Cell Effects of Xenon *In Vitro* under Hypothermal Conditions

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The effects of Xe on cell viability and redox balance in the culture of Wistar rat thymocytes were studied *in vitro* during 24-h storage under hypothermic conditions. The results indicate that after bubbling of cell suspensions (5×10^6 cell/ml, 4 ml medium), the weight of Xe in flasks was 15-43 mg, whereas in cell-free medium no weight increment due to gas accumulation in the system was detected. The content of Xe in cell suspension slightly decreased over 24-h culturing at ambient temperature (by 10% of initial level). Xenon significantly improved cell survival during thermal exposure of all modes. The maximum cytoprotective effect of Xe was observed under rigorous thermal conditions associated with significant cell death without chemical protectors (3°C, -35°C). The effect of Xe was less pronounced at mild temperatures (23-37°C) or in the presence of chemical protectors (-35°C with dimethylsulfoxide). The mechanisms of the effect of the inert gas are determined by its antioxidant or prooxidant action. The capacity of Xe to improve cell survival under hypothermic conditions can be used for the development of new methods for transportation and storage of cell material.

Key Words: *culture of rat thymocytes; bubbling; viability; supernatants; redox status*

Xenon (Xe) exhibits all properties of inert gases (no color, odor, taste, fluorescence during exposure to electric current, *etc.*). On the other hand, being one of the heaviest elements of its subgroup, it is easier dissolved, adsorbed, compressed, transformed into a liquid state compared to other noble gases and forms more chemical compounds in the form of crystalline hydrates. Moreover, Xe is uninflammable, ecologically safe, and can be stored for a long time [2,3].

Narcotic effect of Xe was discovered in 1946, and since 1951 this gas is used in anesthesiology [2,3,10]. Xenon is highly effective in the treatment of adaptation disorders and abstinent syndrome in opiate narcomania [5,8]. Isotopes ^{127}Xe and ^{133}Xe are used in functional diagnosis for detection of respiratory system pathologies and evaluation of bloodflow in various organs (liver, brain, muscles) [3] by computer-aided tomography.

On the other hand, the mechanisms underlying the effects of Xe on cells remain little studied. Xe forms clathrates with water molecules [3], is highly soluble in lipids, and interacts with proteins, therefore accumulation of Xe in cell membranes can be hypothesized [15]. However, ambiguous and sometimes contradictory effects of Xe *in vivo* can be explained by intricate cell-cell interactions based on the balance of cellular, humoral, and hormonal, distant and local, direct and indirect signals. Despite active discussion of the mechanisms of Xe interactions with biological molecules and structures [10], the data on its effects on cells are scanty.

Active use of cell material in laboratory and clinical practice (biotechnology, cell engineering, cell therapy, transplantology, *etc.*) puts forward the problems of cell storage and transportation without loss of their morphological and biochemical char-

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acteristics. Most agents currently used for preservation are toxic compounds. For example, dimethylsulfoxide (DMSO) causes apoptosis, primarily of lymphoid cells [7]. In addition, the maintenance of biological material viability by means of chemical reagents involves numerous manipulations. In addition, even partial loss of cell material is not permissible, because of high probability of transplantation failure [1].

Hence, *in vitro* study of the effect of Xe on cell viability and redox balance under optimal and unfavorable thermal conditions is important for research and practical purposes.

MATERIALS AND METHODS

Experiments were carried out in the fall-winter period. Biological material was obtained from 5 Wistar rats weighing 200-300 g from vivarium of Institute of Cardiology, Tomsk Research Center. Thymocytes, highly sensitive to endogenous and exogenous inductors of cell death [7], were isolated as described previously [11].

For evaluation of the Xe effects at different temperatures, bubbling of cell suspensions was carried out. Xe of 99.999% purity (Laser Diagnosis and Pure Technologies Technocenter) was used.

Cell suspensions (5×10^6 cell/ml in 4 ml medium) were exposed to Xe in a semi-open system (the diameter of inlet needle was greater than that of the outlet one). Xenon was bubbled through the suspension at a pressure of 0.025 MPa and 23°C for 30 sec. The content of gas introduced into the system was evaluated by the weight increment after Xe exposure. High leakage of Xe necessitated evaluation of its release from the flasks over 24 h after bubbling.

The cells were then cultured *in vitro* in 10-ml flasks under different thermal conditions (3 samples per point) for 24 h. The composition of the culture medium was as follows: 95% RPMI-1640 (Sigma), 5% FCS (ISN), 280 mg/liter L-glutamine (Sigma), and 50 mg/liter gentamicin. Cell culture not exposed to Xe served as the control.

In one experimental series, freshly isolated rat thymocytes were frozen in culture medium with DMSO (10% final concentration, intracellular preserving agent). Cell suspension was mixed with cold DMSO solution in 1:1 ratio, placed into a freezer, the temperature was reduced to -35°C over 60 min; the cells were stored for 24 h and then rapidly defrosted in water at 40-42°C.

After 24-h incubation, the viability of nucleated cells was estimated by trypan blue (0.4%) test according to the ISO 10993-5 requirements [11]. The cell suspension was then centrifuged for 10 min at 500*g*, conditioned media were collected, and put into 1.5-ml containers. The concentrations of malonic dialdehyde (MDA) and NO and catalase activity were evaluated in the supernatants.

The concentration of MDA (μM) was evaluated in the test with 0.8% TBA by the formation of stained complex with subsequent colorimetry at λ=532 nm. Catalase activity (ncat/liter) was detected by extinction at 410 nm using ammonium molybdate [4]. The content of NO (mM) in the supernatants was evaluated on an IFA analyzer at λ=550 nm by the content of nitrites after treatment with Griess reagent [11].

Because of low number of measurements ($n<30$) and great differences in the distribution of signs in comparison with the normal, the data were processed using Mann—Whitney nonparametric test.

RESULTS

The content of Xe after bubbling of cell suspension was 15-43 mg in all flasks (Table 1). In cell-free medium, no weight increment due to gas accumulation in the system was detected. This is ex-

TABLE 1. Thymocyte Viability after 24-h Culturing in Xe Atmosphere at Different Temperatures (%)

Temperature	Gas concentration in flask, mg/ml	Group	
		control	experiment
37°C	8.62	70 (*n*=6)	78* (*n*=6)
23°C	7.43	93 (*n*=6)	97* (*n*=6)
3°C	3.76	78 (*n*=10)	89* (*n*=10)
-35°C			
no DMSO	6.83	11 (*n*=15)	35* (*n*=15)
+DMSO	10.72	53 (*n*=15)	60* (*n*=15)

Note. Here and in Table 2: *n*: number of measurements. *$p<0.05$ compared to the control.

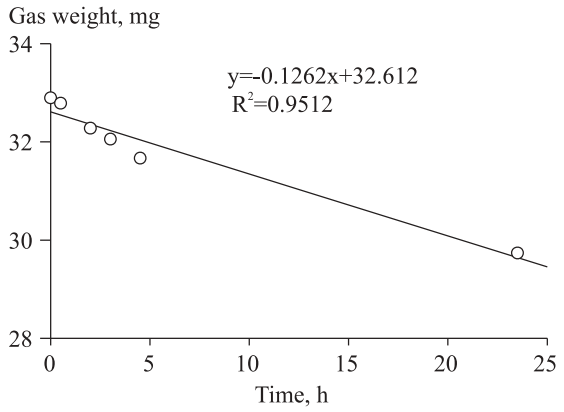

Fig. 1. Dynamics of Xe release from liquid thymocyte culture.

plained by the fact that maximum Xe solubility in the water at atmospheric pressure is no more than 1.2 mg/ml at 0°C and 2.5 times lower at 37°C [14].

In our experiments the content of Xe in the system was higher (Table 1). It seems that Xe accumulation in cell suspension is promoted by its interactions with biological membranes [15]. The detected decrease in Xe content at temperatures approaching the freezing temperature (Table 1) confirms this hypothesis.

Xenon is characterized by high fluidity and solubility in comparison with other inert gases and can penetrate through silicon layers [3], which impedes its practical application.

The study was carried out on 24-h cell cultures, and hence, 24-h dynamics of Xe content in the system was evaluated (Fig. 1). The decrease in Xe content during this period was negligible (3.19 mg, 10% of initial level), the rate of this decrease was 0.13 mg/h; therefore, the initial weight of Xe in the system (1 h after bubbling) was used in subsequent estimations.

Significant deviations from the optimal temperature (33-37°C) disturb vital activity of cells. In our experiments (Table 1) thymocyte survival at different temperatures decreased in the following order: 23°C>3°C>37°C>-35°C (with DMSO)>-35°C (without DMSO). Hence, the problem of antistress protection of cells is important at the extreme thermal conditions.

With temperature decrease from 37°C to 3°C, the oxidative influences on cells decrease (Table 2), which reflects their different physiological status under changing environmental conditions (activation—hypobiosis). It was therefore interesting to try Xe as a cytoprotector fixing the hypobiotic status of the cells [9].

Defrosting causes death of an appreciable part of thymocyte population due to activation of free-radical processes (Table 2). This physical influence on cell membranes can be regarded as an extreme factor, because high catalase activity not characteristic of normal conditions is observed during accumulation of great amounts of H_2O_2.

Xe significantly ($p<0.05$) increased the number of viable cells at all temperatures (Table 1). The maximum increase in thymocyte survival due to the presence of the inert gas was observed after cell cryopreservation without DMSO (318% of control level). These data indicate that the cytoprotective effect of XE is most pronounced under conditions of significant cell damage in media containing no chemical protectors.

Lipid peroxidation (LPO) in cell membranes is a leading mechanism of cell death. This process can be detected by accumulation of MDA [4] and NO [13]. Cell adaptation to extreme conditions can be mediated by different metabolic pathways underlying resistant or tolerant (hypobiotic) adaptation strategies [6], realized through activation or inhibition of cell metabolism.

It seems that due to different initial status of the cells under different thermal conditions, Xe produced a modulating, in fact, reversive effects on redox processes (Table 2). Xe exhibits unambiguous physicochemical properties: hydrophilia and lipophilia, formation of dipoles and clathrate type microcrystals with water molecules, dissolution in lipids, interaction with cations and proteins. Due to this, the predominant effect of Xe on the stimulatory or inhibitory ionic channels with ligand-dependent and potential-dependent gates can determine functional state of cells [10].

TABLE 2. Content of MDA and NO and Catalase Activity in Thymocyte Supernatants after 24-h Culturing in Xe Atmosphere at Different Temperatures

Temperature		Control	Experiment
37°C	MDA	0.979 (*n*=9)	0.456* (*n*=9)
	NO	5.24 (*n*=12)	4.52* (*n*=10)
	catalase	33.67 (*n*=9)	30.67* (*n*=9)
23°C	MDA	0.428 (*n*=8)	0.650 (*n*=9)
	NO	3.33 (*n*=11)	3.57* (*n*=11)
	catalase	24.42 (*n*=9)	23.00 (*n*=9)
3°C	MDA	0.160 (*n*=6)	1.613* (*n*=6)
	NO	1.41 (*n*=1)	0.69 (*n*=3)
	catalase	19.09 (*n*=6)	21.38 (*n*=6)
-35°C	+DMSO		
	MDA	0.671 (*n*=8)	0.285* (*n*=7)
	NO	4.28 (*n*=12)	3.80 (*n*=10)
	catalase	52.67 (*n*=9)	46.92* (*n*=9)

TABLE 3. Vector of Xe Effect on Thymocyte Structure and Function (% of Changes in Parameters per mg Gas Introduced into Cell Culture)

Parameter	Temperature				
	37°C	23°C	3°C	-35°C	
				no DMSO	+DMSO
MDA	-1.550	+1.745*	+60.42*	—	-1.342
Catalase	-0.258	-0.196	+0.798*	—	-0.255
NO	-0.399	+0.242	-3.397	—	-0.262
Cell viability	+0.23	+0.13	+0.69*	+4.96*	+0.18

Note. *$p<0.05$ compared to values at 37°C.

It seems that better understanding of the effects of Xe on cells can be attained, if we consider Xe atom with a radius of 0.218 nm as a subnanometer bioinert particle, because the behavior of bioinert materials in the body largely depends on homeostasis, rather than on the characteristics of the implant. In addition, Xe is an antagonist of NMDA receptor [12] and NO serves as an intracellular transmitter for these receptors. The role of NO as a physiological agent and pro- or antioxidant depends on its concentration and cell status [13].

Changes in Xe content in cell suspension in all experimental series (Table 1) necessitated evaluation of its modulating effect in conversion to 1 mg of gas (Table 3). It was found that the maximum cytoprotective effect was observed under rigorous thermal conditions, associated with significant level of cell death, without chemical protectors. Under comfortable vital conditions (23-37°C) or in the presence of chemical protection factors (-35°C with DMSO) the effects of the noble gas were less pronounced. It is noteworthy that the mechanisms of these effects of the inert gas on cells were associated with its pronounced antioxidant or prooxidant action (Tables 2, 3).

The capacity of Xe, in addition to its unique physicochemical characteristics, to improve cell survival under hypothermal conditions can be used for the development of new methods for cell material transportation and storage. The solution of technological problems of maximum stabilization of cytoprotector levels in the system will significantly improve the efficiency of the method.

REFERENCES

1. K. M. Abdulkadyrov, N. A. Romanenko, and N. N. Starkov, *Vopr. Onkol.*, **46**, No. 5, 513-520 (2000).
2. N. E. Burov, V. N. Potapov, and G. I. Makeev, *Xenon in Anesthesiology. Clinical and Experimental Studies* [in Russian], Moscow (2000).
3. E. A. Damir, N. E. Burov, G. N. Makeev, and D. A. Dzhabarov, *Anesteziol. Reanimatol.*, No. 1, 71-75 (1996).
4. V. S. Kamyshnikov, *Handbook of Clinical Laboratory Diagnosis* [in Russian], Minsk (2000).
5. N. A. Kornetov, M. N. Spisman, S. A. Naumov, *et al.*, *Use of Therapeutic Xenon Narcosis in Combined Therapy of the Abstinent Syndrome in Opiate Narcomania: Handbook for Physicians* [in Russian], Tomsk (2002).
6. V. I. Kulinskii and I. A. Ol'khovskii, *Uspekhi Sovr. Biol.*, **112**, Nos. 5-6, 697-713 (1992).
7. E. F. Lushnikov and A. Yu. Abrosimov, *Cell Death (Apoptosis)* [in Russian], Moscow (2001).
8. S. A. Naumov, N. I. Suslov, V. N. Potapov, *et al.*, *Xenon Therapy of Adaptation Disorders: Methodological Recommendations* [in Russian], Tomsk (2005).
9. B. V. Testov, V. V. Efimov, and A. G. Surnin, *Third Working Conference "New Medical Technologies"* [in Russian], November 12-22, 2000, Tomsk, Moscow (2000), pp. 35-39.
10. I. A. Khlusov, S. A. Naumov, S. M. Vovk, *et al.*, *Vestn. Rossiisk. Akad. Med. Nauk*, No. 9, 32-37 (2003).
11. V. P. Shakhov, I. A. Khlusov, G. Ts. Dambaev, *et al.*, *Introduction in Methods of Cell Culture, Organ and Tissue Bioengineering* [in Russian], Ed. V. V. Novitskii, V. P. Shakhov, and I. A. Khlusov, Tomsk (2004).
12. T. Goto, *Applied Cardiopulmonary Pathophysiol.*, **9**, 129-131 (2000).
13. H. Kobayashi, T. Suzuki, S. Saito, *et al.*, *Toxicol. Ecotoxicol. New Rev.*, **4**, No. 1, 15-19 (1997).
14. T. J. Morrison and N. B. Johnstone, *J. Chem. Soc.*, **101**, No. 10, 3441 (1954).
15. O. Sauer, M. Roth, T. Schirmer, *et al.*, *Acta Crystallogr. D. Biol. Crystallogr.*, **58**, Pt. 1, 60-69 (2002).