

CRYOPRESERVATION OF RED BLOOD CELLS: A REVIEW

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Abstract

In the past years the concern related to transfusional medicine in veterinary practice has thoroughly increased due to the development of emergency medicine and also to veterinarians' practical abilities. Cryopreservation of blood or its products defines the procedures that allow blood to keep its viability in a sterile state for an indefinite period of time without damaging the biological properties.

Key words: blood, cryopreservation

CRYOBIOLOGY OF ERYTHROCYTES

There are numerous cryopreservation methods of blood products known and applied, especially for erythrocytes. Erythrocytes cryopreservation so that all metabolic and biochemical reactions stop, along with the storage at temperatures under 0°C for a long period of time, were intensively researched in 1950-1960, maybe due to the fact that at that time keeping erythrocytes and blood products refrigerated was not possible more than 21 days.

The success of red blood cells preservation for a longer period of time depends on knowing and understanding the physiology of erythrocytes and the biochemical and biomechanical changes that occur during freezing-thawing process.

In 1866, french naturalist Felix Archimede Pouchet was the first that described the fact that after thawing, erythrocytes are being destroyed. For a long period of time it was believed that this was happening because of the mechanical effect produced by ice crystals formed during the freezing process (Pouchet, 1866).

In 1950, Lovelock proved that erythrocytes are destroyed after thawing not mainly because of mechanical effect of ice crystals, but rather because of increased concentration of ions in the extracellular space. This leads to osmotic pressure changes of the extracellular

space followed by dehydration of cells and drastic pH changes. This mechanism causes irreversible lesions of cellular membrane structure before the ice crystals are formed (Huggins et al., 1969; Lecak et al., 2004; Lovelock, 1954; Sputtek, 1996).

Based on these findings, in 1972 Mazur et al. issued the hypothesis that cell survival during freezing process will be maximum if both ice crystals formation in intracellular space and cells dehydration as a result of osmotic shock will be minimized by using an optimal cooling rate (Mazur et al., 1972).

Erythrocytes are cells with highly permeable membrane for water, so the optimal cooling rate is around 3500C/min. The use of cryopreservatives will induce a significant increase of environmental osmolarity allowing lowering the optimal cooling rate. According to their effect, there are two categories of cryopreservatives: intracellular and extracellular.

Intracellular cryoprotectant substances have a relatively simple chemical structure and do not exhibit cytotoxic effects at low concentrations, most used today being glycerol, dimethylsulfoxide (DMSO) or certain types of glycol.

The main mechanism of action consists in reducing the formation of ice crystals by modifying their shape and size. During the cooling phase, they penetrate the intracellular space where they create a hyperosmotic

environment that will determine water removal from intracellular space (Kim et al., 2004).

Therefore, cell progressive dehydration occurs and osmotic stress is reduced by maintaining a balance between the intracellular and the extracellular environment (Pert et al., 1963; Valeri et al., 1966).

Extracellular cryoprotectant substances have a high molecular weight that does not allow them to cross the cellular membrane, the most used today being dextran, modified gelatin, hydroxyethylamidon (HES) or albumin. The mechanism of action for these substances is relatively simple and consists in their ability to stabilize the cellular membrane by interacting with its polar components (McGann, 1978; Sputtek, 2007).

Electrolytes in the frozen extracellular environment will be concentrated in these areas. As a consequence of modifying the osmotic balance in extracellular environment, the water will be slowly eliminated from the intracellular space. Balancing the osmotic difference makes the structural and functional integrity of cellular membrane to be kept (Horn et al., 1997; Sputtek et al., 1995).

CRYOPRESERVATION OF ERYTHROCYTES

The important findings regarding cryoprotectant effect of various groups of substances made possible the development of some efficient preservation techniques by freezing using almost exclusively glycerol. These techniques can be divided into three main groups: cyto agglomeration technique, high glycerol concentration – low rate of freezing and low glycerol concentration – high rate of freezing (Brecher, 2003; Krijnen et al., 1968; Meryman et al., 1972; Rowe, 1973; Rowe et al., 1968).

However the impossibility to add and remove cryoprotective substances in conditions of absolute sterility in a closed system, has made the erythrocytes cryopreservation to be practically applied only at the end of 1950 when Tullis et al. described the first continuous flow centrifuge (Tullis et al., 1966). At the beginning of 1960, Huggins has implemented a set of principles regarding

continuous flow centrifugation and developed a cyto agglomeration technique that allows cryopreservatives to be removed by precipitation of erythrocytes in a hypo-osmotic solution with low pH and re-suspension of erythrocytes in an isotonic environment (Huggins, 1963).

At the beginning of 1970, Meryman and Hornblower simplified and improved the cyto agglomeration technique. Therefore the freezing of erythrocytes units it is made in whole blood collection bags after separation of blood components. Glycerol used as cryopreservative is added in very high concentration (40%) and the freezing is performed slowly at -80°C (around 1°C/min) in mechanical freezers. This way erythrocytes units can be stored up to 21 years (Meryman, 1972; Valeri, 1972). Thawing it is made by immersion of frozen erythrocytes units in 37°C warm water baths for 10 minutes. Removal of glycerol after thawing is made through repeated washings with saline solutions of different concentrations (Valeri et al., 1970).

An alternative technique of freezing red blood cells was described by Pert et al. in 1963 and by Rowe et al. in 1968. The technique is based on the addition of glycerol in low concentrations of 15-20% and applying a rapid cooling rate for freezing (about 100°C/min) by immersion in liquid nitrogen at -196°C and storage of units frozen this way in nitrogen vapors at -165°C. Thawing is made in warm water bath at 42-45°C for 3 minutes and the removal of cryopreservative is made by washing erythrocytes with saline solutions of different concentrations (Pert et al., 1963; Rowe et al., 1968).

A different approach regarding the cryopreservation of erythrocytes refers to the use of extracellular cryoprotectant substances such as hydroxyethylamidon (HES), dextran or polyvinylpyrrolidone (PVP). The major benefits of using them consists in biocompatibility properties in increased concentrations, making possible that erythrocytes units to be transfused immediately after thawing without being necessary any additional processing (Robson, 1970; Sputtek et al., 1993). The idea of using extracellular macromolecules in order to protect erythrocytes of freezing effects was

first implemented by Reinfert (1963). Sputtek et al. were the ones that made thorough studies about the use of HES for the cryopreservation of erythrocytes reporting an acceptable survival rate of erythrocytes after transfusion without any post thawing processing (Sputtek et al., 1993).

Despite the fact that erythrocytes can be frozen and preserved almost indefinitely no matter the technique and the cryopreservatives used, setting an optimal administrative storage time was needed. So, in 1987, for human blood products, the optimal storage period for frozen erythrocytes has been set and accepted to be 10 years according to "Guide to the preparation, use and quality assurance of blood components" (Recommendation No. R (95) 15 Council of Europe). In 2010, European legislation extended the storage period of frozen erythrocytes up to 30 years, depending on storage method (Directive 2044/33/EC) (Lecak et al., 2004).

From a practical point of view, the most important clinical parameter is the usability of erythrocytes after thawing and removing the cryoprotectant substance. Until recently, after thawing and cryoprotectant removal, erythrocytes could be used for very short periods, only 24 hours. This is because of the removal of blood plasma that used to offer erythrocytes buffering capacity and protection against lysis. The washing process to remove the cryoprotectant substance deprives red blood cells of some of its intracellular metabolites. Therefore their metabolic needs are different from whole blood (Moore et al., a, b, 1987).

The discovery and use of additive solutions to meet specific metabolic needs of frozen-thawed erythrocytes may increase applicability of erythrocytes after thawing up to 3 weeks (Hess et al., 2001, Valeri et al., 2001; Valeri et al., 1970).

In conclusion, preservation of erythrocytes by freezing is a feasible method in transfusional medicine due to implementation of modern blood processing techniques in closed system and also due to significant contribution regarding the maximum usability of red blood cells units after thawing by using modern resuspension solutions.

REFERENCES

- Brecher M. E., 2003. Red cell cryopreservation using high-concentration glycerol - Meryman method, in Technical Manual, 14th ed., AABB, Bethesda, MD, pp. 741-743.
- Hess J.R., Hill H.R., Oliver C.K., Lippert L.E., Greenwalt T.J., 2001. The effect of two additive solutions on the postthaw storage of RBCs., *Transfusion*. Jul;41(7), 923-7.
- Horn E.P., Sputtek A., Standl T., Rudolf B., Kühnl P., 1997. Schulte Transfusion of autologous, hydroxyethyl starch-cryopreserved red blood cells, *Anesthesia & Analgesia*, 85, 739-745.
- Huggins C.E., 1969. Frozen Blood, *Europ. Surg. Res.*, 1, 3-12
- Huggins C.E., 1963, Preservation of blood by freezing with dimethyl sulfoxide and its removal by dilution and erythrocyte agglomeration. *Vox Sang*, 8, 99-100.
- Kim H., Tanaka S., Une S., Nakaichi M., Sumida S., Taura Y., 2004, A comparative study of the effects of glycerol and hydroxyethyl starch in canine red blood cell cryopreservation. *J Vet Med Sci*, 66, 1543-7.
- Krijnen, H. W., Kuivenhoven, A. C. J., De Wit, J. J. F. M., 1968, The preservation of blood cells in the frozen state. Experiences and current methods in the Netherlands. *Cryobiology*, 5, 136-143.
- Lecak J., Scott K., Young C., Hannon J., Acker J.P., 2004, Evaluation of red blood cells stored at -80 degrees C in excess of 10 years., *Transfusion*, Sep;44 (9), 1306-13.
- Lionetti F. J., Hunt, S. M., 1975, Cryopreservation of Human Red Cells in Liquid Nitrogen with Hydroxyethyl Starch, *Cryobiology*, 12, 110-118.
- Lovelock J.E., 1954, The Protective Action of Neutral Solutes against Haemolysis by Freezing and Thawing, *Biochem.J.*, 56, 265-270.
- Mazur P., Leibo S.P., Chu E.H.Y., 1972, A two-factor hypothesis of freezing injury: evidence from Chinese hamster tissue-culture cells, *Exp Cell Res* 71, 345-355.
- McGann, L.E., 1978, Differing actions of penetrating and nonpenetrating cryoprotective agents, *Cryobiology*, 15, 382-390.
- Meryman H.T., Hornblower M, 1972, A method for freezing and washing red blood cells using a high glycerol concentration, *Transfusion*, 12, 145-156.
- Moore G.L., Ledford M.E., Mathewson P.J., Hankins, D.J., 1987a, *Transfusion* 27, 496-498.
- Moore G.L., Ledford, M.E., Mathewson P.J., Hankins D.J., Shah, S.B., 1987b, *Vox Sang*. 53, 15-18.
- Pert JH, Schork PK, Moore R, 1963, A new method of low temperature blood preservation using liquid nitrogen and glycerol sucrose additive, *Clinical Research*, 11, 197.
- Pouchet MFA, 1966, Recherches expérimentales sur la congélation des animaux, *Journ. L'Anat. et Physiol*, iii, 1-36.

- Rinfret A.P., 1963, Some aspects of preservation of blood by rapid freeze-thaw procedures, *Fed Proc* 22, 94-101.
- Robson D. C., 1970, Rapid freezing and thawing techniques using extra-cellular agents. In: *Modern Problems of Blood Preservation*, (Spielmann, W. and Seidl, S., eds.), Fischer, Stuttgart, Germany, pp. 204-208.
- Rowe A. W. , 1973, Preservation of blood by the low glycerol-rapid freeze process, In: *Red Cell Freezing*, American Association of Blood Banks (ed.), Washington, DC, pp. 55-71.
- Rowe A. W., Eyster E., Kellner A., 1968, Liquid nitrogen preservation of red blood cells for transfusion: a low glycerol-rapid freeze procedure. *Cryobiology*, 5, 119-128.
- Sputtek A, *Kryokonservierung von Blutzellen*, 1996 *Transfusionsmedizin*, 2nd.ed. (Ed.: C. Mueller-Eckhardt), 125-167, Springer, Berlin.
- Sputtek A, Roling C, Singbartl G, et al, 1993, First autologous retransfusion of human red blood cells (450 mL) cryopreserved in the presence 11.5% HES without post-thaw washing. *Cryobiology*, 30, 657.
- Sputtek A, Singbartl G., Langer R., Schleinzer W., Henrich HA, Künl P., 1995, Cryopreservation of red blood cells with the non-penetrating cyoprotectant hydroxyethylstarch, *Cryo Letters* 16, 283-288.
- Sputtek A., 2007, Cryopreservation of red blood cells and platelets. In Day JG, Stacey GN (ed): *Cryopreservation and freeze-drying protocols*. Totowa, Humana Press, pp 283-302.
- Tullis J.L., Kethel M.M., Pyle H.M., et al, 1958, Studies on the in vivo survival of glycerolized and frozen human red blood cells, *JAMA* ,168, 399-404.
- Valeri C.R., 1996, *Frozen Blood*, *New Engl. J. Med.*, 25, 425-431.
- Valeri C.R., Ragno G., Pivacek L.E., Srey R., Hess J.R., Lippert L.E., Mettillie F., Fahie R., O'Neill E.M., Szymanski I.O., 2001, *Transfusion*, Jul; 41(7), 933-9.
- Valeri C.R., Szymanski I.O., Runck A.H., 1970, Therapeutic effectiveness of homologous erythrocyte transfusion following frozen storage at -80°C for up to seven years, *Transfusion*, 10, 102-112.
- Valeri C.R., Pivace L.E., Cassidy G.P., Rango G., 2000, The survival, funktion, and hemolysis of human RBCs stored at 4°C in additive solution (AS-1, AS-3, or AS-5) for 42 days and then bochemimically modified, frozen, thawed, washed, and stored at 4°C in sodium chloride and glucose solution for 24 hours, *Transfusion*, Nov; 40(11), 1341-5.
- Valeri C.R., 1972, Viability, function and rejuvenation of previously frozen washed red blood cells. In Chaplin HJ, Jaffe ER, Lenfant C, Valeri CR (ed): *Preservation of red blood cells*. Washington, DC, National Academy of Science, pp 265-297.
- *** - Commission Directive 2004/33/EC of March 2004, Annex IV.
- *** - Guide to the preparation, use and quality assurance of blood components, Council of Europe, Recommendation No.R (95), 15.