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**EFFECT OF CRYOPRESERVED FRAGMENTS OF PIGLET'S SKIN
EXTRACT OR AQUEOUS COLLOIDAL SOLUTION OF FULLERENE C₆₀
ON WOUND HEALING AFTER SKIN CRYOABLATION**

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Introductions. Cryoablation is widely used in surgery to treat skin tumors. After skin cryoablation, a wound always appears, the healing rate of which strongly affects the treatment results. The extracts of cryopreserved pig organ fragments and newborn piglets are known to stimulate reparative regeneration under experimental pathological conditions. C₆₀ fullerenes have unique physicochemical properties, they are able to interact with lipid biological membranes and penetrate into a cell. Now it is commonly known that fullerenes exhibit immunomodulatory, anti-inflammatory, antibacterial, antiviral activities, effectively capture free radicals and act as powerful antioxidants. Taking onto account the unique properties of C₆₀ fullerenes, exploring

their possible use to improve wound healing after skin cryodestruction looks promising.

Aim. The research purpose was to evaluate macroscopically the wound healing after cryoablation of skin and introduction of cryopreserved fragments of piglet's skin or aqueous colloidal solution of fullerene C₆₀.

Materials and methods. The studies were performed in 6-month-old white male rats in accordance with the Law of Ukraine "On the Protection of Animals Against Cruelty" (No. 3447-IV dated of February 21st, 2006), meeting the requirements of the Committee in Bioethics of the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv), agreed with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The experimental animals were divided into 3 groups of 10 animals in each.

The skin cryoablation was performed on lateral thigh surface of hairless rats using a cryoinstrument (with 10 mm applicator diameter, 120 s exposure time). The temperature of the working surface of the cryoapplicator did not increase above - 50°C during low-temperature exposure. The animals of one experimental group were administered with an extract of cryopreserved piglets' skin fragments at a dose of 500 µg / kg once a day throughout the experiment. Animals of the second experimental group were administered with an aqueous colloidal solution of fullerene C₆₀ for 5 days at a dose of 0.1 mg / kg. Rats of the control group were injected with a saline. All the solutions were injected into abdominal cavity. Macroscopic evaluation of wounds was performed on days 7, 14 and 21 of the experiment. The area of wounds was determined using the digital images with the Image J 1.5 software. Of the obtained results were statistically processed using the Statistica 6.0 and Microsoft Excel 2003. When comparing the parameters we used nonparametric methods (Kruskal-Wallis test). Significance of differences between group mean values was assumed at significance level of $p < 0.05$

Results and discussion. In the control group on day 7 after cryodestruction the moderate hyperemia and edema, infiltration of the edges of wounds, inflow of purulent secretion from under the scab of yellow-green color with a characteristic odor were observed, when checking at the bottom of the wounds boundary accumulations of pus were found. In the group with the introduced extract there were observed the wounds under the scab of red-brown color, with no signs of suppuration. The edges of the wound were quite straight, there was a moderate hyperemia somewhere, no swelling was found. With the introduced solution of fullerenes on day 7 the wounds were under the scab of red-brown color with no signs of suppuration. Edges of the wound were quite leveled, no hyperemia and edema were observed. No significant differences in the area of wound defects at day 7 were revealed.

On day 14 the wounds in the control group were of round and oval shapes under the scab of yellow-brown color, the local signs of inflammation were slightly expressed, at times there was hyperemia of the edges of the wound defect, somewhere there were the sites of serous discharge from the scab, in the revision of the wounds no fluid and purulent accumulations were found.

In the group with the introduced extract on day 14 the wounds had oval shape under the red-brown scab, the edges of the scab were raised. Hyperemia, swelling and infiltration of the wounds were not observed, there was no discharge from the wounds. The area of wounds on day 14 in the group with the introduced extract did not differ from the control values.

On day 14 in the group with the introduced solution of fullerenes of the wounds were of oval shape under a thin brown scab with raised edges or just without scab. Hyperemia, swelling and infiltration of the wounds were absent, no secretion from the wounds.

The area of wounds in the group with the introduced fullerene solution was 1.7 times smaller than that of the control group and 1.65 times smaller than that of the group with the introduced extract.

On day 21 in the control group of the oval wound under a thin vein no local signs of inflammation and discharge were observed. In the experimental groups, a

complete epithelialization of wounds of round or oval shapes was observed, no scab or just thin one were found. Local signs of inflammation and secretion were not observed. At this time of the experiment, the area of wounds in the group with the introduced extract or solution of fullerenes in comparison with the control was smaller in 1.9 and 1.4 times, respectively.

Conclusions. Thus, the use of cryopreserved fragments of piglets' skin or an aqueous colloidal solution of C60 fullerene promoted the acceleration of cold wounds. The results obtained indicate the potential of cryopreserved fragments of piglets' skin or an aqueous colloidal solution of C60 fullerene be used for stimulation of the wound healing.