БІОФІЗИКА КЛІТИНИ

UDC 566.352.462: 611-018.46: 547.42

MEASUREMENT OF THE PERMEABILITY OF PLASMA MEMBRANE OF MURINE BONE MARROW CELLS TO WATER AND CRYOPROTECTANTS

V.S. Kholodnyy, L.F. Rozanov

Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of the Ukraine, 23, Pereyaslavskaya st., Kharkov, 61015, e-mail: cryo@online.kharkov.ua

Received April 22, 2002

Authors studied the osmotic behaviour of murine bone marrow cells in the hypertonic solutions of permeating cryoprotectants dimethyl sulfoxide (Me_2SO), propylene glycol and glycerol. Using the Kedem Katchalsky formalism the permeability parameters of plasma membrane for water and mentioned cryoprotectants were determined. The results demonstrate an hydraulic conductivity, L_p in the presence of cryoprotectant of 1.00 ± 0.09, 1.07 ± 0.12 and 1.19 ± 0.15 ($\times 10^{-13}$ $m^3/s/N$) and cryoprotectant permeability coefficient, K_1 of 6.92 ± 0.08, 11.83±0.11 and 0.94 ± 0.13 ($\times 10^{-8}$ m/s) for Me_2SO, propylene glycol and glycerol, respectively.

KEY WORDS: murine bone marrow cells, osmotic behaviour, Kedem Katchalsky formalism, permeability parameters, dimethyl sulfoxide, propylene glycol, glycerol

Haemopoietic stem and progenitor cell transplantation has been used to successfully treat the broad spectrum of malignant and non-malignant diseases. Sources of these cells include bone marrow, peripheral blood and placental/umbilical cord blood. Now the low temperature storage is the main method in creating the banks of this transplantation material.

Recently the elaboration of the methods for bone marrow cryopreservation was mainly based on empirical approach. Insufficient attention was paid to theoretical substantiation of such an important stage as equilibration of the cells in the solutions of cryoprotectant additive (CPA), meanwhile non-optimal procedures of CPA addition can be the cause of the reduction in cell survival during and after their low temperature storage. An important information about this stage can be obtained from the analysis of the kinetics of interaction of cells with the solution of cryoprotectants.

Osmotic phenomenon, connected with transfer of various substances via selective permeable cell membranes, brought significant contribution in cell membrane structures damage and its protection on various cryopreservation stages.

Study of such biophysical parameters as cell membrane permeability for water and cryoprotectant molecules is an important problem, since these characteristics (equally with cell's geometric parameters) determine optimal values of a number of regimen parameters of cell suspensions cryopreservation. It is known that such biophysical parameters as cell membrane permeability for water and cryoprotectant molecules determine the optimal values of a number of regimen parameters of cell suspensions cryopreservation.

Mentioned characteristics determine the cell osmotic behaviour during its contact with cryoprotectant solutions. Experimental modelling of various cryobiological conditions allows to become the base cryobiological parameters of cells and to study the influence of medium factors on these parameters, and from another side it allows understanding the processes of mass-transfer between cell and surrounding solution. Such approach makes the base of science forecasting the conditions of cryopreservation regimens optimisation.

The osmotically induced changes in cell volume associated with addition and removal of permeating cryoprotectants can be obtained theoretically using the biophysical equations derived from the methods of irreversible thermodynamics established by Kedem and Katchalsky [1]. This formalism can be used to describe the changes in cell volume in relation to the fluxes of water and solutes, providing the cells act as ideal osmometer, if the osmotically inactive volume (α) is known, and if three transport parameters are known: the hydraulic conductivity (L_p), the permeability coefficient of the cryoprotectant additive (K_1), and the associated reflection coefficient (σ_1).

Using the procedure of fitting the equations to the experimental data we can determine the permeability coefficients.

MATERIALS AND METHODS

Collection of bone marrow cells.

Bone marrow cells were obtained by washing the femur bones of (CBA×C57Bl) F_1 line mice, aged 8-12 weeks. Following thorough resuspending the suspension was exposed for 5 min to precipitate the conglomerates, and then it was subsequently used in the experiments.

Measurement of cell osmotic response

Investigations were carried out with an inverted microscope MBI-13, basing on which at the Laboratory of theoretical bases of low temperature preservation of IPC&C of the Natl. Acad. Sci. of Ukraine the model assembly was developed, which includes the microscope and the camera, allowing the recording the processes occurring in the studied sample.

Using this assembly we have evaluated the osmotic response of murine bone marrow cells to variable osmotic stresses. For determining the cell osmotic inactive volume, α, individual cellular volumes were measured following an abrupt dilution to 285, 600, 1000 and 1200 mOsm/kg solutions of NaCl at 18°C by analysing the images obtained with light microscopy. We studied the osmotic response of mononuclears with diameter of 8-10 mcm, which include haemopoietic stem cells, lymphoid cells, basophylic, oxiphylic and polychromatophylic normoblasts [2]. Equilibrated cell volumes were normalised to their respective isotonic values and then plotted versus the reciprocal of normalised osmolality (Boyle van't Hoff plot). Linear regression was calculated to fit the Boyle van't Hoff equation to the data. The Boyle van't Hoff equation is defined by

$$\frac{V}{V_0} = \frac{\pi_0}{\pi}\left(1 - \frac{\alpha}{V_0}\right) + \frac{\alpha}{V_0}, \qquad (1)$$

where V is the cell volume at osmolality π, V_0 is the cell volume in isotonic solution (π_0), α is the osmotically inactive volume. The α was determined by the y-intercept of the regression line, corresponding to $\frac{\alpha}{V_0}$.

Determination of permeability parameters

The mentioned assembly was used to measure cell volumes as described above. Volume excursions were measured following an abrupt (e.g., one-step) adding of cells to dimethyl sulfoxide (Me_2SO) and propylene glycol solutions in concentrations of 1M and glycerol solutions in concentration 0.75M. The solutions were prepared on 0.15 M NaCl solution. The cell kinetic data were analysed using the non-linear equations first introduced by Kedem and Katchalsky [1] to determine the permeability of cell membranes to the solution consisting of permeable solute (cryoprotectant), an impermeable solute (NaCl), and water.

Processes of mass transfer via cellular membranes in the presence of one penetrating through the membrane substance and (n-1) non-penetrating substances are described with the equation system:

$$\frac{dV}{dt} = SL_p\left(\sigma_1\Delta\pi_1 + \sum_{k=2}^{n}\Delta\pi_k - \Delta p\right) \qquad (2)$$

$$\frac{d\pi_1^{in}}{dt} = -\left[\gamma K_1\Delta\pi_1 - \sigma_1\pi_1^{in}\frac{dV}{dt}\right]\frac{1}{y-\alpha} \qquad (3)$$

$$\pi_k^{in} = \frac{\pi_k^{in}(0)(1-\alpha)}{y-\alpha} \qquad (4)$$

where σ_1 - reflection coefficient for penetrating through membrane substance; $\Delta\pi_1=\pi_1^{in}-\pi_1^{out}$, π_1^{out} and π_1^{in} - osmotic pressure of penetrating substance outside and inside the cell, correspondingly; $\Delta\pi_k$ – (k=2,..., n)-transmembrane differential of osmotic pressure of the k-th non-penetrating via membrane substance; $\gamma = \frac{S}{V_0}$-surface-volume ratio of a cell; V_0 - initial cell volume; K_1 - permeability coefficient of cell membrane for penetrating substance; $\pi_k^{in}(0)$ - initial value of osmotic pressure of the k-th non-penetrating intracellular substance; α - cell osmotic inactive volume; $y = \frac{V}{V_0}$ - relative cell volume.

The differential of pressure on membrane Δp can be neglected due to the fact that when reducing the cell volume for plasmatic membrane there is an energetic benefit to be deformed by bending, but not extending or shrinking. For such a type of deformation the area of membrane surface remains unchanged, and the pressure differential on it is insignificantly low when compared with that, appearing during membrane extending.

Let's introduce the parameters:

$$\tau_0 = \frac{1}{L_p\gamma\pi_0^{in}}; \quad \tau_1 = \frac{1}{K_1\gamma}$$

where π_0^{in} – 7.8 atm. – osmotic pressure of isotonic solution.

Equations (2-4) will be as follows:

$$\frac{dy}{dt} = \frac{1}{\tau_0}\left[\sigma_1\left(\tilde{\pi}_1^{in} - \tilde{\pi}_1^{out}\right) + \frac{1-\alpha}{y-\alpha}\right]; \qquad (5)$$

$$\frac{d\pi_1^{in}}{dt} = -\frac{1}{\tau_1}\left[\left(\tilde{\pi}_1^{in} - \tilde{\pi}_1^{out}\right) + \sigma\tilde{\pi}_1^{in}\frac{dy}{dt}\right] \Big/ (y-\alpha); \quad (6)$$

$$\tilde{\pi}_1^{in} = \tilde{\pi}_1^{in}(0)\left(\frac{1-\alpha}{y-\alpha}\right); k = 2,...,n, \quad (7)$$

where $\tilde{\pi}_1^{in} = \frac{\pi_1^{in}}{\pi_0^{in}}; \tilde{\pi}_1^{out} = \frac{\pi_1^{out}}{\pi_0^{in}}$;

It should be considered that the coefficients L_p and K_s can depend themselves on cryoprotectant's concentrations, that was demonstrated for different types of cells.

These equations were solved by the method of Runge - Kutt using the software elaborated at the Department of Low Temperature Preservation of the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of the Ukraine. A fixed value of α, determined from the Boyle van't Hoff plot, was used in the fitting calculation. The value of σ we considered as 0.95. Varying the τ_0 and τ_1 parameters was used to fit the equations to the experimental data and determine the values of L_p and K_s.

The fitting was performed separately for each experiment and then the obtained results were statistically processed with standard Student method.

RESULTS AND DISCUSSION

Bone marrow cells responded as ideal osmometers over a discrete range of osmolalities. The construction of a Boyle van't Hoff plot (Fig.1) for murine bone marrow cells allows determination of the osmotically inactive volume of bone marrow cells. Its value is ($0.34\times V_0$) and is within the range observed for a variety of cell types ($0.2\text{-}0.36\times V_0$) [3-5].

Permeability is generally defined as the function relating chemical flux to chemical potential gradient. In the case of multiple chemical species flux, there is not only a permeability function for each species but also a set of functions representing the interactions of the different fluxes. In the present study, two fluxes are of primary interest: water and cryoprotectant. As most cells are highly permeable to water as well as the cryoprotectant under investigation, a coupled flow of both occurs. The dynamics of this coupled transport dictates cell volume and intracellular concentrations of cryoprotectant during the addition portion of the cryopreservation process and thus has a fundamental role in the success of the cryopreservation procedure used [6].

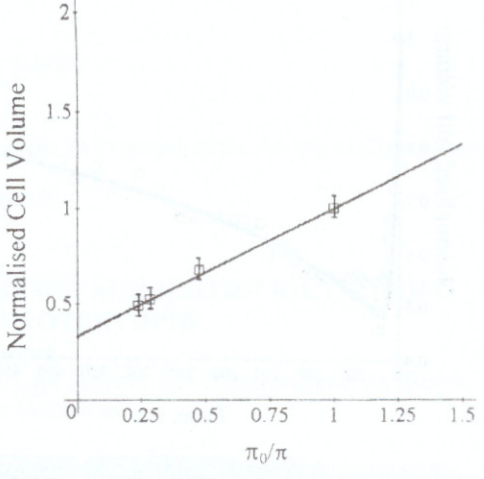

Fig.1. Boyle van't Hoff plot for murine bone marrow cells with diameter of 8-10 mcm. Open squares represent mean cell volume, included in the regression line to determine the osmotically inactive volume

Fig.2 shows representative results of curve fitting procedure of experimental and theoretically derived data for 1M Me_2SO, 1M propylene glycol and 0.75M glycerol. Fig.3 shows the photographs describing cell volume changes in 1M Me_2SO.

It is seen, that when transferring cells from isotonic solution to the permeating cryoprotectant hypertonic solution its volume at first fast decreases up to some value, and then quite enough slowly recovers. Such behaviour of cell is interpreted as follows: to achieve the thermodynamic equilibrium the concentrations of permeating substances out of cell and in it should be equal. The getting equal of these concentrations may occur in two ways: a) because of the outflux of part of water from the cell; b) on account of penetrating of part of cryoprotectant from surrounding solution into cell. Since the water molecules penetrate via cell membrane faster than cryoprotectant molecules the equalising process of outer and inner concentrations on first stage occurs on account of cell's dehydration and its volume decrease down to some minimum value. On the next stage the cryoprotectant molecules slowly penetrate into cell. In the same time some amount of water entrances into cell, that provides the osmotic equilibrium of outer- and intracellular solutions. Cell volume continually decreases here. The maximum volume value on this stage is determined by out-of-cell concentration of impermeable substance (usually NaCl). In case of NaCl isotonic concentration the maximum cell volume must not exceed the initial volume.

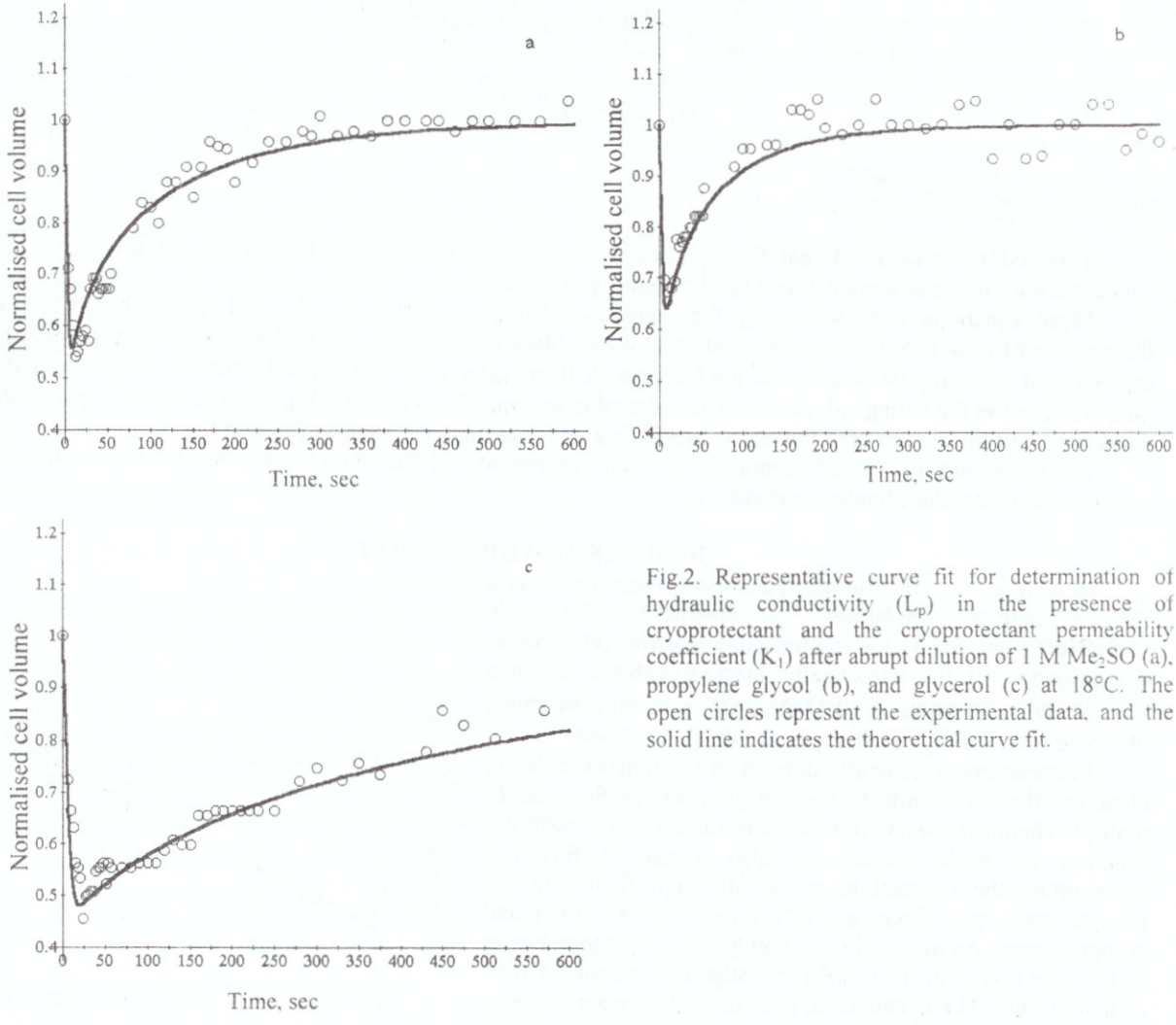

Fig.2. Representative curve fit for determination of hydraulic conductivity (L_p) in the presence of cryoprotectant and the cryoprotectant permeability coefficient (K_1) after abrupt dilution of 1 M Me_2SO (a), propylene glycol (b), and glycerol (c) at 18°C. The open circles represent the experimental data, and the solid line indicates the theoretical curve fit.

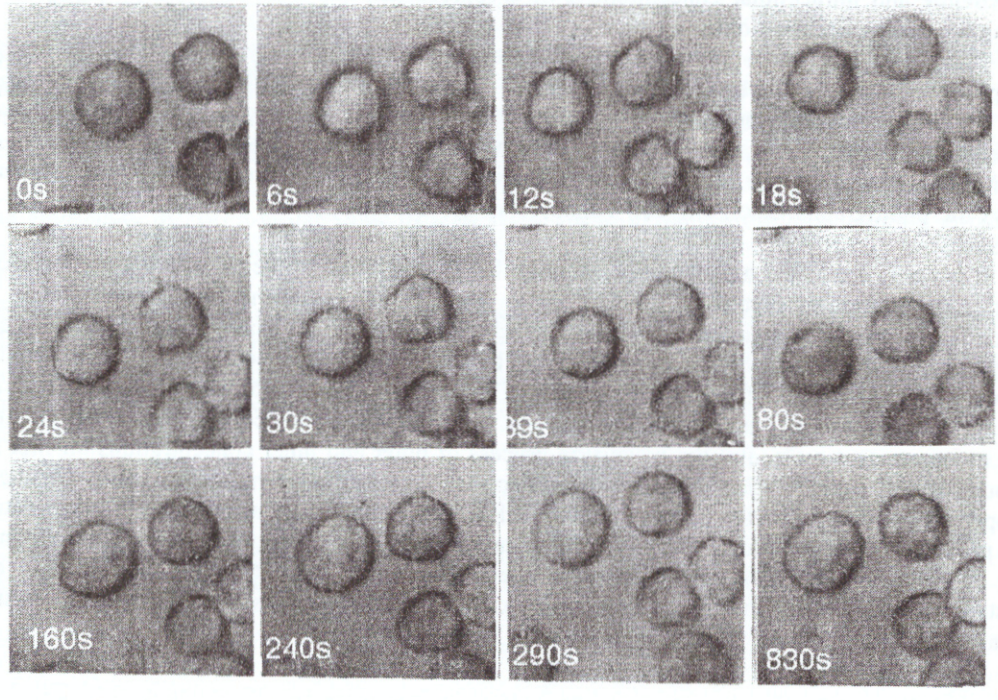

Fig.3. Volume excursions of murine bone marrow cells following an abrupt dilution with 1M Me_2SO solution at 18°C.

Table shows the mean values of determined plasma membrane permeability coefficients of bone marrow cells in cryoprotectant solutions. It is seen that the hydraulic conductivity does not significantly changed when varying the cryoprotectant type. The permeability coefficients for cryoprotectants determined in present study are within the ranges observed for other cell types in which permeability for these cryoprotectant was measured. So, permeability of bull sperm membrane for Me_2SO, propylene glycol and glycerol makes 1.65×10^{-7}, 2.32×10^{-7} and 6.49×10^{-8} m/s, correspondingly [7]. Permeability of human platelets for propylene glycol and Me_2SO is 2.68×10^{-7} and 1.12×10^{-7} m/s, correspondingly [8].

Table. Plasma membrane permeability characteristics of murine bone marrow cells, hydraulic conductivity, L_p, and permeability coefficient for cryoprotectant, K_1, measured following abrupt dilution with hypertonic cryoprotectant solution at 18°C (mean±SD)

Cryoprotectant	L_p, 10^{-13} $m^3/s/N$	K_1, , 10^{-7} m/s
Me_2SO	1,00 ± 0,09	6,92 ± 0,08
Propylene glycol	1,07 ± 0,12	11,83 ± 0,11
Glycerol	1,19 ± 0,15	0,94 ± 0,13

REFERENCES

1. Kedem O., Katchalsky A. // Biochem. Biophys. Acta. 1997. 27. P. 229-246.
2. Gavrilov O.K., Kozinets G.I., Chernyak N.B. Cells of the bone marrow and the peripheral blood. Moscow. "Meditsina", 1985, 288 p.
3. Blinks J.R. // J. Physiol. 1965. 177. P. 42-57.
4. Hepling H.G., Thompson D. and Dupre A. // J. Cell Physiol. 1977. P. 293-302.
5. Liu J., Zieger M.A.J., Lakey J.R.T. et al. // Cryobiology. 1997. P. 1-13.
6. McGrath J. // Cryobiology. 1997. P. 315-334
7. Chaveiro A.E.N., Liu J., Woelders H. and Critser J.K. // Proceedings of the 38th Meeting of the Society of Cryobiology. University of Edinburgh, 2001. P. 170
8. Woods E.J., Liu J., Gilmore J.A. et al // Cryobiology. 1999. 38. P. 200-208.

ВИМІРЮВАННЯ ПРОНИКНОСТІ ПЛАЗМАТИЧНОЇ МЕМБРАНИ КЛІТИН КІСТКОВОГО МОЗКУ МИШІ ДЛЯ ВОДИ І КРІОПРОТЕКТОРІВ

В.С. Холодний, Л.Ф. Розанов

Інститут проблем кріобіології і кріомедицини Національної академії наук України, вул. Переяславська, 23, Харків, 61015, Україна, e-mail: cryo@online.kharkov.ua

Автори вивчали осмотичну поведінку клітин кісткового мозку миші в гіпертонічних розчинах проникаючих кріопротекторів ДМСО, 1,2 – пропандіолу та гліцерину. З використанням моделі Kedem Katchalsky було визначено коефіцієнти проникності плазматичної мембрани для молекул води і означених кріопротекторів. Коефіцієнти проникності для води L_p в присутності кріопротекторів дорівнюють 1,00 ± 0,09, 1,07 ± 0,12 і 1,19 ± 0,15 ($\times10^{-13}$ м^3/с/Н), коефіцієнти проникності для кріопротекторів K_1 - 6,92 ± 0,08, 11,83 ± 0,11 і 0,94 ± 0,13 ($\times10^{-8}$ м/с) для ДМСО, 1,2 – пропандіолу та гліцерину, відповідно.

КЛЮЧОВІ СЛОВА: клітини кісткового мозку миші, осмотична поведінка, модель Kedem Katchalsky, коефіцієнти проникності, ДМСО, 1,2 – пропандіол, гліцерин.