Time Limiting Boundaries of Reversible Clinical Death in Rats Subjected to Ultra-Deep Hypothermia

Evgeniy L Gagarinskiy, Aleksey S Averin, Viktor K Uteshev, Pavel V Sherbakov, Vladimir I Telpuhov, Nikolay E Shvirst, Yulya A Karpova, Artem E Gurin, Aleksandr V Varlachev, Anatoliy L Kovtun, Eugeny E Fesenko Jr
Institute of Cell Biophysics of the Russian Academy of Sciences, PSCBR RAS, Pushchino, Advanced Research Foundation, Moscow, Russia

ABSTRACT

Background: It is well known that body temperature maintenance between 20 and 35°C prevents hypoxic damage. However, data regarding the ideal duration and permissible temperature boundaries for ultra-deep hypothermia below 20°C are rather fragmentary. The aim of the present study was to determine the time limits of reversible clinical death in rats subjected to ultra-deep hypothermia at 1–8°C.

Results: Rat survival rates were directly dependent on the duration of clinical death. If clinical death did not exceed 35 min, animal viability could be restored. Extending the duration of clinical death longer than 45 min led to rat death, and cardiac functioning in these animals was not recovered. The rewarming rate and the lowest temperature of hypothermia experienced did not directly influence survival rates.

Conclusions: In a rat model, reversible ultra-deep hypothermia as low as 1–8°C could be achieved without the application of hypercapnia or pharmacological support. The survival of animals was dependent on the duration of clinical death, which should not exceed 35 min.

Keywords: Cardiac arrest, clinical death, survival rate, suspended animation, ultra-deep hypothermia

BACKGROUND

Hypothermia is a state in which the body temperature of an organism drops lower than is required to maintain normal metabolic functioning. During hypothermia, metabolic rates decrease, which result in the reduction of oxygen consumption. Different stages of the core temperature drop are classified as mild (33–35°C), moderate (30–32°C), deep (20–30°C), and ultra-deep (<20°C) hypothermia. At present, the most studied stages of hypothermia are mild and moderate. Small overcooling to 32–34°C has been widely used for decreasing the risk of undesirable complications of insufficient blood supply or trauma. Furthermore, in limited published studies of deep hypothermia, authors have described mainly the negative consequences of cooling warm-blooded animals to temperatures that have ranged from 18 to 28°C. It has been noted that such cooling is accompanied by biochemical and visceral disturbances, which may result in the death of the organism. Among the primary causes of death associated with deep hypothermia are intracranial pressure rise caused by cerebral edema, disturbance of cardiac performance, and renal damage. Medications used to facilitate the entrance to and exit from a deep hypothermia state include L-type Ca-channel blockers, antioxidants, polyethylene oxides, and urea and its analogues.
The majority of studies investigating ultra-deep hypothermia have largely concentrated on the development of emergency preservation and resuscitation (EPR) technology to extend the golden hour (a period after a traumatic injury, when emergency treatment is most likely to be successful) in life-threatening situations. Controlled ultra-deep hypothermia enables successful resuscitation in cases involving serious injuries of the chest cavity that are accompanied by blood loss with a fatality rates as high as 90%. The EPR protocol provides the time required for surgical care by cooling the body to 10°C.

The aim of the present study was to a) investigate the possibility of reaching ultra-deep hypothermia at temperature level below 10°C in rats under conditions of forced external cooling following Shcherbakov’s method with modifications (Shcherbakov et al., 1989) without the application of hypercapnia and pharmacological drugs to obtain initial data on capacities of an organism on which the hypothermia control and management methods could be based; b) evaluate the duration throughout which ultra-deep hypothermia is tolerated and normal vital functions are restored, and c) define optimal cooling and rewarming procedures.

**METHODS**

Experiments were conducted using male Wistar rats (200–250 g) that were obtained from the vivarium of ICB RAS. In total, 53 rats were used in three series of experiments. The study protocol was approved by the Institutional Animal Care and Use Committee of ICB RAS.

In the first series of experiments, we studied the possibility of reaching reversible ultra-deep hypothermia in rats. We performed fast cooling of animals using an external cold-water flow and achieved temperatures 1–8°C. Animals were subsequently rewarmed to 30°C at a rate of 1.2°C × min⁻¹ [Figure 1]. Changes in cardiac function were evaluated by assessing heart rate (HR) dynamics during the cooling–rewarming processes. Forced deep cooling of animals was performed in accordance with Shcherbakov et al. (1989) with some modifications (the surgical introduction of a pressure sensor into the subclavian artery was excluded to reduce the occurrence of traumatic injury; rewarming rates were increased). Rats were anaesthetized with ether in a transparent glass jar. Then, anaesthetized rats were placed on a surgical table on their backs. To perform artificial pulmonary ventilation (APV), animals were intubated with original tubes (D = 1.8–2.2 mm). The rat ligamentous apparatus reflexively compressed the intubation tube, thus protecting the lungs from water entering airway.

To record an electrocardiogram (ECG), needle-shaped electrodes were attached to the paws of each animal. APV was performed with an EPM-2 device (1st MGMU, Russia) using a tidal volume of 1 ml × 100 g⁻¹ and a frequency of 70 min⁻¹. Rectal temperature was measured with an electronic thermometer (Actacom-ATT, Taiwan). ECG in the second standard lead was recorded using a veterinary monitor IM-10 (ZooMed, China). Each rat was placed into the plastic chamber with circulating water at 1–4°C at a water level 2.
cm higher than the uppermost portion of the body of the animal. Water cooling and circulation were carried out using a Ministat 230 W cryothermostat [Figure 1; Huber, Germany]. Our experiments showed that after reaching a rectal temperature of 10°C, cardiac functioning of rats ceased and only slight electrical activity and some ECG spikes were observed. At 8–9°C, cardiac functioning was completely terminated. Therefore, 10°C was accepted as a starting point for reversible clinical death. Spontaneous recovery of a heartbeat throughout the rewarming period was also observed after reaching a rectal temperature of 10°C. The time period that extended from the point at which the body temperature reached 10°C in the cooling process to 10°C when the animal was rewarmed was designated as the duration of reversible clinical death. APV intensity was gradually reduced to 20 min⁻¹ as the rectal temperature decreased. After the cardiac arrest, APV was turned off. The lowest point of hypothermia was regulated by cooling time with indicators of rectal temperature used as a beacon. From 2 to 3.5°C, before the required hypothermic level was reached, the cryothermostat was switched to a heating mode at a given speed to compensate for the inertia that occurred while cooling/rewarming rat body.

At the rewarming stage, each rat was removed from the water on the basis of three indicators that included a rectal temperature of 27–30°C, heart rate above 378 ± 22 beats × min⁻¹ and visual signs of motor activity. At this time, the intubation tube, ECG electrodes, and rectal thermometer were removed. Final rewarming was carried out with a dryer by blowing hot air (~50°C) on each animal.

In the second series of experiments (paragraph 3.3 in the results section), we studied the influence of the rewarming rate on the recovery of vital functions in rats cooled to 3°C (group 1; 12 animals) and 8°C (group 2; 12 animals). The rewarming speed was 0.6 (6 animals) and 1.2°C × min⁻¹ (6 animals) for each of 2 groups.

In the third series of experiments (paragraph 3.4 in the results section), 12 rats were cooled to 8°C and 6 of the cooled rats were kept at the temperature for 10 min, while the other 6 rats were kept at 8°C for 30–75 min. To stabilize the entrance to the hypothermic temperature plateau, a cryothermostat N2 preheated to 8°C was additionally used. All animals were rewarmed at a rate of 1.2°C × min⁻¹.

Fully recovered animals were returned to vivarium and observed for a month. Animals were housed in standard cages and were provided food and water ad libitum for an equal duration (12 h) of the light–dark cycle. Data analysis was performed using Sigma Plot 12.5 software (Systat Software Inc, US) and all data were expressed as mean ± standard deviation (SD).

RESULTS

Survival of rats cooled between 1 and 8°C

The survival of rats cooled between 1 and 8°C varied according to the level and duration of hypothermia achieved. The heart functioning of animals cooled below 3°C did not recover upon rewarming. The heart functioning of rats cooled to temperatures above 3°C was capable of spontaneously recovering as rectal temperatures increased to temperatures above 10°C, except in animals that were exposed to temperatures of 3–8°C longer than 45 min. Overall, in the first series of experiments, 12 of 17 rats fully recovered after being clinically declared dead. Rectal temperature changes during cooling and rewarming steps are shown in Figure 2.

Heart rate dynamics of rats that survived rewarming

Heart rate dynamics during cooling and subsequent rewarming of surviving rats are shown in Figure 3. As expected, throughout the cooling process, HR decreased as rectal temperature declined. As the figure illustrates, at the same body temperature, HRs were higher during the rewarming than cooling. For instance, at 20°C, the average HRs during cooling and rewarming steps were 110 ± 9 and 150 ± 10 beats × min⁻¹, respectively, and at 30°C, the average HRs observed were 295 ± 33 and 378 ± 22 beats × min⁻¹, respectively.

Influence of rewarming rate on the recovery of vital functions

In order to study the influence of rewarming rate on the restoration of vital functioning, we carried out experiments...
in which rats were cooled to 3°C and 8°C and subsequently rewarmed at different speeds [Figure 4]. In the first case, when cooled to 3°C [Figure 4a], vital activity of animals was recovered after rewarming at a speed of 1.2°C min⁻¹. On the other hand, the cardiac function of rats in the second group, which were rewarmed at 0.6°C min⁻¹ was not restored, despite attempts to reanimate the animals. In the second case, when animals were cooled to 8°C, rewarmed speed did not affect survival rates of rats in either group, and the vital activities of all animals were restored [Figure 4b].

**Influence of the duration of hypothermia on animal survival**

The analysis of rat survival in the third series of experiments showed that the duration of clinical death determines whether restoration of the vital activity is possible. Six of six rats survived in the subgroup that was cooled and maintained at 8°C for 10 min, which produced a total period of clinical death that did not exceed 30 min. However, 0 out of 6 rats that were maintained same hypothermic temperature for 30–60 min survived. In this case, the duration of clinical death exceeded 45 min [Figure 5].

**DISCUSSION**

Results regarding hypothermic rat survival obtained in this study can be explained in terms of the duration of clinical death. The duration of the silent heart, in our view, was a critical factor, which defined whether the animal could be recovered after being exposed to ultra-deep hypothermia at a temperatures 1–8°C. The rewarming rate and the lowest temperature of hypothermia did not affect revitalization. Animal viability could be restored as long as the period of clinical death did not exceed 30–35 min. A period of clinical death that exceeded 45 min led to the death of experimental animals, and cardiac functioning in these animals was unable to be recovered.

Assuming that there is a critical duration of clinical death that determines the capacity of animals to be rewarmed successfully explains observed failures to cool rats to temperatures of 1°C, since it takes about 90 min to reach this temperature. The time required significantly exceeds the duration allowed, and leads inevitably to animal death. Similar results were obtained in experiments with spinal cord neurons, which showed that the duration of hypothermia was the main factor affecting nerve cell survival.²⁰

The importance of the duration of clinical death to successful recovery of the vital functions of cooled rats has also been assessed by Andzhus and Hozich (1965). They found that greatest duration of clinical death that facilitated the recovery of the vital functions of rats was...
We believe that it is not enough to
inhibitory mediator of the central nervous system.
accumulation of animals.
mechanisms similar to those that exist in hibernating
or during initial stages of cooling in rats activates defence
At the same time, tolerating hypoxia–hypercapnia prior to,
which vital functions were not restored (n = 6) (clinical death duration exceeded 45 min)

60 min. Increasing time spent in a state of clinical death led to decreases in the number of surviving animals. Observed difference in the maximum duration allowed in our study and the abovementioned work may be linked to the additional use of the hypoxia–hypercapnia method by Andzhus and Hozich prior to the cooling of the animals, which could have increased their resistance to ultra-low temperatures.

A similar approach has adopted Niazi (1957) who used gas mixtures with an increased CO₂ concentration during lung ventilation of monkeys cooled between 4°C and 9°C. In his experiments, animals restored activity after a 2-hour period of clinical death. During prolonged clinical death under ultra-deep hypothermic conditions, metabolic processes slow, but do not fully stop. This leads to energy and oxygen depletion in the organism. Hypoxia that develops during hypothermia leads to direct damage of the brain and heart through disruption of the NA/K pump, accumulation of intracellular Ca, edema, and acidosis. Also, it leads to the accumulation of reduced oxygen equivalents in the mitochondrial electron transport chain, which can stimulate increased formation of reactive oxygen species and oxidative stress process that causes lipid, protein, and nucleic acid dysfunction.

At the same time, tolerating hypoxia–hypercapnia prior to, or during, initial stages of cooling in rats activates defence mechanisms similar to those that exist in hibernating animals. Evoked hypoxia–hypercapnia leads to the accumulation of γ-aminobutyric acid, which is the main inhibitory mediator of the central nervous system. Suppression of neuronal activity increases brain tolerance to hypoxia, decreases oxygen consumption and contributes to survival during hypothermia. Overall, it allows the body to withstand longer periods in a state of clinical death involving ultra-deep hypothermia.

HR dynamics observed in our study show that there is a gradual decrease in heart rate following a temperature drop. When temperature dropped to below 13°C, ECG tracings showed in most cases a decrease in the amplitude of heart contractions, a gradual smoothing of the P waves, inversion of the QRS complex, and arrhythmia of ventricular origin. There are data demonstrating that an increase of catecholamine is observed in the blood during cooling in experiments involving moderate hypothermia of 33–34°C. We believe that it is not enough to compensate for heart rate decreases under intensive cooling conditions used here. During rewarming, a slight increase in HR was observed compared to cooling step, which is in accordance with previous studies. Th The arrhythmia arising at the time of rewarming quickly stabilized, however, in some rats, single extrasystoles of the ventricle persisted until they were removed from the water. We suppose that the occurrence of more serious cardiac arrhythmias during cooling and rewarming steps is prevented by lung ventilation which was turned off or started after reaching a rectal temperature of 10°C. It protects the heart from hypoxia, which occurs during the natural respiratory arrest at a temperature of 16.2 ± 0.6°C.

It should be noted that in nature, the problem of reversible ultra-deep hypothermia has successfully been solved. In a state of deep dormancy, the body temperature of the Yakut ground squirrel (Spermophilus undulatus) decreases to 0°C. The animals can remain for 3 weeks in a state in which their heart rates slow to 3–4 beats min⁻¹. The similarity of morpho-physiological changes found in hibernators and non-hibernating animals entering a low-temperature state indicates the presence of common mechanisms that affecting a switch to a low metabolic rates under certain conditions.

Artificial deep and ultra-deep hypothermia is of particular interest for medicine, since it can be used in intensive surgical treatment of seriously injured or ill (e.g., aortic aneurysms) patients to protect organs during circulatory arrest. Emergency Preservation and Resuscitation Technology involves the use of a cold aortic flush to replace blood with saline solution and induce a deep hypothermic state at 10°C for up to 2 h, and facilitates the repair of traumatic injuries. Whether hypothermic temperatures can be lowered further than 10°C is the subject of debate. Our
data suggest the possibility of the successful revitalization of rats subjected to cooling to temperatures as low as 3°C, without pharmacological support. Deeper hypothermia may contribute to a further decrease in metabolic activity by an additional 50−70%. Oxygen consumption of the brain decreases 1.8−3.5 fold (depending on the temperature interval) every 10°C.[38,39] The overall metabolic rate of hibernating animals at near-zero temperatures decreases to 1/10−1/100 of the normal physiological levels.[40] Also, decreasing hypothermic temperatures to below 10°C will not significantly prolong the duration of reversible clinical death, according to our data. Theoretically, it may increase the protection of organs against ischemia and decrease possible complications that occur after rewarming.

CONCLUSIONS

The obtained results of this work suggest the following:
1. The probability of the successful recovery of vital functions in deeply cooled rats does not depend directly on the minimum temperature reached (in the range of 1−8°C).
2. The rate of rewarming of deeply cooled rats is not the defining factor for the recovery of vital functions.
3. The successful recovery of vital functions of deeply cooled rats depends on the duration of clinical death. If the duration of clinical death did not exceed 35 min, all animals were able to recover from cooling. The reanimation of rewarmed animals was not observed when the duration of clinical death exceeded 45 min.

The limitation of this study was the emphasis on the time limiting boundaries of reversible clinical death during ultra-deep hypothermia. According to our data, the period from 35 to 45 minutes was borderline in terms of the organism’s survival. In borderline group, at the stage of rewarming, in addition to death and recovery, some animals showed a delayed death in a period from several hours to 2 days. It was accompanied by shortness of breath and frostbite signs on extremities. The difference in survival in borderline group probably depends on the individual resistance to hypothermia of a particular animal and does not fit all or nothing boundaries we aimed for. We consider that another limitation of this work is the lack of complex assessment of cognitive functions in rats subjected to ultra-deep hypothermia which could be a subject of further research.

Ethical approval and consent to participate
All animal procedures performed in this study were approved by the Biological Safety and Ethics Committee (Institute ICB RAS) in accord with Directive 2010/63/EU.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Kuffer DP. Maximizing neuroprotection: Where do we stand. Ther Clin Risk Manag 2012;8:185–94.
2. Polderman KH. Mechanisms of action, physiological effects, and complications of hypothermia. Crit Care Med 2009;37:186–202.
3. Fox JI, Vu EN, Doyle‑Waters M, Brubacher JR, Bélanger R, Hu Z. Prophylactic hypothermia for traumatic brain injury: A quantitative systematic review. Can J Emerg Med 2010;12:355–64.
4. Testori C, Sterz F, Behringer W, Haugk M, Uray T, Zeinert A, et al. Mild therapeutic hypothermia is associated with favorable outcome in patients after cardiac arrest with no‑shockable rhythms. Resuscitation 2011;81:1162–7.
5. Abdurahmanov RG, Pinyaskina EV, Gitinmagomedova MM. Influence of nifedipine on electrical brain activity during hypothermia rats, Fundamental research 2014;8:620–3.
6. Kondratiev TV, Wold RM, Aasum E, Tveita T. Myocardial mechanical dysfunction and calcium overload following rewarming from experimental hypothermia in vivo. Cryobiology 2008;56:15–21.
7. Tsai MS, Huang CH, Yu PH, Tsai CY, Chen HW, Cheng HJ, et al. Prolonged cooling duration mitigates myocardial and cerebral damage in cardiac arrest. Am J Emerg Med 2015;33:1374–81.
8. Loveeitch IV, Gunnikov AI, Davydova LG. Hypothermia as a method of neuroprotection of patients with damage of structures of postcrania! fetal. Sixth NPK “Safety of patients in anaesthesiology and resuscitation” 2008. p. 43–4.
9. Mallet ML. Pathophysiology of accidental hypothermia. QJM 2002;95:775–85.
10. Prandini MN, Neves FA, Lapa AJ, Stavale JN. Mild hypothermia reduces polymorphonuclear leukocytes infiltration in induced brain inflammation. Anq neuropsiquiatr 2005;63:779–84.
11. Marazov VK, Vyazovskaya OV, Ovsyannikov SE, Kompaniets AM. The dynamics of intracranial pressure after acute general deep hypothermia and possible strategies of its normalization. The Journal of V.N.Karazin Kharkiv National University. Series "Biology" 2008;7:161–9.
12. Gurabi Z, Koncz I, Patocsakai B, Nesterenki VV, Antzelevitch C. Cellular mechanism underlying hypothermia-induced ventricular tachycardia/ventricular fibrillation in the setting of early repolarization and the protective effect of quinidine, cilostazol, and milrinone. Cire Arrhythm Electrophysiol 2014;7:134–42.
13. De Rosa S, Antonelli M, Ronco C. Hypothermia and kidney: A focus on ischemia‑reperfusion injury. Nephrol Dial Transplant 2016;32:241–7.
14. Kuriyama S, Tomonari H, Numata M, Imasawa T, Hosoya T. Clinical characteristics of renal damage in patients with accidental hypothermia. Nihon Jinzo Gakkai Shi 1999;41:493–8.
15. Gitinmagomedova MM, Pinyaskina EV, Abdurahmanov RG. Influence of thiourea on the electrical activity of rat brain during hypothermia. Fundamental research 2013;11:357–60.
16. Rhee PM, Acosta J, Bridgeman A, Wang D, Jordan M, Rich N. Survival after emergency department thoracotomy: Review of published data from the past 25 years. J Am Coll Surg 2000;190:288–98.
17. Tisherman SA. The yin and yang of hypothermia in trauma. J Intensive Care Med 2010;25:240–2.
18. Alam HB, Bowyer MW, Kousta! V, Gushchin V, Anderson D, Stanton K, et al. Learning and memory is preserved after induced asanguineous hyperkalemic hypothermic arrest in a swine model of traumatic exsanguination. Surgery 2002;132:278–88.
19. Shcherbakov PV, Telpuhov VI, Holdo AV. Reversible deep hypothermia of whole organism of rats. Bull Exp Biol Med 1989;107:543-5.
20. Lucas JH, Emery DG, Wang G, Rosenberg-Schaffer JJ, Jordan RS, Gross GW. In situ investigations of the effects of nonfreezing low temperatures on lesioned and uninjured mammalian spinal neurons. J Neurotrauma 1994;11:35-61.
21. Andzhus R, Hozich N. On the boundaries of reversible clinical death in some hibernating and non-hibernating animals at a body temperature of 0 degrees and on the possibility of artificial prolongation of this state. Bull Exp Biol Med 1965;9:38-42.
22. Niazi SA, Lewis FJ. Profound hypothermia in the monkey with recovery after long periods of cardiac standstill. J Appl Physiol 1957;10:137-8.
23. Klichkhanov NK, Ismailova ZG, Astaeva MD. Intensity of free radical processes in rats’ blood while deep hypothermia and self-warming. Bulletin of Eastern-Siberian scientific center 2016;1:111.
24. Ignatiev DA, Fialkovskaya LA, Perепелкина NI, Markevich LN, Kraev IV, Kolomyiceva IK. Influence of hypothermia on radioresistance of rats. Radiat Biol Radioecol 2016;46:1-7.
25. Nilsson GE, Lutz PL. Role of GABA in hypoxia tolerance, metabolic depression and hibernation—possible links to neurotransmitter evolution. Comp Biochem Physiol C Comp Pharmacol Toxicol 1993;105:329-36.
26. Kozyreva TV, Tkachenko EY, Kozaruk VP, Latsyheva TV, Gilinsky MA. Effects of slow and rapid cooling on catecholamine concentration in arterial plasma and the skin. Am J Physiol 1999;276:1668-72.
27. Kozyreva TV, Tkachenko EY, Kozaruk VP, Latsyheva TV, Gilinsky MN. Features of reaction of sympathoadrenal system of rats at different types of cooling. Ros Physiol J 1999;85:1434-9.
28. Arokina NK Luchakov YI, Zilov VG, Nesmeyanov AA. Influence of the apnea duration under deep hypothermia on restoring of the rats heart. Journal of New Medical Technologies 2019;1:7-11.
29. Ignatiev DA, Suhova GS, Suhov VP. Dependence of heart rate on external environment temperature. J Evol Biochem Physiol 1992;28:582-90.
30. Andrews M, Russeth K, Drewes I, Henry P. Adaptive mechanisms regulate prefered utilization of ketones in the heart and brain of a hibernating mammal during arousal from torpor. Am J Physiol 2009;296:383-93.
31. Carey HV, Andrews MT, Martin SL. Mammalian hibernation: Cellular and molecular responses to depressed metabolism and low temperature. Physiol Rev 2003;83:153-81.
32. Heinis FI, Vermillion KL, Andrews MT, Metzger JM. Myocardial performance and adaptive energy pathways in a torpid mammalian hibernator. Am J Physiol Regul Integr Comp Physiol 2015;309:368-77.
33. Zegunov GF. Electrophysiological parameters of heart functioning of ground squirrels Citellusundulatus in the process of awakening from winter sleeping. Cryobiology 1986;1:31-4.
34. Dumont E, Carrier M, Cartier R, Poirier N, Bouchard D, et al. Repair of aortic false aneurysm using deep hypothermia and circulatory arrest. Ann Thorac Surg 2004;78:117-20.
35. Svensson LG, Crawford ES, Hess KR, Coselli JS, Raskin S, Shenq SA, et al. Deep hypothermia with circulatory arrest: Determinants of stroke and early mortality in 656 patients. J Thorac Cardiovasc Surg 1993;106:19-31.
36. Kochanek PM, Wu X, Fisherman SA, Stezoski SW, Yaffe L. Emergency preservation and resuscitation methods. Patent № US8628512B2 2014.
37. Fisherman SA, Alam HB, Rhee PM, Scalea TM, Drabek T, Forsythe RM, et al. Development of the emergency preservation and resuscitation for cardiac arrest from trauma clinical trial. J Trauma Acute Care Surg 2017;83:803-9.
38. Bernard SA, Buist M. Induced hypothermia in critical care medicine: A review. Crit Care Med 2003;31:2041-51.
39. McCullough JN, Zhang N, Reich DL, Juvonen TS, Klein JJ, Spievolgel D, et al. Cerebral metabolic suppression during hypothermic circulatory arrest in humans. Ann Thorac Surg 1999;67:1895-9.
40. Anufriev AI. Mechanisms of hibernation and cold resistance of hibernating squirrels of Yakutia. Science and education 2015;1:77.