CRYOPRESERVATION OF PLACENTAL CELLS IN MEDIA BASED ON PHARMACOPOEIAL PREPARATIONS

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The study of cryosensitivity and the preservation of the morpho-functional characteristics of placental derivatives is an urgent area of modern cryobiology and cryomedicine. These studies are impossible without effective cryopreservation, one of the most important factors of which is the composition of the cryoprotective medium (CM). CM must have cryoprotective properties, be non-toxic and approved for clinical use, that is, comply with GMP requirements. Thus, the aim of the work was to study the effect of cryoprotective medium based on pharmacopoeial preparations on the preservation of placental cells (PC) during cryopreservation.

Experiments were carried out on Wistar rats in accordance with the "General Principles of Animal Experiments" approved by the V National Congress on Bioethics and agreed with the provisions of the "IV European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (ETS 123, Strasbourg, France, 1986). Cells from the rat placenta were obtained by the explant method [1]. The number of PC was counted in hemocytometer, viability was assessed by 7AAD and trypan blue staining. The metabolic activity of PC was assessed by the MTT test. The adhesive properties were studied by plating PC on a 6-well plate in the amount of 2×10^5 cells per well. After a day, the number of adhered PC was counted. Placental cells obtained by the above method were equilibrated in CM (DMEM with cryoprotectant) at a concentration of 6×10^5/ml at a temperature of 20°C for 15 minutes, frozen at a rate of 1°C/min down to -70°C on a programmed freezer, immersed in liquid nitrogen for storage for 1-7 days in a low-temperature storage, thawed in a water bath at a temperature of 37°C.

For the selection of CM with the content of pharmacopoeial preparations, DMEM was replaced with Hanks or Ringer's solution. Pharmacopoeial preparations registered in Ukraine were also used, which include substances with cryoprotective properties: "Polyglucin" (6% dextran), "Neohemodez" (6% polyvinylpyrrolidone), "Stabizol" (6% hydroxyethyl starch) and "Reosorbilact" (6% sorbitol). DMSO at a
concentration of 5%, 10%, and 15% was used as the standard and the most appropriate cryoprotective agent for PC [2, 3].

Active cell migration was observed 2 days after the start of culturing placenta explants. After 4 days of cultivation, a confluent monolayer was formed. PC viability was 95.3±3.3% by trypan blue test, 8.9±4.2% by 7AAD+ test, the concentration of cells was 7.5±0.2×10^5/ml, 91.8±4.2% of cells adhered to the surface of the well, metabolic activity index according to MTT-test was 0.801±0.03 conv.units.

When cryopreserving cells with 5%, 10% or 15% DMSO, all studied parameters veraciously did not differ from the positive control.

When using Ringer’s, Hanks’ solutions, 6% dextran or 6% polyvinylpyrrolidone as CM, an insignificant decrease in cell concentration, viability, and the rate of monolayer formation was found, while PC metabolic and adhesive activity, and the ability to form monolayer remained at the control level.

When using 6% sorbitol or 6% hydroxyethyl starch, a more significant, compared with dextran and polyvinylpyrrolidone, decrease in cell concentration, viability, metabolic activity, adhesive properties and the rate of monolayer formation was found.

Thus, it was found that the maximum preservation of the rat placental cells is achieved by cryopreservation under the protection of 10% DMSO solution. Among pharmacopoeial preparations, sufficiently high cell preservation was also observed when using “Polyglukin” (6% dextran) or “Neohemodez” (6% polyvinylpyrrolidone).

References:
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