Evaluation of cerebral pathologic changes and long-term behavioral disorder after deep hypothermic circulatory arrest in dogs

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Abstract

The purpose of this study is to evaluate the extent of brain damage following deep hypothermic circulatory arrest (DHCA), using behavior and pathological findings. The dogs underwent 60, 90 or 120 min of DHCA. After 72 h or 6 months, their cerebrum pathological findings were examined. No neurological deficit was found in any of the dogs. After 72 h, hippocampus cells (CA1) were TUNEL positive in 120-min-DHCA dogs. This study demonstrates that 90-min-DHCA-dogs can survive healthily over 6 months, and apoptotic cell death occurs in canine hippocampus following 120 min of DHCA at 15 °C.

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1. Introduction

Deep hypothermic circulatory arrest (DHCA) has proven to be of great benefit in cardiovascular surgery [1]. It is generally used as a cerebroprotective technique in patients with aortic aneurysm [2,3], chronic pulmonary thromboembolism [4,5], or complex congenital anomalies in neonates [6]. However, some questions related to hypothermic circulatory arrest are still unresolved; one such question is that of the safe period for circulatory arrest. It has been thought, on the basis of past empirical findings, that the outer limit for hypothermic circulatory arrest is 45–60 min [7]. The purpose of this study is to evaluate the extent of brain damage following deep hypothermic circulatory arrest (DHCA), using behavioral and cerebrum pathological findings.

2. Materials and methods

2.1. Materials

Fourteen male beagle dogs, 12–24 months of age, weighing 10–15 kg, were each assigned to one of four groups, after cooling to a rectal temperature of 15 °C: one group received 60 min of DHCA (long-term model: n = 3), another received 90 min of DHCA (long-term model: n = 3, 72 h model: n = 4), and a third received 120 min of DHCA (72 h model: n = 4).

2.2. Animal care

All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press (revised 1996). The protocol for these experiments was approved by the Chiba Animal Care Committee.

2.3. Anesthesia

After being sedated with intravenous injections of thiopental sodium (20 mg/kg), the dogs were intubated with a 7-F cuffed endotracheal tube. Each dog was ventilated mechanically with a ventilator rate of 12 breaths/min, a tidal volume of 10 ml/kg, and an inspired oxygen fraction of 1.0 by means of a volume-controlled ventilator. Anesthesia was maintained using 1.0–2.0% halothane with 100% oxygen.
Muscular paralysis was not used. Temperature probes (Nihon-Kohden, Tokyo, Japan) were placed in the esophagus and the rectum to monitor core temperatures. Appropriate catheters were placed in the left external jugular vein and the left femoral artery to monitor central venous pressure, arterial blood pressure, and arterial blood gases. Arterial blood gas analysis (GEM-STAT; Mallinckrodt, MO, USA) was performed using the a-stat method. Oxygen tension was maintained at more than 100 mmHg.

2.4. Cardiopulmonary bypass

The right external jugular vein was exposed surgically in order to place venous cannulas for cardiopulmonary bypass. In the same way, the right femoral vein and artery were exposed surgically for venous and arterial cannulation. After heparinization (300 IU/kg), the femoral artery was cannulated with a 10-F arterial cannula, and the femoral vein with a 12-F venous cannula, while the external jugular vein was cannulated with a 14- or 16-F venous cannula. Non-pulsatile cardiopulmonary bypass (CPB) was initiated at a flow rate of 100 ml/kg per min, and then adjusted to maintain a minimum mean arterial pressure of 50 mmHg.

The CPB circuit used a nonpulsatile roller pump (model 7000; Sarns Inc., MI, USA), a membrane oxygenator (Midiflow D705; Dideco, Mirandola, Italy), a 32-μm arterial filter (CX-AF02; Terumo, Tokyo, Japan). The circuit was primed with 600 ml of a solution including 20% mannitol (50 ml), Ringer’s lactated solution (500 ml), sodium bicarbonate (50 ml), and cefazolin (30 mg/kg). The oxygenator received 1.0% halothane with 100% oxygen at 1 l/min to maintain anesthetic depth during CPB. CPB was continued until a rectum temperature of 15°C was achieved. The heart was arrested using intravenous administration of potassium chloride as necessary at 20°C. The dogs underwent 60, 90, or 120 min of DHCA. When the pump was turned off, venous blood was drained into the reservoir. After DHCA, the animals were rewarmed to 37°C on CPB. The heart was defibrillated as necessary at 30°C. After decannulation and skin closure, anesthesia was discontinued, and protamine (3 mg/kg) and cefazolin sodium (30 mg/kg), were administrated intravenously. All dogs were mechanically ventilated and monitored until extubation. Extubation criteria were determined by confirming spontaneous breathing and reaction to the tube in all dogs.

2.5. Postoperative management

Neurological and behavioral evaluations were performed 24 h after DHCA. These included level of consciousness, breathing pattern, motor and sensory function, ability to walk, and ability to eat.

At the elective sacrifice day, the dogs were anesthetized and a median sternotomy was performed. Heparin (300 IU/kg) was administered intravenously prior to insertion of a 20-F cannula into the ascending aorta, and a 30-F drainage cannula into right atrial appendage. Five liters of 4% buffered paraformaldehyde (for hematoxylin–eosin (H&E), Nissl staining and the TUNEL method) or 1.5% paraformaldehyde with 1.0% glutaraldehyde (for transmission electron microscopy (TEM)) were infused into the aorta to perfuse and fix the brain in situ. The brain was removed immediately, and stored at 4°C in buffered formalin for 24 h.

2.6. Histopathological analysis

All brains were bisected along the sagittal plane, and the left hemisphere was processed for subsequent histological analysis. Coronal samples, 5 mm thick, were sliced from the parietal lobe, olfactory field, hippocampus, and cerebellum. Three sections of 5 μm thick cut from each tissue block were mounted onto slides. One section was stained with H&E; one section was stained with Nissl method; another section was prepared for terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) to detect in situ DNA fragmentation.

2.7. Transmission electron microscopy

The sections were fixed for 1 h in 2.5% glutaraldehyde with 0.1 M phosphate buffer (PB), followed by 1% OsO4 in 0.1 M PB for 1 h at 4°C. Following dehydration, the sections were embedded flatly in Spurr’s resin. Ultrathin sections were stained with uranyl acetate and examined with a JEOL 1200 EX electron microscope.

2.8. Statistical analysis

Data are shown as mean and standard deviation. Differences between groups at baseline were tested by Student’s t-test. Differences were considered significant at P < 0.05.

3. Results

3.1. Long-term model

3.1.1. Surgical result and physiological data

The mean weight of the dogs was 11.0 ± 2.6 kg in the 60-min-DHCA group and 8.0 ± 1.8 kg in the 90-min-DHCA group. The mean CPB cooling time was 52 ± 26 min in the 60-min-DHCA group and 58 ± 16 min in the 90-min-DHCA group. Rewarming times were 61 ± 2 min and 63 ± 6 min, respectively. Temperatures during the DHCA did not differ between the groups. All dogs could be weaned from cardiopulmonary bypass, and could be extubated about 2 h after CPB. Postoperative hematocrit values were 29 ± 5% and 27 ± 4%, respectively.
Fig. 1. Nissl-stained section from dentate gyrus cells (A–C), pyramidal cells of hippocampus CA1 region (D–F), cerebral cortex (G–I), cerebellar cortex (J–L). (A,D,G,J) Normal untreated control dog. (B,E,H,K) 60-min-DHCA dog. (C,F,I,L) 90-min-DHCA dog. There are no remarkable changes in number of cells, or morphology of cells between groups.

Fig. 2. Pyramidal cells of hippocampus CA1 region stained according to the TUNEL method. Hippocampus pyramidal cells (CA1) showed TUNEL positive in 120-min-DHCA dogs (B). There are no apoptotic cells in 90-min-DHCA dogs (A). Apoptotic cells stained dark brown as opposed to normal cells, which stained green.
3.1.2. Behavior

All dogs were stable during the surgical procedures and survived until the day on which they were killed. No neurological deficit was found in any of the dogs. They were able to feed themselves, walk, and play with other dogs. We did not see any abnormalities in the dogs up until sacrifice. There were no significant differences in behavior between the groups at any point in time. The behavioral performance of each dog is shown in Table 1.

3.1.3. Cerebrum pathological findings

None of the dogs showed histopathological evidence of neuronal damage. None of the dogs had areas of necrosis or infarction in the neocortex, olfactory field, hippocampus, or cerebellum (Fig. 1).

3.2. Seventy-two-hour model

3.2.1. Surgical results and physiological data

The mean weight of the dogs was 11.5 \( \pm \) 3.8 kg in the 90-min-DHCA group and 14.0 \( \pm \) 3.0 kg in the 120-min-DHCA group. The mean CPB cooling time was 42 \( \pm \) 9 min in the 90-min-DHCA group and 35 \( \pm \) 7 min in the 120-min-DHCA group. Rewarming times were 55 \( \pm \) 8 min and 44 \( \pm \) 5 min, respectively. Temperatures during DHCA did not differ between the groups. All dogs could be weaned from cardiopulmonary bypass, and could be extubated about 100 min after CPB. Postoperative hematocrit values were 27 \( \pm \) 5% and 27 \( \pm \) 6%, respectively.

3.2.2. Behavior

All dogs were stable during the surgical procedures and survived until the day on which they were killed. No neurological deficit was found in any of the dogs. They were able to feed themselves and to walk. We did not see any abnormalities in the dogs up until sacrifice. There were no significant differences in behavior between the groups at any point in time. The behavioral performance of each dog is shown in Table 1.
3.2.3. Cerebrum pathological findings

After 72 h, hippocampus cells (CA1) showed up as TUNEL-positive in the 120-min-DHCA dogs (Fig. 2B). No morphological changes were observed in CA1 among the 90-min-DHCA dogs (Fig. 2A). Using TEM, pyramidal cells (CA1) showed condensation of the nucleus, expansion of the nuclear body, blebbing of the nuclear envelope, chromatin granules, and phagocytic vacuoles (Fig. 3A–C). There were no changes in dentate gyrus cells. Their ultrastructure was normal among 90-min-DHCA dogs (Fig. 3D).

4. Discussion

This study demonstrates that dogs can survive in good health for over 6 months after 90 min of DHCA, and that apoptotic cell death occurs in canine hippocampus following 120 min of DHCA at 15°C. It suggests that control of apoptosis in acute phase leads to improvement in outcome after DHCA.

To determine the safe period for circulatory arrest, we have developed a canine model of hypothermic circulatory arrest during nonthoracotomic cardiopulmonary bypass. Bleeding was minimized thereby, and we were able to carry out the ECC without blood transfusion. This was important because severe hemodilution during cardiopulmonary bypass may cause inadequate oxygen delivery during early cooling [8].

Ischemic delayed neuronal cell death in the brain of gerbils under normothermic conditions has been studied for a long time [9]. Recent studies have demonstrated that apoptosis participates in the mechanism of this type of delayed neuronal death [10]. Apoptosis involves an intrinsic suicide mechanism. This process depends on ATP for expression of new genes. Apoptosis demonstrates characteristic cell degeneration, in which the cell shows chromatin condensation patterns, and consequently breaks up into vesicles, which are phagocytosed by local macrophage systems. Accordingly, apoptosis is deficient in cases involving inflammation and secondary cell necrosis. Delayed neuronal cell death through apoptotic pathways is of special attention because of the possibility that modification may suppress this process.

Recent studies have demonstrated that apoptosis occurs in the mechanism of the type of neuronal cell death that follows DHCA. Kin et al. [11] demonstrated that neuronal death observed in CA1 pyramidal cells of canine hippocampus at 72 h after 90 min of deep hypothermic circulatory arrest at 15°C, the tympanic temperature, involves apoptosis, and suggests that the maximum allowable time for ischemia in deep hypothermic circulatory arrest at 15°C is <60 min. These results differ from our results, which indicate that apoptotic cell death does not occur in canine hippocampus following 90 min of DHCA at 15°C. This may be chiefly due to differences as to anesthetic drugs and temperature measurement sites during DHCA. The other studies used ketamine hydrochloride and fentanyl citrate, whereas we used thiopental sodium and halothane, the former of which is known as a neuroprotective agent [12].

We adopted rectal temperature as the criterion for DHCA; therefore the brain temperature of the dogs might have been lower than 15°C. In a pilot study, we measured direct brain temperature under the same CPB system in three dogs. These were 12.8–13.4°C when the rectal temperature was 15°C.

There is little literature describing long-term survival after DHCA in experiments with canines or swine. Many investigators [13–15] have surveyed the brain directly after a few days after DHCA. But to apply their findings to clinical circumstances, it is essential to conduct studies on models of long-term survival following DHCA. We established a canine long-term survival model for hypothermic circulatory arrest. With this model, we can estimate the correlation between short-term pathological changes and long-term neurological functions.

5. Conclusion

From these experiments, we revealed that 120 min of DHCA might cause apoptosis (programmed cell death) of brain cells, in the same way as ischemia at normal temperatures, and that without such apoptosis dogs can survive over 6 months after 90 min of DHCA.

References


