argon stream (the duration of one procedure was 1 minute), rats of the third and fourth groups were treated with argon cold plasma (1 and 2 minutes, respectively). Cold plasma generation was performed using a device using the principle of microwave ionization of a gas stream. Argon of high purity (99.99%) was used as the latter. The dielectric parameters of the skin of animals in the treated area (in control group rats – at a similar point in the back) were evaluated upon completion of a full course of exposure. For this purpose, a specialized software and hardware complex was used, providing near-field resonant microwave probing of biological tissues. It was found that the course treatment of the skin of the back of rats with gas streams with different ionization causes the formation of a specific functional-metabolic and morpho-structural response. Its character is determined by the parameters of the gas flow used: non-ionized argon significantly reduces the dielectric parameters (permeability and conductivity), and the result of the action of cold argon plasma depends on the exposure. During one-minute treatment, tissue permeability was observed to remain intact with a moderate decrease in conductivity. In the case of an increase in the exposure time to 2 minutes, the dielectric constant increased, and the conductivity remained unchanged.

1 Introduction

To date, it has been convincingly shown that cold plasma is a universal bioregulator with a wide range of biological effects [1-3]. Among them, in particular, are antibacterial1, [4-6], antiviral and fungicidal action [7-9], the ability to activate hemocoagulation [10], a positive

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effect on regenerative processes [5,11,12], etc. On this basis, it is assumed that the cold plasma of atmospheric pressure can be used as a basis for the creation of innovative biomedical and veterinary technologies, as well as biotechnologies (for example, to increase the yield of some crops, combat plant diseases, etc. [13,14]).

On the other hand, plasma medicine, given its short history of development as a scientific field, still has a large number of unresolved issues. Thus, the biophysical aspects of plasma interaction with living objects are not fully understood, the molecular mechanisms of this process, as well as its limiting parameters, are not fully disclosed [15,16]. It can be assumed that the latter include the characteristics of the plasma itself (the conditions and medium of generation, the type of ionizing source, the voltage supplied), the presence of modulators (ionization of the air flow or monocomponent inert gases, the use of oxygen, nitrogen, etc.). In addition, the key indicator that can vary the operator conducting the impact on the biosystem is the treatment exposure, since the previously specified parameters in most cases are unregulated and serve as the initial "rigid constants" of a particular device for generating cold plasma. At the same time, the optimal modes of application of the factor in question remain controversial.

To control the role of these parameters in determining the characteristics of the response of the biological system to the action of cold plasma, it is necessary to use highly informative, but non-invasive diagnostic methods. Such, in particular, is near-field resonant microwave sounding, which allows integral study of the structure of tissues, as well as their layered examination (tomography) [17-19]. This diagnostic technology continues to develop, but the effectiveness of its use for assessing the condition of the skin (dermatoses, tumors of the integumentary tissues, burns, etc.) has already been demonstrated [17-22]. Consequently, near-field microwave sensing can act as a diagnostic search tool and to reveal the local effects of cold plasma in in vitro and in vivo experiments, as well as their features depending on the exposure parameters [18,21-24].

Therefore, the purpose of this study was a comparative study of the dielectric parameters of rat skin when treated with argon and argon cold plasma.

2 Material and methods

The experiment was performed on 40 sexually mature male Wistar rats (body weight - 250-300 g.), divided into four groups of equal numbers. The first group of animals (n=10) was a control (intact), no manipulations were performed with its representatives, except for a single study of the dielectric properties of the skin. The rats of the remaining groups (n=10 in each) were treated daily with a pre-epilated area of the skin of the back (area = 1 x 1 cm). The duration of the course was the same for all the experimental groups and was 10 procedures. Animals of the second group were treated with a non–ionized argon stream (the duration of one procedure was 1 minute), rats of the third and fourth groups were treated with argon cold plasma (the duration of each exposure was 1 and 2 minutes, respectively).

Cold plasma generation was performed using a device of an original design using the principle of microwave ionization of a gas stream [25,26]. The gas supply rate was strictly controlled for all procedures. As the latter, high purity argon (99.99%) was used to exclude the influence of impurity gases on the effects of the studied monocomponent plasma.

The dielectric parameters of the skin of animals in the treated area (in control group rats – at a similar point in the back) were evaluated upon completion of the full course of exposure. For this purpose, a specialized software and hardware complex was used, providing near-field resonant microwave probing of biological tissues [21,23]. The spectrum of the main evaluation indicators includes the dielectric permittivity of the medium and its conductivity, calculated by the degree of shift of the resonant sounding frequency [20,21,23]. Probing was carried out by a single integrated sensor to a depth of 5 mm. under the surface of the skin.

Conducting research is regulated by the permission of the local ethics committee of the Privolzhsky Research Medical University of the Ministry of Health of the Russian Federation.

Statistical processing of the results was carried out using the Statistica 6.0 program. All parameters were expressed as (mean ± SD). T-test was used for comparison among the four studied groups. The P-vales <0.05 were considered statistically.

3 Results

It is established that the considered physical factors have a multidirectional effect on the dielectric parameters of the rat skin (Fig. 1 and 2). Thus, according to the level of dielectric permittivity of tissues, it was shown that the course treatment of the skin area of the animal's back with a stream of non-ionized argon moderately reduces this indicator (by 1.15 times; $p<0.05$ relative to rats with which no manipulations were performed).

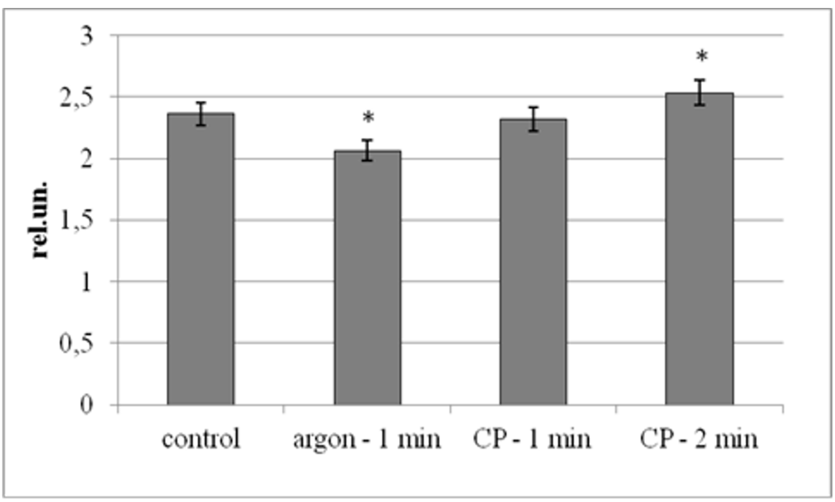

Fig. 1. The level of dielectric permittivity of the skin of the back of animals under various types of treatment (CP - argon cold plasma; "*" - differences relative to rats of the control group are statistically significant, $p<0.05$)

The use of an ionized flow of the used carrier gas, at least, kept the dielectric permittivity of the skin at the level of animals of the control group, however, in this case, the dependence of the response on the duration of treatment was recorded. At an exposure of 1 minute, the parameter value did not differ from the control, while doubling the duration of exposure contributed to its moderate, statistically significant increase (by 7.2%; $p<0.05$ compared with rats of the intact group). Taking into account the fact that the dielectric permittivity of the tissue, being determined by its morphostructural characteristics, significantly depends on the degree of hydration, it can be assumed that the course treatment with non-ionized argon reduces the intensity of local circulation in this area of the skin. On the contrary, argon cold plasma either keeps it at its initial values (with a minimum duration of exposure), or moderately stimulates it (with an increase in the duration of the procedure). To clarify this effect, a direct determination of the state of microcirculation at the treatment site is required.

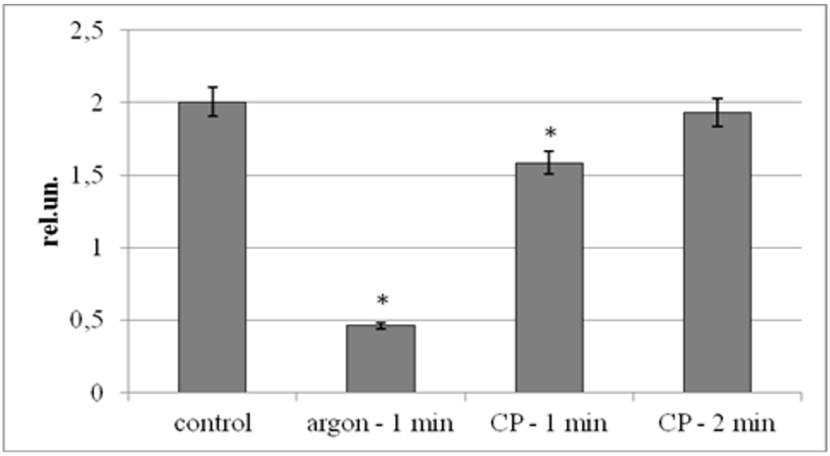

Fig. 2. The level of dielectric conductivity of the skin of the back of animals under various types of treatment (CP - argon cold plasma; "*" - differences relative to rats of the control group are statistically significant, $p<0.05$)

4 Conclusion

The experiments made it possible to establish that the course treatment of the skin of the back of rats with gas streams with different ionization causes the formation of a specific functional-metabolic and morphostructural response. Its character is determined by the parameters of the gas flow used: non-ionized argon significantly reduces the dielectric parameters (permeability and conductivity), and the result of the action of cold argon plasma depends on the exposure. During one-minute treatment, tissue permeability was observed to remain intact with a moderate decrease in conductivity. In the case of an increase in the exposure time to 2 minutes, the dielectric constant increased, and the conductivity remained unchanged. This creates prerequisites for choosing the optimal mode of application of cold argon plasma in regenerative biomedicine and veterinary medicine.

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