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Дослідження впливу біологічних домішок на кріопротекторну дію багатокомпонентних середовищ на основі комбінацій гліцерину та полівінілового спирту під час заморожування еритроцитів людини

Я. Гвоздюк, А. Компанієць
Інститут проблем кріобіології і кріомедицини НАН України, м. Харків

Study of Impact of Biological Additives on Cryoprotective Action of Multicomponent Media Based on Combinations of Glycerol and Polyvinyl Alcohol When Freezing Human Erythrocytes

Y. Hvozdiuk, A. Kompaniets
Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv

The conventional methods used worldwide for erythrocyte cryopreservation at low and ultra-low freezing temperatures have their drawbacks. They include the need for a multi-stage process of cryoprotectant washing out from blood cells prior to use after thawing. This process may involve additional expenses and damages, thereby complicating the cryopreservation technique. Therefore, the attempts are currently being made to produce a cryopreservative agent with the content that would simplify its removal from cell suspension. In this regard, to increase a cryoprotective effect of the solutions, there is suggested to supplement them with various synthetic antifreeze compounds and biologically active additives such as: proteins, carbohydrates, polyhydric alcohols, vitamins *etc.* [Menshikov, 1987; Wagner, 2002; Quan, 2008].

Here, we have studied the effect of carbohydrates (glucose, sucrose) and mannitol on a cryoprotective effect of the glycerol- and polyvinyl alcohol (MW 9 kDa)-based combined solutions.

The research material was the erythrocyte concentrate procured from donated human A(II) blood, prepared with Glugicir blood preservative at the Kharkiv Blood Service Center, and stored for no longer than 48 hrs at a temperature of (4 ± 2)°C. The erythrocyte concentrate was obtained by centrifugation of preserved donor blood at 1,250 g for 25 min. Glycerol (Czech Republic), polyvinyl alcohol MW 9 kDa (Sigma Aldrich, USA), glucose, sugar and mannitol (Ukraine) were used as components of cryoprotective solutions. They were prepared by the weight method based on 0.1 M PBS (pH 7.4) expressed as a percentage by mass (wt.%). Solutions were used after a 24-hr exposure at a temperature of (20 ± 2)°C. The concentrations of free hemoglobin in the supernatant and total hemoglobin of cell suspension were measured by hemoglobin-cyanide method using the 'Hemoglobin SpL-200' kit (Ukraine), hematocrit values were assessed with the CM-70 centrifuge, the percentage of erythrocyte hemolysis in supernatant was calculated by the formula reported in [Huggins, 1963], the number of cells recovered after washing was calculated according to the formula [Valery, 1997].

The studied biologically active substances (sucrose, glucose, mannitol) affected a cryoprotective effect of multicomponent media. Their impact is determined by the type of biologically active substance, and the cryoprotectant, being the base of a multicomponent cryoprotective medium. The prospects of using sucrose or glucose carbohydrates within combined media, containing glycerol as a cryoprotectant and synthetic polymer of polyvinyl alcohol, have been established.

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Порівняльна оцінка антиульцерогенної дії кріоконсервованого екстракту плаценти за різних способів введення

М. Чиж[1], І. Кошурба[2]
[1] Інститут проблем кріобіології і кріомедицини НАН України, м. Харків, Україна
[2] КНП "Чернівецький обласний перинатальний центр", м. Чернівці, Україна

Comparative Evaluation of Antiulcerogenic Action of Cryopreserved Placenta Extract Under Different Modes of Introduction

M. Chyzh[1], I. Koshurba[2]
[1] Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine
[2] Communal Non-Profit Enterprise "Chernivtsi Regional Perinatal Center", Chernivtsi, Ukraine

In the research of Hladkykh F.V. *et al.* (2021) the cryopreserved placental extract (CPE) was found to have an antiulcer activity in ulcerative lesions of the digestive tract induced by nonsteroidal anti-inflammatory drugs. This is a prerequisite for further in-depth study of gastroprotective activity of CPE under ulcerogenesis of other etiologies to assess the feasibility of using this cryoextract in classical peptic ulcer disease.

The research aim was to carry out a comparative assessment of the antiulcerogenic effect of CPE when administered preventively and for therapy in a model of alcohol-prednisolone gastric lesions in rats.

The study was conducted in 28 male rats weighing 200–220 grams. After 24 hours of starvation, the rats were administered intragastrically with prednisolone (20 mg/kg) dissolved in 80.0% ethyl alcohol (0.6 ml/100 g of animal body weight). The use of an alcohol-prednisolone mixture (APM) is justified by synergism of ulcerogenic action of the components, *i. e.* the corticosteroid prednisolone inhibits the prostaglandin biosynthesis, that weakens the gastric mucosa (GM) resistance. The CPE was administered intramuscularly at a dose of 0.16 ml/kg body weight preventively (1 time per day for 5 days before APM administration) and for therapy (1 hour after APM administration). Twenty-four hours after APM administration, rats were sacrificed, a macroscopic scoring of the GM status was performed, and an ulcer index (UI) was calculated.

It was found that a preventive administration of CPE had a pronounced antiulcerogenic effect in the model of alcohol-prednisolone gastric ulcer in rats, as evidenced by a significant ($p < 0.05$) UI decrease by 7.4 times in rats injected with CPE if compared with control animals. In addition, it should be noted that the gastroprotective effect of CPE exceeded by 4 times the similar activity of the proton pump inhibitor esomeprazole (reference drug), that was likely due to the peculiarities of the chosen administration mode. At the same time, a therapeutic administration of CPE was significantly inferior to the effectiveness of esomeprazole. Thus, when using the selected cryoextract, the UI was 9.3 times higher than in rats received esomeprazole.

CPE has a pronounced anti-ulcerogenic effect in preventive administration, but is significantly inferior to the reference drug esomeprazole in a therapeutic one. It is expedient to study an antiulcer effect of CPE under combined (therapeutic and preventive) administration.