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REPORT
ON PATHOLOGIC MORPHOLOGIC STUDY OF RATS CEREBRAL CORTEX AFTER COLD PLASMA COAGULATION PERFORMED WITH “SORING” Co APPARATUS

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Materials and analysis

The experimrnt was done on male rats of Vistar line with body weight 200-250 g. The animals were divided into 8 groups: 1 and 5 comprised intact animals; 2 and 6 was formed of the rats undergone craniotrypsis; 3 and 7 made up rats whose brain after craneotomy has been exposed to cold plasma coagulation in the 3d mode during 2 sec.on apparatus made by “Soring” Co. (cold plasma 15, gas flow 2,0); 4 and 8 was composed of animals whose brain after craneotomy has been exposed to cold plasma coagulation in the 5th mode during 2 sec.on apparatus made by “Soring” Co. (cold plasma 25, gas flow 3,0).

Before performing the main experiments we have analysed rats’brain after coagulation 20 min. later. The coagulation was performed in the 3d mode and lasted 2 sec.

Craneotomy was realized under etherization in accordance with our technique [4]. The skin was lanced along the middle line with cut length of 1-1,5 cm. By means of a bur made from a hollow metal tube with 0,5 cm diameter, tectum of mesencephalon being exposed in place of sinus union was drilled to form an opening. (See the drawing)

A plan of craneotomy performing.

Trephine opening was covered with bone fragment extracted from the bur. Scalp incision was sutured with sterile surgical silk. The animals were withdrawn from the experiment on the 7th and 21st day under thiopentone anaesthesia (20 mg/kg). The terms of the experiment correspond to initial stages and reparation time in neural tissue [1,3].Total amount of animals used in experiment made 24 rats. Brain fragments were put into Buen acid liquor for 24 hours. Paraffin sections of 7 mcm thickness were placed on object-plates covered with poly-L-lysine (“Sigma”). General histology of brain tissue was examined on sections coloured with hematoxylin and eosin. To study cells proliferative activity mice monoclonal antibodies to PCNA diluted 1:50 (clone PC10, Calbiochem, USA) and set of avidin-biotin-peroxidase for mice antibody detection (Vectastain, USA) were used.

Immunohistochemical antigen detection from histological sections was carried out according main requirements for immuneperoxidase methods [2,8] following the scheme:

1. Sections heated up in thermostat during 10 min. at 56^0 C were deparaffinized through xylol, ethanol and brought to distilled water.
2. Endogenous peroxidase was disabled by 3% hydrogen peroxide during 10 min.
3. The sections were washed with physiologic saline with addition of triton X-100 (Sigma).
4. Nonspecific binding of immunoglobulins were blockaded by 1% normal serum of 2 antibodies from animal- donor during 20 min.
5. After removal of normal serum excess (without washing) first antibodies (against detected antigene) were put on the sections while diluting suitable for optimal immune colouring. Object-plates were placed into moist chamber, the incubation of the sections in refrigerator at 4^0 C lasted 18 hours.
6. After incubation with first antibodies the sections were washed in physiologic saline (3 times during 5 min.), then deposited biotin covered antibodies to immunoglobulines of animal-donor first antibodies and incubated in compliance with the protocol of the commercial set.
7. The sections were washed in physiologic saline (3 times during 5 min.), marking enzyme (peroxidase) was visualized in incubating mixture with diamine- benzidine and hydrogen peroxide during 10 min. (DAB-system, Immunotech).

7. Preparations were washed first in physiologic saline, then in distilled water, later they were dehydrated in alcohols, decolorised in xylol and confined in Entellan (Merck.).

At present immunehistochemical localization of PCNA is widely used in experimental and diagnostic histopathology to study proliferative activity of cells [6,7]. Moreover, there is evidence that PCNA takes part in the reparation of DNA damaged under different actions on tissues [5].

The investigation findings
Macroscopic analysis
All animals were active with adequate response to exogenous irritants. All of them had a correct body-built of normal nutritional state and neat bright black hair. Visible mucous tunics were of pink colour and wet. Anal and urethral surfaces were clean. On the head surface of rats from 2,3,4,6,7 and 8 groups a dry clen operative suture was visible. After skin flap removal a sign of trephine opening was disclosed on the surface of parietal bone. The trephine opening was covered with a bone fragment in intimate mating of the surrounding bone stock.
Brain surface after removal of calvarium was smooth and bright without any visible damages of pia mater of brain. Rats from the 3d and 4th groups showed minor edema, i.e. brain swelling in the area of perforation and coagulation.

Microscopic study
Groups 1 and 5. Control.
Cortex of cerebrum histoarchitectonics were in agreement with variants of norm (Fig.1). Pia mater of brain was of normal thickness and abundantly vascularized. Neuropile of grey matter was homogenuous and of pale blue colour.
Reaction to PCNA of temperate or intensive colour was revealed in single nuclei of endothelioma cells in capillars located in pia matter of brain. Pale coloured nuclei of neurocytes and neuroglii were rare in occurence (fig.2).

Group 0 – rats' brain 20 min. later craneotomy
Pia matter of brain in the coagulation area was destroyed, capillars in close location were bleeding and produced small hemorrhage zones. Neuropile in the damage site of conical form and penetrating deep into cortical substance was edematic (Fig.3).

Group 2 – rats' brain 7 days later craneotomy
Brain structure of rats from both groups was the same. However, in some places a slight thickening of pia matter of brain and its hyperemia was observed. Such a reaction could be attributed to craneotomy (Fig.4).
PCNA response: around operation opening in thickness area coloured nuclei were seen along with increased number of PCNA- positive nuclei in the neuropile of grey matter (Fig. 5).

Fig. 1. Intact rats' cerebrum coloured with hematoxylin and eosin x 350.

Fig.2. Intact rats' cerebrum. Immunohistochemical PCNA response x 65.

Fig.3. Rats' cerebrum 20 min. later cold plasma application coloured with hematoxylin and eosin x 350.

Fig.4. Rats' cerebrum 7 days later craneotomy. Immunohistochemical PCNA response x 65.

Group 3 – 7 days later craneotomy and cold plasma coagulation during 2 sec. in the 3d mode.
Within this term histologic cerebrum view had been cosiderably changed in comparison with group 0 and control group. Neuropile edema area was stricted forming a narrow channel leading from pia matter and ending by oval cavity in lower layers of grey matter. Hyperplasia and vascularization of pia matter was observed. Pia matter of one rat from this group as a result of hyperplasia served as a plug closing coagulation channel (Fig.8).

Basphilic cells area along the channel was essentially stricted but the number of PCNA-positive nuclei was enlarged (Fig. 9). Colouring power rate of nuclei varied from pale to intensive. Some rats had only single PCNA-immune positive nuclei of neuroglial cells along the channel. As in previous group enlargement of coloured nuclei was observed in local thickenings of pia matter and in underlying areas of grey matter (Fig.7).

Group 4 – 7 days later craneotomy and coagulation during 1 sec, in the 5th mode.

The thickening of pia matter due to vascularization was observed and an opening into long, narrow channel penetrating deep into grey matter. As a rule, the channel closed a small round cavity. The area of hyperchromic neuroglia and erythrocytes nuclei surrounding the channel was some wider than in previous group (Fig.10). Small in size PCNA-positive nuclei of different colour power tended to group asymmetric allocation in relation to the length of the channel. Same as in the 3d group the number of PCNA-positive nuclei was a bit enlarged in the upper area of cortical substance in compliance with trephine opening location.

Fig. 6. Rats' cerebrum 7 days later plasma coagulation and craneotomy coloured with hematoxylin and eosin x 65.

Fig. 7. Rats' cerebrum 7 days later plasma coagulation and craneotomy. Immunohistochemical PCNA response x 65.

Fig.8. Rats' cerebrum 7 days later plasma coagulation and craneotomy coloured with hematoxylin and eosin x 65.

Fig. 9. Rats' cerebrum 7 days later plasma coagulation and craneotomy. Immunohistochemical PCNA response x 350.

Group 6 – 21st day after craneotomy.
Histologic stugy findings and the results of immunohistochemical PCNA response in animals' brain cortex were practically the same as in control group apart from slightly increased PCNA-immune positive nuclei of connective tissue cells in pia matter.

Group 7 – 21st day later craneotomy and coagulation during 2 sec. in the 3d mode.
Pia matter of brain was regenerated on the site of coagulation, it was thickened and vascularized. The regeneration process was realized in a very narrow channel up to its end within brain cortex area. The width of basophilic neuroglia cells and neurocytes was minimal in comparison with the 3d group (Fig. 11).
The allocation region of PCNA-positive nuclei around coagulatin channel was essentially cut-back and limited by rather shallow funnel-shaped opening in pia matter area.
The width of PCNA-positive nuclei around the channel was too much less than in the 3d group. Outside this region one could see only single, small in size PCNA-positive nuclei.

Group 8 - 21st day later craneotomy and coagulation during 1 sec. in the 5th mode.
Histologic view was quite the same as in the 7th group. The coagulation channel was hardly dicserned, the allocation area of the cells with basophilia phenomena was very narrow. At the

end of the channel some rats showed microcavity of irregular shape with smooth walls that was filled in homogenious contents with rather few eosinophiles.

Fig. 10. Rats' cerebrum 7 days later plasma coagulation and craneotomy coloured with hematoxylin and eosin x 65.

Fig. 11. Rats' cerebrum 21 days later plasma coagulation and craneotomy coloured with hematoxylin and eosin x 65.

Conclusion

The data obtained from the study testify that there is a certain sequence of the destructive changes process following the coagulation pattern in cerebrum of rats subjected to cold plasma coagulation. In neuropile of rats from group 0 immediately after coagulation an area of edema-swelling was formed and basophilia of cells located in this area. 7 days later coagulation (groups 3 and 4) the edema was dissipated. At this place the channel outgoing from damaged pia matter appeared. The channel penetrated deep into grey matter. Hyperplasia and vascularization of pia matter were the signs of the regenerative processes. The quantity of PCNA-positive nuclei was enlarged that made possible to confirm the beginning of the restorative processes in the destructed area.

On the 21- st day the reparative process of rats subjected to cold plasma coagulation in the same mode as group 3 came to the end as demonstrated by maximum narrowing, basphilic cells reduction and decrease in PCNA-positive nuclei. The same sequence of reparative processes was observed in comparison of histologic indices in rats from 4 and 8 groups subjected to cold plasma coagulation during 1 sec. In the 5th mode. The swelling was decreased, the channel width was narrowed, neuropile area with basphilic cells was diminished. Connective tissue and vaccular structures of pia matter penetrated into cerebral cortex damaged regions and provided its reparation process as evidenced by the enlargement of PCNA-positive nuclei in cerebral cortex. Immunehistochemical examination data confirmed that coagulation eliminating conditions for necrosis development in tissues, the process of glial structure reparation took place. On the 7th day general width of neuropile region containing activated nuclei of glial cells correlated the intensity of restorative process within this period. Decrease in PCNA-positive nuclei of cells located around the channel on the 21 day after CPC exposure was the evidence of progress in cerebral cortex structures restoration. The restoration, however, had not been completed by this time. In some cases a small cavity was formed deep inside cerebral cortex. Rats'normal functioning state in postoperative period testified general positive results in restoration of cerebral cortex structures and functions.

Thus, pathologic study of rats' cerebrum cortex after the exposure to cold plasma coagulation by means of the apparatus made by "Soring" allowed to conclude the following:

1. destructive changes in grey matter of rats' cerebrum developed according to coagulation pattern;
2. these changes didn't cause any inflammation and necrotic processes in tissues surrounding coagulation zone;
3. the rate of brain tissue damage after coagulation depended on the mode of exposure to coagulation beam;
4. Complete restoration of brain tissue structure was basically determined by terms expired after the exposure.basphilic cells