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L-carnitine as an antioxidant to improve the efficiency of cord blood hematopoietic progenitor cell cryopreservation

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ABSTRACT

Cryopreservation of hematopoietic progenitor cells (HPCs) derived from cord blood is a critical step prior to transplantation, ensuring the long-term availability of viable cells for therapeutic use. However, oxidative stress during the freezing and thawing process can compromise cell survival and function, highlighting the need to improve cryopreservation protocols.

***PURPOSE**. This study is aimed to evaluate the effectiveness of L-carnitine, an antioxidant, when added to DMSO-based cryoprotective solutions in enhancing the post-thaw survival and viability of human cord blood-derived HPCs under in vitro conditions simulating transfusion.*

***MATERIALS AND METHODS**. Cord blood-derived nucleated cells, including $CD45^{dim}CD34^{+}$ HPCs, were collected after normal deliveries (n = 5). Cryopreservation was performed using DMSO at concentrations of 2.5 %, 5 %, and 7.5 %, supplemented with L-carnitine in the range of 1-50 mM. Cell viability was assessed immediately after thawing and following in vitro simulated transfusion (incubation in Hanks' solution at 37 °C for 1 hour) using 7-aminoactinomycin D (7-AAD) staining and flow cytometry.*

***RESULTS**. The addition of L-carnitine to the cryopreservation medium significantly increased the viable yield of $CD34^{+}$ cells, particularly at concentrations of 15-20 mM, both immediately after thawing and following incubation at physiological temperature. These results indicate that L-carnitine confers protective effects against oxidative stress-induced damage during cryopreservation.*

***CONCLUSION**. The results obtained indicate that L-carnitine increases the efficiency of cryopreservation of human cord blood hematopoietic progenitor cells, contributing to an increase in their preservation and viability. The determined optimal cryoprotective medium may be useful for the development of new and improvement of existing protocols for the preservation of human cord blood-derived cellular components.*

***KEY WORDS:** cord blood; hematopoietic progenitor cells; cell cryopreservation; L-carnitine.*

The use of hematopoietic progenitor cells (HPCs) has become one of the key methods in modern medicine in developed countries for the treatment or alleviation of blood diseases, immune disorders, metabolic disorders, oncological and other serious pathologies [2]. Due to its unique properties, ease and safety of collection, cord blood has taken an important place among the sources of HPCs. The significant clinical effectiveness of cord blood preparations has led to the need to create cryobanks, where samples can be stored for a long time at a temperature of -196 °C without losing their biological properties, and be used at any time if necessary. Since the volume of each dose of cord blood is limited, an important task is to preserve as many cells as possible in a viable state during cryopreservation. This problem can only be solved by conducting comprehensive studies aimed at studying the role of factors affecting the preservation and viability of cord blood-derived HPCs at each stage of freezing-thawing. This will allow identifying critical points of structural component disruption and conducting targeted selection of conditions for cell stabilization during freezing with subsequent development of effective cryopreservation methods.

For cryopreservation of cord blood-derived HPCs, the most widely used cryoprotectant is dimethyl sulfoxide (DMSO) in final concentrations of 7.5÷10 % [3, 4]. These specific concentrations of DMSO have been experimentally recognized as the most effective for cell protection. However, despite its effectiveness, DMSO also has pronounced cytotoxicity [5]. Therefore, recent efforts by researchers [6, 7] have been aimed at finding approaches to reducing the concentration of DMSO in cryoprotective mixtures in order to reduce the negative impact on both cells and the recipient's body. However, reducing the concentration of the cryoprotectant requires careful research, since this can negatively affect the structural and functional parameters of cells both immediately after thawing and, which is also of great importance, after transfer to the recipient's body.

It is known that during freezing and thawing, cells are exposed to a complex of stress factors, among which dehydration, changes in the ionic strength of the solution, pH, structural rearrangements of biopolymers, and intracellular crystallization/recrystallization play a significant role. These factors can lead to the accumulation of reactive oxygen species (ROS) and the development of significant oxidative stress [8]. Disruption of the prooxidant-antioxidant balance inhibits the antioxidant system of cells, which, in turn, triggers apoptosis processes and significantly reduces cell viability [9, 10, 11, 12]. Therefore, the use of substances such as antioxidants which can inhibit oxidative stress caused by cryopreservation factors may be a promising approach to reduce the formation of ROS in cells and prevent the development of oxidative stress in them to increase the level of preservation and viability of cryopreserved cells, including after transfer to the recipient's body. In recent years, much attention has been paid to the antioxidant L-carnitine (β-hydroxy-γ-trimethylaminobutyric acid) [13, 14]. The antioxidant L-carnitine demonstrates a complex protective effect in terms of the ability to neutralize ROS [15, 16]. This substance actively reduces the intensity of apoptotic processes, which, ultimately, should have a positive effect on the number of viable cells after cryopreservation.

There are also suggestions [17] that during cryopreservation, cells receive certain signals that make them "targeted" for death, but these destructive processes are not initiated at this point. Therefore, a very important task is to determine the long-term survival of cells, and not only to assess their structural and functional state immediately after thawing.

THE AIM of this study was to evaluate the effectiveness of using the antioxidant L-carnitine in cryoprotective mixtures to enhance the preservation of human cord blood-derived HPCs in a viable state after cryopreservation and *in vitro* transfusion simulation.

MATERIALS AND METHODS

Human cord blood was used, obtained from the umbilical vein after normal delivery (38-40 weeks of gestation, n = 5) with informed consent of the woman in labor in accordance with the requirements of the Bioethics Committee at the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine. For detecting $CD45^{dim}CD34^{+}$ cells anti-human FITC-labeled CD45 (clone 2D1, *BD Biosciences*, USA) and PE-labeled CD34 (clone 8G12, *BD Biosciences*, USA) monoclonal antibodies were used. Isolation of the nucleated cell fraction, which also includes hematopoietic progenitor ($CD45^{dim}CD34^{+}$) cells from whole cord blood (70-100 mL) was carried out by sedimentation in polyglucine (6 % dextran solution) (*Yuria Pharm*, Ukraine) with a molecular weight of 60,000 [18]. For this purpose, polyglucine was added to whole cord blood in a ratio of 1:1 and allowed to settle until a clear separation of the erythrocyte layer and supernatant was observed. The sedimentation time was from 30 to 50 min, after which the supernatant was collected and centrifuged for 15 min at 800 ×g to obtain a concentrate of nucleated cells. Treatment of cord blood nucleated cells with a cryoprotectant was carried out by adding a 25 % solution of dimethyl sulfoxide (DMSO), prepared in a 6 % solution of polyglucine [19], to the early obtained nucleated cells suspension dropwise in an ice bath for 20 minutes to final concentrations in the sample of 2.5; 5; 7.5 %. The antioxidant L-carnitine was prepared on the saline solution and used in the work at concentrations of 1; 5, 10; 15; 20; 50 mM (*Sigma-Aldrich*, USA). Cryopreservation of cord blood cells was carried out using 1 mL cryovials at a rate of 1 °C per minute to -80 °C in a programmable freezer (*Cryoson*, Germany) with subsequent immersion in liquid nitrogen. Thawing of the samples after 1-4 week storage in liquid nitrogen was carried out at a temperature of 37-40 °C in a water bath with constant shaking until the solid phase disappeared. All subsequent studies were performed without washing away the cryoprotectant both immediately after thawing and after *in vitro* transfusion simulation. For this, an aliquot of the cell suspension was transferred to Hanks' solution (1:10 by volume) with the incubation temperature maintained (37 °C) for 1 hour. Determination of the absolute number of nucleated cells was calculated using the Goryaev chamber according to the standard technique [20]. The losses of cells during the freezing and thawing (preservation of nucleated cells) were counted based on the following ratio:

$$\text{Nucleated cells (or HPCs) preservation} = \frac{\text{Number of cells after cryopreservation or after } \textit{in vitro} \text{ transfusion simulation}}{\text{Initial number of cells before cryopreservation}} \times 100\ \%$$

Determination of the absolute number of HPCs and their viability ($CD45^{dim}CD34^{+}7AAD^{-}$) was performed according to the standard protocol for testing cord blood preparations by ISHAGE (International Society of Hematotherapy and Graft Engineering) using monoclonal antibodies CD45-FITC, CD34-PE and DNA dye 7-aminoactinomycin D (7AAD) (*BD Biosciences*, USA) by flow cytometry with a flow cytometer FACS Calibur (*BD Biosciences*, USA). To minimize error, 20,000 events were analyzed.

Samples were analyzed using CELLQuest Pro software (*BD Biosciences*, USA). The yield of viable HPCs ($CD45^{dim}CD34^{+}7AAD^{-}$) after cryopreservation was determined by the ratio of the number of viable cells after cryopreservation (*in vitro* transfusion simulation) to their initial level before cryopreservation.

Statistical processing of the results was performed using the "Statgraphics plus 2.1" software package (*Manugistics Corp.*, USA). Data are presented as M ± SE (Mean ± Standard Error). The statistical significance of the results was determined by the nonparametric Mann-Whitney test at $p < 0.05$.

RESULTS AND DISCUSSION

At the first stage of the researching the effectiveness of adding antioxidant L-carnitine to the cryoprotective mixture during the preservation of cord blood hematopoietic progenitor cells, the preservation of $CD45^{dim}CD34^{+}$ cells after cryopreservation was determined (**Table 1**).

The results of the studies showed that the greatest loss of $CD45^{dim}CD34^{+}$ cells was, as expected, in samples cryopreserved under the protection of 2.5 % DMSO, where the preservation was at the level of 50 %. The addition of L-carnitine provided an increase in this parameter: in samples containing 1-10 mM and 50 mM of the antioxidant, the increase was not very pronounced, while in samples containing 15-20 mM the changes were significantly increased up to 22.0 % ($p < 0.05$) (**Table 1**). Freezing in solutions with 5 % DMSO ensured the preservation of control samples at the level of 75 %. The adding of L-carnitine did not significantly change the preservation. The maximum number of cells was preserved in samples frozen with 7.5 % DMSO. In the control and with an L-carnitine content of 1-5 mM, this indicator was about 83 %. Increasing the antioxidant concentration provided a significant increase in preservation with maximum values in cryoprotectant solutions containing 20 mM L-carnitine (90.9 ± 2.3 %). That is, only 10 % of the HPCs is lost at all stages of cryopreservation, starting with treatment with the cryoprotectant solution and ending with freeze-thaw.

Table 1. Preservation and viability of cord blood CD34⁺ cells after cryopreservation in solutions containing DMSO and L-carnitine of different concentrations, % (M ± SE, n = 5).

DMSO concentration, %	L-carnitine concentration, mM	Hematopoietic progenitor cells (CD34⁺)	
		Preservation	Viability
2,5	0 (control)	50.6 ± 3.2	50.4 ± 3.8
	1	51.7 ± 3.9	50.3 ± 2.7
	5	54.8 ± 3.5	52.1 ± 2.6
	10	58.5 ± 6.2	55.8 ± 2.6
	15	61.5 ± 5.0*	55.0 ± 3.0
	20	62.1 ± 4.8*	58.2 ± 2.5*
	50	58.1 ± 4.9	58.1 ± 2.6*
5	0 (control)	74.0 ± 3.2	61.9 ± 3.2
	1	73.5 ± 2.4	63.0 ± 3.4
	5	76.3 ± 3.2	64.5 ± 2.4
	10	78.9 ± 3.1	64.4 ± 2.9
	15	77.8 ± 1.8	66.1 ± 3.0
	20	77.8 ± 2.3	66.3 ± 3.1
	50	77.6 ± 1.6	66.8 ± 2.8
7,5	0 (control)	81.4 ± 2.9	68.9 ± 2.2
	1	83.2 ± 2.4	71.0 ± 2.8
	5	87.7 ± 2.7	72.0 ± 2.0
	10	88.6 ± 2.2*	72.0 ± 3.3
	15	89.3 ± 1.9*	71.4 ± 2.6
	20	90.9 ± 2.3*	72.4 ± 3.1
	50	88.1 ± 1.8*	73.1 ± 3.3

*Note: * – differences are significant compared to the control group without the addition of L-carnitine ($p < 0.05$).*

The use of flow cytometry using the vital DNA dye 7-AAD, which binds only to the uncoiled DNA molecule, allows, as shown by additional tests (including culture), to identify viable functionally active cells [21]. Analysis of the viability of HPCs cryopreserved with the addition of 20 mM L-carnitine demonstrated a higher number of viable CD34⁺ cells in samples with an ultra-low concentration of DMSO (2.5 %). Although at this content of the cryoprotectant there was significant cell destruction (as evidenced by the preservation data), L-carnitine, due to its cytoprotective properties, is able not only to increase the preservation indicators, but also viability by 16 % (**Table 1**). During the cryopreservation of HPCs in solutions containing 5 or 7.5 % DMSO and L-carnitine, no significant changes were observed. However, a trend towards increased cell viability can be noted in samples containing the antioxidant.

It should be noted that such an indicator as cell viability is characterized by its relativity, since in each sample any number of remaining cells is taken as 100 %. At the same time, quantitative indicators, in particular the absolute number of cells, are not taken into account in this analysis. And since we determined the absolute number of cells in all experimental samples and calculated the percentage of viable $CD45^{dim}CD34^{+}7AAD^{-}$ cells, for the most objective analysis of the effectiveness of the selected antioxidant concentrations, the yield of viable cells after freezing-thawing was determined at the next stage. This indicator can become an integral parameter for determining the effectiveness of cryopreservation technology.

Determination of the yield of viable cord blood CD34⁺ cells after cryopreservation in solutions with different concentrations of DMSO and L-carnitine showed that this indicator was the lowest in samples containing 2.5 % DMSO. Even under the conditions of L-carnitine addition, the yield of viable cells remained low, which indicates insufficient cryoprotection of this cryoprotective medium. However, it is possible to indicate a significant increase in the number of viable cells in samples containing 10-50 mM of the antioxidant (**Fig. 1**).

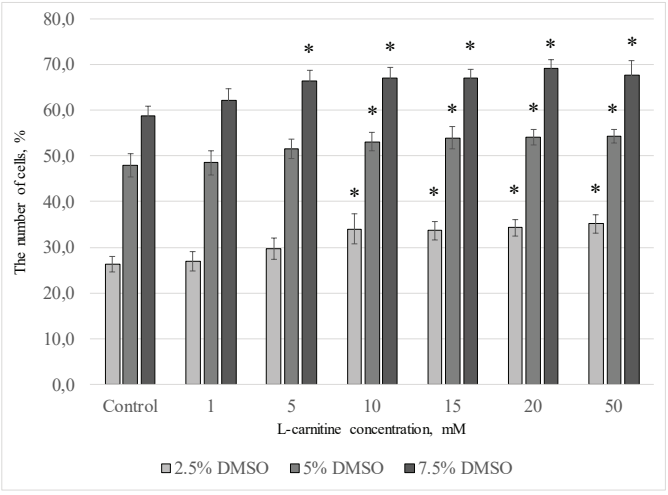


Fig. 1. The viable CD34⁺ cell yield after cryopreservation in solutions containing DMSO and L-carnitine of different concentrations, relative to their initial level before freezing.
Note: * – differences are significant compared to the control group without the addition of L-carnitine (M ± SE, n = 5, $p < 0.05$).

Increasing the cryoprotectant concentration to 5 % provided a significant increase in the yield of viable cells compared to 2.5 DMSO in all experimental groups. In the control group, the yield was 48 %, and when adding the antioxidant L-carnitine to the cryoprotective solution, this indicator was on average 55 % (the data are significant for samples containing 15-50 mM L-carnitine). Analysis of samples containing 7.5 % DMSO and L-carnitine in the cryoprotective solution demonstrated significant differences from the two other DMSO concentrations. In the control,

the yield of viable CD34+ cells was about 59 % of their number before cryopreservation, and when adding 10-50 mM L-carnitine, this parameter was up to 72 %. That is, the yield of viable cells in these samples was higher by 12.8-17.5 %, which indicates the effectiveness of this cryoprotective medium.

As already noted, during cryopreservation, the disruption of the antioxidant system and the structural ordering of cell membranes due to the development of lipid peroxidation [16] does not occur immediately after thawing, but after a few hours, when natural antioxidant mechanisms, in particular the glutathione peroxidase system, are already partially disrupted. This process is a characteristic manifestation of the so-called latency of cold damage, when in the early stages ultrastructural membrane disorders are not noticeable yet, but irreversible oxidative processes are already triggered. In this regard, to predict the therapeutic efficacy of cord blood preparations, an important task is not only to assess them immediately after thawing, but also to determine delayed cell survival. Therefore, at the next stage of the work, we assessed delayed cell death after thawing by simulation of physiological conditions *in vitro* [22]. To do this, we used a simple model in the experiment, reproducing only the basic principles of transfusion: dilution of the thawed cell suspension, which occurs naturally in the recipient's bloodstream, isoosmoticity of the medium and incubation temperature (37 °C). Maintaining a temperature of 37 °C throughout the entire period of cell incubation is an important factor that allows us to detect metabolic disorders, since the preservation of cells at lower temperatures masks a possible imbalance in cellular metabolism due to the slowdown of almost all processes with a decrease in temperature. During transfusion simulation, a 10-fold dilution of the suspension of cryopreserved NCs was adopted, isoosmoticity was provided by Hanks' solution with the inclusion of a buffer system that maintains pH 7.4.

For the analysis of data regarding the effect of cryoprotectant solutions with the antioxidant L-carnitine on the condition of cord blood CD34+ cells after thawing and *in vitro* transfusion simulation, experiments were conducted to determine their preservation and viability.

As shown in **Table 2**, cell preservation exhibited significant differences depending on the DMSO content in the samples. This parameter was minimal in samples frozen with 2.5 % DMSO and maximal (60-73 %) in samples with 7.5 % DMSO concentration in the cryoprotectant solution. The presence of the antioxidant in the samples resulted in the preservation of a greater number of hematopoietic cells. When cryopreserved with 2.5 % DMSO and L-carnitine, significant differences were observed only in samples with 20 mM antioxidant, where preservation increased by almost 30 %. In solutions with 5 % DMSO, the effect of the antioxidant adding was more pronounced: the presence of L-carnitine ensured a significant increase in the number of preserved HPCs starting from L-carnitine concentration of 5 mM. In samples containing 7.5 % DMSO and 10-50 mM L-carnitine, the number of preserved cells was 15-20 % higher compared to the control group.

Table 2. Preservation and viability of cord blood CD34+ cells after cryopreservation in solutions containing DMSO and L-carnitine of different concentrations and *in vitro* transfusion simulation, % (M ± SE, n = 5).

DMSO concentration, %	L-carnitine concentration, mM	Hematopoietic progenitor cells (CD34+)	
		Preservation	Viability
2,5	0 (control)	32.9 ± 4.0	59.7 ± 6.1
	1	34.3 ± 3.3	59.1 ± 5.7
	5	35.2 ± 3.9	60.9 ± 5.3
	10	36.3 ± 5.0	63.0 ± 5.2
	15	38.0 ± 3.2	65.7 ± 5.5
	20	42.3 ± 3.8*	65.1 ± 4.2
	50	39.6 ± 4.9	63.7 ± 4.7
5	0 (control)	52.8 ± 2.7	63.4 ± 3.6
	1	55.9 ± 2.0	63.8 ± 3.1
	5	60.4 ± 2.5*	66.4 ± 3.6
	10	61.0 ± 1.9*	64.9 ± 4.0
	15	59.7 ± 1.7*	66.6 ± 3.1
	20	61.1 ± 3.6*	67.1 ± 2.5
	50	63.2 ± 1.5*	66.1 ± 2.9
7,5	0 (control)	60.3 ± 4.8	66.7 ± 2.7
	1	62.8 ± 5.6	68.2 ± 1.8
	5	68.2 ± 3.9	66.3 ± 2.1
	10	69.8 ± 3.3*	70.3 ± 2.9
	15	71.7 ± 3.0*	68.6 ± 2.3
	20	72.7 ± 3.8*	70.4 ± 1.1
	50	73.1 ± 2.3*	69.1 ± 0.5

*Note: * – differences are significant compared to the control group without the addition of L-carnitine ($p < 0.05$).*

The analysis of the viability of cryopreserved with DMSO and L-carnitine cord blood-derived HPCs after *in vitro* transfusion simulation did not reveal any significant differences between the different experimental groups (**Table 2**). This once again emphasizes the importance of determining the yield of viable cord blood CD34+ cells. Thus, in samples with 2.5 % DMSO, with an increase in the concentration of L-carnitine, a tendency to an increase in the viable cell yield was observed compared to control samples (**Fig. 2**). Significant differences were observed in samples containing 2.5 % DMSO and 20 mM antioxidant in the cryoprotective solution, where the maximum of the viable cell yield was observed, which indicates a significant antioxidant effect of this concentration. With a further increase in the amount of L-carnitine in the samples (up to 50 mM), no noticeable changes were observed, which may indicate the achievement of saturation or even the possible toxicity of high doses of this antioxidant.

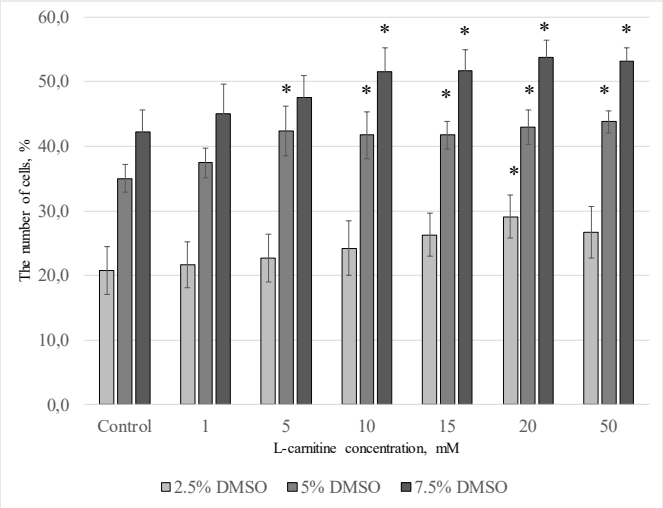


Fig. 2. The viable CD34+ cell yield after cryopreservation in solutions containing DMSO and L-carnitine of different concentrations and *in vitro* transfusion simulation, relative to their initial level before freezing.
Note: * – differences are significant compared to the control group without the addition of L-carnitine (M ± SE, n = 5, $p < 0.05$).

In samples containing 5 % DMSO, a significantly higher cell yield was recorded compared to 2.5 % DMSO. And this increase was 1.5-1.9-fold compared to 2.5 % DMSO. The presence of L-carnitine in the samples, starting from 5 mM, provided a significant increase in the viable HPC yield by 20-26 %. The maximum indicators were in samples containing 20 mM. *In vitro* transfusion simulation of samples cryopreserved with 7.5 % DMSO and L-carnitine revealed that significant differences were observed with the addition of the antioxidant at concentrations of 10-50 mM.

Thus, cryopreservation of cord blood-derived hematopoietic progenitor cells under the protection of different concentrations of DMSO without the use of antioxidants causes a decrease in cell yield, especially when the optimal concentration of cryoprotectant is reduced. Oxidative stress, caused by the accumulation of reactive oxygen species, plays a key role in post-thaw damage. Numerous studies have shown that cryopreservation leads to an increase in the level of superoxide anion, hydrogen peroxide and other active molecules, which triggers the apoptotic cascade [10, 23, 24].

In addition, it is known that during cryopreservation, cells receive certain damage or signals that make them "prone" to the development of apoptotic processes, but do not initiate their development at this point [17], i.e., delayed cell death after thawing may be associated with metabolic imbalance, energy depletion, caspase activation, and oxidative stress. As noted by Scott et al., Bissoyi et al. post-cryopreservation apoptosis is significant for many cell types and is most intense during the first 24 hours after thawing [25, 26]. This phenomenon, known as Cryopreservation-induced, delayed-onset cell death (CIDOCD) [27], was also recorded in our study: a decrease in cell viability was observed not only immediately after thawing, but also, especially, after an hour-long transfusion simulation *in vitro*.

All this confirms the importance of using substances with antioxidant properties to reduce ROS levels and improve cryopreservation results. In particular, Kadekar et al., Motta et al. showed that a medium enriched with antioxidants significantly reduces oxidative stress during cryopreservation of cord blood cells, resulting in an increase in the number of viable cells after thawing [8, 28].

The results of our study confirm the effectiveness of using an antioxidant such as L-carnitine to improve the preservation and viability of hematopoietic progenitor cells of cord blood after cryopreservation. Literature data indicate the use of different concentrations of this antioxidant, in particular, in *in vitro* experiments 10-80 mM of L-carnitine [29, 30] is used, in the study of Manee-In et al. 25 mM L-carnitine is added to the cryopreservation medium [31]. In our experiments, it was shown that the addition of L-carnitine at concentrations of 15-20 mM significantly increased cell survival, especially when using 7.5 % DMSO.

Therefore, the optimal combination of cryoprotectant and antioxidant contributes to a significant increase in cell viability after cryopreservation and *in vitro* transfusion simulation, which confirms the effectiveness of L-carnitine as a component of the cryoprotective medium.

CONCLUSION

The study of the effect of L-carnitine on the preservation of viable cord blood hematopoietic progenitor cells after cryopreservation with different concentrations of DMSO and subsequent in vitro transfusion simulation showed that the addition of this antioxidant to the cryopreservation medium contributes to increased cell preservation and viability. The results confirm the promising use of antioxidants in cryopreservation technologies to minimize cell damage caused by cryopreservation factors and ensure long-term survival of hematopoietic progenitor cells, which will certainly contribute to increasing the clinical effectiveness of cord blood cell preparations.

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L-карнітин як антиоксидант для підвищення ефективності кріоконсервування гемопоетичних клітин-попередників пуповинної крові

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РЕЗЮМЕ

Кріоконсервування гемопоетичних клітин-попередників пуповинної крові є вирішальним етапом перед їх подальшою трансплантацією, що забезпечує довгострокову доступність життєздатних клітин для терапевтичного застосування. Однак окислювальний стрес під час процесу заморожуванні і розморожування може поставити під загрозу виживання та функціонування клітин, що підкреслює необхідність вдосконалення протоколів кріоконсервування.

***МЕТА**. Дослідження спрямоване на оцінку ефективності додавання антиоксиданту L-карнітину до кріопротекторних розчинів на основі ДМСО з метою підвищення збереженості та життєздатності отриманих з пуповинної крові людини гемопоетичних клітин-попередників після кріоконсервування та моделювання трансфузії in vitro.*

***МАТЕРІАЛИ ТА МЕТОДИ**. Отримані з пуповинної крові ядровмісні клітини, включно з $CD45^{dim}CD34^{+}$ HPC, збирали після нормальних пологів (n = 5). Кріоконсервування проводили з використанням ДМСО у концентраціях 2,5 %, 5 % та 7,5 % з додаванням L-карнітину в діапазоні концентрацій 1-50 мМ. Життєздатність клітин оцінювали, як відразу після розморожування, так і після моделювання трансфузії in vitro (інкубація в розчині Хенкс при 37 °C протягом 1 години) за допомогою фарбування 7-аміноактиноміцином (7-AAD) методом проточної цитометрії.*

***РЕЗУЛЬТАТИ**. Додавання L-карнітину до середовища кріоконсервування сприяло підвищенню виходу життєздатних $CD34^{+}$-клітин, особливо при концентраціях 15-20 мМ, як відразу після розморожування, так і після годинної інкубації за фізіологічної температури. Ці результати свідчать про захисну дію L-карнітину проти пошкодженнь, спричинених оксидативним стресом під час кріоконсервування.*

***ВИСНОВКИ**. Отримані результати свідчать про те, що L-карнітин підвищує ефективність кріоконсервування гемопоетичних клітин-попередників пуповинної крові людини, сприяючи збільшенню їхньої збереженості та життєздатності. Визначене оптимальне кріопротекторне середовище може бути корисним для розробки нових та вдосконалення існуючих протоколів консервування клітинних компонентів, отриманих з пуповинної крові людини.*

***КЛЮЧОВІ СЛОВА**: пуповинна кров; гемопоетичні клітини-попередники; кріоконсервування клітин; L-карнітин*