Retrograde cerebral perfusion of oxygenated, compacted red blood cells attenuates brain damage after hypothermia circulation arrest of rat

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Background: It was proved that higher haematocrit (Hct) might improve the function of brain after hypothermia circulation arrest (HCA). In the present study we established a new rat HCA model and investigated whether retrograde cerebral perfusion of oxygenated, compacted red blood cells (RBC) could attenuate brain injury after HCA.

Methods: A new rat HCA model was developed and rats were randomly distributed into three groups: HCA group, HCA combined with retrograde cerebral perfusion of oxygenated, compacted red blood cell group (HCArcp group), and sham operation group (sham op. group). Animals both in the HCA group and in the HCArcp group underwent HCA 90 min at 18°C. Brain damage after HCA was evaluated with light microscopy and electron microscopy. Immunohistochemistry and RT-PCR techniques were used to measured the different expressions of the C-Fos, Bcl-2, Bax mRNA and protein among the groups. Additionally we measured the wet/dry ratio of the brain in order to evaluate the oedema degree after HCA.

Results: The new HCA model of rat we developed was comparable to the clinical setting not only in terms of the intubation, anaesthesia method and materials employed but also in terms of the priming volume in relation to body weight. The number of injured neurones in the hippocampus CA1 and parietal cortex, but not in the thalamus of the HCA group, was significantly greater than that of the HCArcp group (P < 0.05). The mean score of mitochondrion of the hippocampus CA1 in the HCA group was significantly higher than in the HCArcp group (P < 0.05). The expression of C-Fos, Bax mRNA and protein in the hippocampus CA1 and/or parietal cortex area was higher in the HCA group than in the HCArcp group (P < 0.05). Expression of the mRNA and protein of Bcl-2 was higher in the HCArcp group than in the HCA group (P < 0.05). The degree of oedema after HCA between the HCA group and HCArcp group had no significant difference (P > 0.05).

Conclusions: We established a new rat model of HCA comparable to the clinical setting. Retrograde cerebral perfusion of oxygenated, compacted RBC is a simple, effective, and safe method to protect the brain during HCA. Adjusting the gene expression in relation to apoptosis might contribute to the neuroprotective effects of a retrograde cerebral infusion of oxygenated, compacted RBC.

Accepted for publication 1 March 2005

Key words: Apoptosis; brain; cardiac arrest; hypothermia; rats retrograde cerebral perfusion.

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neurological injury after HCA. In the present study a new rat HCA model was developed to test the hypothesis.

Methods
The study was approved by Shanghai Second Medical University Animal Care and Use Committee. All procedures herein described met the guidelines of the NIH for animal care (Guide for the Care and Use of Laboratory Animals, Health and Human Services, NIH Publication no. 86–23, revised in 1985).

CPB circuit preparation
The cardiopulmonary bypass (CPB) circuit consisted of a non-pulsatile roller pump (Masterflex®; Cole-Parmer Instrument Co., Vernon Hills, IL) and sterile silicone tubing, with an inner diameter of 1.6 mm (Tygon®; Cole-Parmer Instrument Co.). Cardiopulmonary bypass was established at a flow rate of 50 ml min\(^{-1}\). Venous return was drained by gravity into a 10-ml sterile reservoir (a 10-ml syringe). The membrane oxygenator used was designed deliberately for rats with a surface area of 0.1 m\(^2\). The heat exchanger was fixed to the oxygenator with a surface area of 0.03 m\(^2\) (Fudan Biomaterials Co. Ltd, Shanghai, China), Fig. 1. Overview of the CPB circuit connected to the rat is illustrated in Fig. 2. Total prime volume of the circuit was 20 ml, of which the oxygenator constituted 8 ml. Priming was carried out with 10 ml of Ringer’s lactate solution, 1 ml of 20% mannite, 1 ml of heparin (100 IU) and 8 ml of 6% Hetastarch (200/0.05, HEAS-Steril Fresenius Kabi Co. Ltd., Bad Homburg, Germany).

Anaesthesia and monitoring
Male Sprague-Dawley rats (400–450 g) were used for all experiments. They were anaesthetized with mixed drugs (fentanyl citrate 0.005%, ketamine hydrochloride 5%, droperidol 0.25%, 2.4 ml kg\(^{-1}\)). After endotracheal intubation, the animals were maintained on positive-pressure ventilation with 100% oxygen; anaesthesia was maintained with the same solution (0.8 ml kg\(^{-1}\) h\(^{-1}\)). The right femoral arteries were exposed and cannulated with a 22-gauge i.v. catheter (BD Angiocath™, Franklin Lakes, NJ) to monitor mean arterial blood pressure (MAP) and arterial blood gas analysis (Staprofile M, NOVA Biomedical, Waltham, MA). The rats received 150 IU heparin i.v. after placement of the first intravascular catheter. Through a 0.5-cm horizontal neck incision, the right internal jugular vein was cannulated with the 22-gauge i.v. catheter toward the cranium to monitor oxygen saturation jugular vein (SjvO\(_2\)). Electrocardiography (ECG) was performed with three subcutaneous needle electrodes, and rectal temperature was monitored.

Surgical procedure
The left femoral artery was identified, encircled with suture and cannulated with the 22-gauge i.v. catheter, which served as the arterial outflow for the CPB circuit. Through it the oxygenated blood was pumped into the circulation of the rat. The right common jugular vein was identified and encircled with suture. A 14-G i.v. catheter modified to make a multiorificed was inserted and advanced 3.5 cm until the cannula tip was placed near the junction of the superior vena cava and right atrium, which served as the venal inflow for the CPB circuit. Through it blood was drawn from right atrium to

Fig. 1. Membrane oxygenator deigned deliberately for this study. The oxygenator employs gas exchange technology, which is similar to that employed for clinical devices. The blood enters the device from the entrance (1) and passes through the fibre bundle (3) into the heat exchanger fibre bundle tube (6) and exits at the exit port (8). Gas flows through the entrance (2) and passes over the fibres and exits at the exit port (4). Water flows through the entrance (5) and passes over the fibres and exits at the exit port (7).
the CPB circuit. In the sham op. group the cannula was advanced only half the length in order to avoid an effect on the heart.

Oxygenated, compacted RBC preparing
The donor rats was anaesthetized with the same mixed drugs (2.4 ml kg$^{-1}$) and the left femoral artery was cannulated with the 22-gauge i.v. catheter. After an infusion of heparins (100 IU), blood was drawn. Then the blood was centrifugated in 4°C, 2800 g, for 10 min. The precipitate was the compacted RBC. The Hct of the compacted RBC was about 70–75%. Then the compacted RBC was oxygenated with the said CPB circuit.

Experimental protocol
Rats were randomly assigned into three groups: sham operation group (sham op.); HCA alone group (HCA group) and retrograde cerebral perfusion of oxygenated, compacted RBC group (HCArcp group). Anaesthesia, ventilation, cannulation and heparinization were identical in the three groups throughout all experiments. But the rats in the sham op. group did not undergo CPB.

Animals both in the HCA group and in the HCArcp group were cooled to rectum temperature of 16°C to 18°C over 20 min, and then the CPB was stopped; the temperature was maintained by surface cooling for 90 min. Ventilation was stopped after the establishment of CPB. In the HCArcp group, 5 ml of oxygenated, compacted RBC was infused through the internal jugular vein catheter in 5 min with a infusion pump (Perfusor Compact BRAUN, Melsungen, Germany) just at the beginning of the CPB arrest. The CPB was restarted after 90 min of arrest and the animals were warmed to 37°C within 30 min. Animals were weaned from the CPB and the left femoral arterial cannulae were removed. Ventilation (100% oxygen) was started 10 min before the weaning from the CPB. Dopamine was administered as necessary to maintain haemodynamically stable. After 60 min the rats were sacrificed and their brains were dissected and prepared for further processing.

Histopathologic and immunohistochemistry analysis
After success of HCA (or after the same time in the sham op. group), six rats from each group were transcardially perfused with 4% paraformaldehyde and phosphate-buffered saline solution (at 4°C) for 4–5 min after a brief saline wash. Animals were then decapitated and the brains were dissected and cut into two hemispheres along the sagittal midline. The left hemisphere was immersed in a 2.5% glutaral and phosphate-buffered saline solution (at 4°C) for ≥24 h and then prepared for measurement of electronic microscopy. The right hemisphere was immersed in a 4% formalin and phosphate-buffered saline solution (at 4°C) for ≥24 h until further processing. Later, Coronal brain blocks were processed for paraffin embedding. Coronal sections 5 μm in thickness were cut and stained with haematoxylin and eosin for histopathological analysis.

Coronal sections of another six rats were prepared for immunohistochemistry analysis, which was performed using an avidin-biotin peroxidase method as described previously (10). Briefly, sections were washed in 0.1 mol l$^{-1}$ phosphate-buffered saline (PBS), incubated in 0.1 mol l$^{-1}$ PBS containing 1% bovine serum albumin and 0.2% Triton-X-100 for
30 min, and subsequently incubated overnight with specific first antibody (C-fos, Sigma (St Louis, MO), 1:10,000 dilution; Bcl-2, Sigma, 1:1000 dilution; Bax, Sigma, 1:5000 dilution). On the next day, the sections were incubated with appropriate secondary IgG (1:200 dilution) for 1 h and avidin-biotin peroxidase for 1 h in a humidified chamber. Phosphate-buffered saline (0.1 mol l⁻¹, pH 7.4) containing 0.5% bovine serum albumin was used to wash sections on slides between all steps. The antigen-antibody complexes were visualized by incubation for 5 min in 0.05% 3,3’-diaminobenzidine and 0.003% H₂O₂.

Then the sections were examined using standard light microscopy. Images of them were digitized with a video camera, and the number of injured neurones (stained with haematoxylin and eosin), or the number of C-fos, Bcl-2 and Bax immunoreactive cells in the hippocampus CA1, parietal cortex and thalamus within the fixed 109634 pixels were measured using image-analysis software (DMBL QWin, Leica, Switzerland).

Electron microscopy measurement

Hippocampus CA1 was prepared as an ultrathin section following its dissection from the left brain hemisphere immersed in the glutaral. With the transmission electron microscopy (HITACHI H-500 (Hitachi, Tokyo, Japan)), we classified the mitochondria into five scores (0–4): 0, normal mitochondria, full of granula; 1, normal structure but less granula; 2, swelling and matrix transparence; 3, crista broken and matrix compact or transparent; and 4, crista broken and the inner membrane or outer membrane broken as well. Five pictures of visual fields were randomly chosen under electron microscopy. Ten mitochondria in one picture were scored, and the sum of the score was considered the score of the picture. The average score of five pictures represented the score of a specimen. Then the data were analyzed by SPSS 10.0 (Chicago, IL) with the ANOVA, and P < 0.05 as the indicator of significance.

RT-PCR

After the success of the HCA (or after the same time in the sham op. group), four rats from each group were decapitated. Each brain was dissected and cut into two hemisphere along the sagittal midline. The weight of left hemisphere was recorded and then placed in a thermostat container at 90°C for 48 h. Weight was again recorded and the ratio of wet/dry was calculated and considered as the degree of the brain oedema after HCA. Hippocampus of the right hemisphere was separated and stored in liquid nitrogen for further processing. Total tissue RNA was extracted by the Trizol solution (Invitrogen Co., Tokyo, Japan) and subjected to the subsequent semiquantitative RT-PCR. The reverse transcription was performed in a total volume of 20 μl: total RNA 1 μl, 5×buffer 4 μl, dNTP (10 μM) 2 μl, 50 μM Oligo dT Primer 1 μl, 40 U μl⁻¹ RNase Inhibitor (Promega Co., Madison, WI) 0.5 μl, 200 U μl⁻¹ M-MLV RT enzyme (Promega Co.) 1 μl and RNA-free H₂O₂. Taking 10 μl of the reverse transcription product as a template, the PCR amplification was performed under conditions of 94°C at 180 s, 60°C at 30 s, 72°C at 300 s, 27 cycles in C-fos, 29 cycles in Bcl-2 and 29 cycles in Bax. The quantity of the template and cycle was examined to be within a line figure limit. PCR products were separated on agarose gels and visualized by ethidium bromide staining and ultraviolet (UV) transillumination. Using two types of β-actin as an inner parameter, final numeric values were expressed as a ratio of the gene of interest to the internal gene, β-actin.

The primer pairs for the target genes were as follows: C-fos forward, 5′-CCT CGA GGG GTT CCC GTA GA-3′; C-fos reverse, 5′-ACA GTA CGT GGA TAT AGC GA-3′; Bcl-2 forward, 5′-CTGGTGGACAACATCGCTCTG-3′; Bcl-2 reverse, 5′-GGTCTGCTGACCTACTTGTG-3′; Bax forward, 5′-TCCAGGATCGAGCA-3′; Bax reverse, 5′-AAGTAGAAGGCGAACC-3′; β-actin forward, 5′-TCT CTACAT CTT CGCTTGTG-3′; β-actin reverse, 5′-TGCCGATAGTGTAGTAC CTCTT-3′.

Statistics analysis

All data were presented as the means ± SEM and analyzed using ANOVA or a t-test with SPSS 10.0. P < 0.05 was considered statistically significant.

Results

Oxygenator performance

The oxygenator worked well in all cases and provided sufficient blood gas and temperature exchange during CPB. Data taken from the rat model compared very favourably with the clinical data. In the results from the rat model, the mean of PO₂ was >277.97 mmHg and the mean of SO₂% was >99.07%. The statistical analysis tends to support the oxygenator employed in the rat model as offering at least similar levels as those observed...
with its clinical counterpart. Detailed data are in Table 1.

**Haemodilution**
Maintaining an acceptable and clinically comparable level of haemodilution was an essential component of this model and was recognized as being difficult in the rat without resorting to employing large volumes of donor blood. In the present study, a oxygenator designed deliberately for the rat was used, and because of its small prime volume (8 ml) the level of Hct encountered with this model was similar to that encountered clinically, showing a decrease to about 20%. Detailed data are shown in Table 1.

**Acidosis during HCA**
Significant acidosis could be found when the rewarming started. The value of pH both in the HCA group and in the HCArp group deceased significantly, but the degree in the HCA group was greater than that of the HCArp group ($P < 0.05$). The concentration of blood lactic acid in the HCA group was higher than that in the HCArp group ($P < 0.05$). In another phase of the CPB, the value of pH was not different between the HCA and HCArp groups. Detailed data are shown in Table 1.

**Histopathology and immunohistochemistry analysis results**
With the haematoxylin and eosin stain, many injured neurones could be found in the hippocampus CA1, parietal cortex and thalamus both in the HCA and HCArp groups but not in the sham op. group. However, the numbers of injured neurones in the hippocampus CA1 and parietal cortex but not

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**Table 1**

<table>
<thead>
<tr>
<th>Blood gas analysis</th>
<th>DHCA (n = 6)</th>
<th>DHCArcp (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct preoperation</td>
<td>41.33 ± 2.16</td>
<td>42.17 ± 2.32</td>
</tr>
<tr>
<td>CPB start</td>
<td>19.67 ± 1.75</td>
<td>20.50 ± 1.87</td>
</tr>
<tr>
<td>Rewarming start</td>
<td>20.50 ± 1.87</td>
<td>20.83 ± 1.47</td>
</tr>
<tr>
<td>pH cooling period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>7.40 ± 0.05</td>
<td>7.38 ± 0.06</td>
</tr>
<tr>
<td>20 min</td>
<td>7.39 ± 0.04</td>
<td>7.41 ± 0.05</td>
</tr>
<tr>
<td>Rewarming period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>7.03 ± 0.17</td>
<td>7.25 ± 0.13*</td>
</tr>
<tr>
<td>30 min</td>
<td>7.37 ± 0.04</td>
<td>7.39 ± 0.05</td>
</tr>
<tr>
<td>PO$_2$ (kPa) cooling period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>288.52 ± 18.56</td>
<td>281.87 ± 5.71</td>
</tr>
<tr>
<td>20 min</td>
<td>285.02 ± 12.24</td>
<td>284.05 ± 11.08</td>
</tr>
<tr>
<td>Rewarming period</td>
<td></td>
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<tr>
<td>0 min</td>
<td>288.50 ± 6.36</td>
<td>280.80 ± 8.42</td>
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<tr>
<td>30 min</td>
<td>277.97 ± 1.59</td>
<td>282.70 ± 11.69</td>
</tr>
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<td>SO$_2$ (%) cooling period</td>
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<tr>
<td>0 min</td>
<td>99.07 ± 0.76</td>
<td>99.18 ± 0.43</td>
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<tr>
<td>20 min</td>
<td>99.07 ± 0.52</td>
<td>99.43 ± 0.38</td>
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<tr>
<td>Rewarming period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>99.12 ± 0.86</td>
<td>99.15 ± 0.58</td>
</tr>
<tr>
<td>30 min</td>
<td>99.33 ± 0.54</td>
<td>99.25 ± 0.73</td>
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<td>SjvO$_2$ (%) cooling period</td>
<td></td>
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<tr>
<td>0 min</td>
<td>77.15 ± 0.81</td>
<td>76.07 ± 2.70</td>
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<tr>
<td>20 min</td>
<td>97.20 ± 0.49</td>
<td>96.83 ± 0.64</td>
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<tr>
<td>Rewarming period</td>
<td></td>
<td></td>
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<tr>
<td>0 min</td>
<td>48.07 ± 1.49</td>
<td>46.10 ± 1.41</td>
</tr>
<tr>
<td>30 min</td>
<td>77.05 ± 0.80</td>
<td>77.85 ± 0.49</td>
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<tr>
<td>Lac cooling period (mmol l$^{-1}$)</td>
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<tr>
<td>0 min</td>
<td>4.67 ± 0.31</td>
<td>4.77 ± 0.41</td>
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<tr>
<td>20 min</td>
<td>4.85 ± 0.19</td>
<td>4.90 ± 0.22</td>
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<tr>
<td>Rewarming period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>12.93 ± 1.86</td>
<td>8.58 ± 1.11*</td>
</tr>
<tr>
<td>30 min</td>
<td>4.85 ± 0.24</td>
<td>4.97 ± 0.40</td>
</tr>
</tbody>
</table>

Mean ± SEM.
Hct = haematocrit; PO$_2$ = arterial oxygen pressure; SO$_2$ = arterial oxygen saturation; SjvO$_2$ = oxygen saturation of jugular venous blood; Lac = blood lactic acid.

*T-test: $P < 0.05$. 

1176
in the thalamus of the HCA group were significantly greater than those in the DHCArcp group ($P < 0.05$) (Fig. 3). Detailed data are shown in Table 2.

Figure 4 shows representative microscopic fields of c-Fos, Bcl-2 and Bax immunoreactive brain sections in the hippocampus CA1. The results of image analysis indicated that the number of c-Fos and Bax immunoreactive neurones in the hippocampus CA1 and parietal cortex after HCA in the HCA group was significantly higher than that in the HCArcp group ($P < 0.05$). In contrast, the number of Bcl-2 immunoreactive cells in the hippocampus CA1 and parietal cortex were less in the HCA group than in the HCArcp group. However, in the thalamus area, the numbers of C-fos, Bcl-2 and Bax immunoreactive cells were of no significant difference between the HCA and HCArcp groups. In the sham op. group no immunoreactive cells were detected. Table 2 presents detailed data about the C-fos, Bcl-2 and Bax immunoreactive cells in the three brain areas of the HCA and HCArcp groups.

Ultramicrostructure changes and oedema
The ultramicrostructure changes could have been very clearly found under the transmission electron microscopy. In the sham op. group, the nucleus was nearly round; nuclear membrane and chromatosphere were discriminated clearly; heterochromatin had the characters of neurones; rich cytoplasm, polyribosome and rough endoplasmic reticulum; a little neurofilament could be found in the cytoplasm; and the mitochondrion was normal.

In the HCA group, the nuclear membrane of the neurones in the hippocampus CA1 shrunk; an irregular shape and local notch could be found; mitochondrion was swelled moderately; crista was broken but granules could still be observed on the integrated mitochondrial outer membrane; and its score was 2–3.

In the HCArcp group, despite the shrinking and irregular nuclear membrane of the neurones, the degree of the local notch was lighter; much cytopharyngeal apparatus could be found in the cytoplasm; mitochondrion swelled lightly, part of crista was broken and the granules decreased; and its score was 1–2. The results of the image analysis indicated that the mean score of the mitochondrion in the HCA group was significantly higher than that in the HCArcp group ($P < 0.05$) (Figure 5). Detailed data are presented in Table 3.

In the present study we found that the wet/dry ratio both in the HCA group and in the HCArcp group obviously increased after the HCA compared with the sham op. group ($P < 0.05$). However, no significant difference in the ratio of wet/dry could be found between the HCA group and the HCArcp group ($P > 0.05$) (Table 3).

RT-PCR
As shown in the Fig. 6, HCA could induce C-fos, Bcl-2 and Bax mRNA expression in the hippocampus. The semiquantitative measurement by the Alpha Imager 2000 (Alpha Innotech, San Leandro, CA) is shown in Table 4. The expression of the c-fos, Bcl-2 and Bax mRNAs increased after the HCA in the hippocampus compared with the sham op. group ($P < 0.05$). However, the increased degree was different. The expression level of the C-fos and the Bax mRNA in the HCA group was the highest among the three groups ($P < 0.05$). In contrast, the expression of Bcl-2 mRNA in the HCArcp group was the highest one ($P < 0.05$). A detailed ratio of the gene of interest to the internal gene $\beta$-actin is listed in Table 4.

Discussion
Since the introduction of the first heart–lung bypass machine by Gibbon in 1937 (11), CPB has improved tremendously. However, complications associated with its application persist, even after nearly 70 years of research, development and practice (12). Further improvements depend largely on the
utilization of a proper animal model of bypass. The first rat model of CPB was reported by Subramanian and colleagues (13), with a priming volume of 120 ml. Perfusion was established between the right atrium and the femoral artery. Bypass was performed for 30 min with a bubble oxygenator. However, significant haemolysis was also noted. Since then, numerous rat models have been developed. Improvements include prolonging the CPB time (14), fastening the flow rate (15), or reducing the priming volume (16). But none of the models present an ideal.

The primary aim of any model used to investigate the pathophysiology of CPB must be to maintain a study environment largely similar to that encountered clinically. In our current model, however, a membrane oxygenator was deliberately designed for rat CPB and total priming volume was only 20 ml with no donor blood in it. This miniaturized oxygenator provided excellent oxygenation during the perfusion, as confirmed by the blood-gas analysis. In this model all rats were intubated endotracheally to maximally reduce possible injury. Animals in the study were anaesthetized with an application of fentanyl, ketamine and droperidol, which was comparable to the clinical setting. The model functioned well throughout the experiments. No accidental death was encountered during perfusion. The animals exhibited a haemodilution profile similar to that encountered clinically, and there was only a minimal need for fluid administration during the experiments. Supporting the above bases, this model is comparable to the clinical setting in most important aspects.

<table>
<thead>
<tr>
<th></th>
<th>Hippocampus CA1</th>
<th>Parietal cortex</th>
<th>Thalamus</th>
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<tr>
<td><strong>Histopathology</strong></td>
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<tr>
<td>HCA</td>
<td>8.29±1.31</td>
<td>10.68±1.24</td>
<td>6.26±2.41</td>
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<tr>
<td>HCArcp</td>
<td>4.85±1.00*</td>
<td>7.06±1.81*</td>
<td>5.57±1.72</td>
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<tr>
<td><strong>C-fos</strong></td>
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<tr>
<td>HCA</td>
<td>10.83±1.94</td>
<td>12.50±1.87</td>
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<tr>
<td>HCArcp</td>
<td>5.50±1.05*</td>
<td>8.50±1.05*</td>
<td>8.17±1.17</td>
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<tr>
<td><strong>Bcl-2</strong></td>
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<tr>
<td>HCA</td>
<td>11.00±1.41</td>
<td>11.83±2.04</td>
<td>9.50±1.87</td>
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<td>13.17±1.17*</td>
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<td>8.50±1.05</td>
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<tr>
<td><strong>Bax</strong></td>
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<tr>
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<td>14.67±2.94</td>
<td>14.00±2.83</td>
<td>10.00±2.61</td>
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<tr>
<td>HCArcp</td>
<td>10.50±1.87*</td>
<td>8.83±1.72*</td>
<td>10.83±1.47</td>
</tr>
</tbody>
</table>

Mean ± SEM.
Histopathology: stained with haematoxylin and eosin, and the injury neurones were counted with an image-analysis system (Alpha Innotech, San Leandro, CA).

*P < 0.05 compared with the hypothermia circulation arrest (HCA) group.

Fig. 4. C-fos, Bcl-2 and Bax immunoreactive cells in the hippocampus CA1 of the two experiment groups. (A) C-fos-immunoreactive cells in the hypothermia circulation arrest (HCA) group; (B) C-fos-immunoreactive cells in the HCArcp group; (C) Bcl-2-immunoreactive cells in the HCA group; (D) Bcl-2-immunoreactive cells in the HCArcp group; (E) Bax-immunoreactive cells in the HCA group; and (F) Bax-immunoreactive cells in the HCArcp group.

Fig. 5. Ultrastucture changes of the hippocampus CA1 after hypothermia circulation arrest (HCA). (A) ×20,000, HCA alone group, mitochondrial swelled moderately, and the cristae was broken or disappeared. Granules could still be observed on the integrated mitochondrial outer membrane; and (B) ×8000 in the HCArcp group, mitochondrial only lightly swelled.
Since the first introduction of hypothermic circulatory arrest in the 1960s, its limitations have been recognized, that longer periods of circulatory arrest are sometimes associated with neurological complications such as neurocognitive deficits, delirium, confusion, or generalized seizures (5, 17, 18). Improving brain protection during HCA in order to decrease the risk of adverse neurological outcomes becomes the focus of research.

The end-point pathology and the electron microscopy measurement both demonstrated that retrograde cerebral perfusion of oxygenated, compacted RBC had a neuroprotective effect after HCA. The expression of C-Fos mRNA and protein was a marker of the brain injury (19, 20). In the present study they were higher in the HCA group than in the HCArcp group, suggesting that the brain injury was more severe in HCA group. However, in the area of the thalamus, there was no significant difference between the two groups, which was consistent with the result of the HE stain, but the reason is unknown.

Apoptosis is a well-documented series of events that results in the programmed self-destruction of cells (21), which is an important method of neuronal death. The initiation of an apoptotic process that results in a delayed loss of neurones in specific regions may be a possible explanation for the occurrence of brain injury after HCA (22, 23). There are several plausible reasons why HCA might be expected to induce apoptotic pathways in the brain. Anoxemia during circulation arrest was the main cause. Some genes regulated the pathway of apoptosis, Bax, Bcl-2 and Bad for proapoptotic pathways, whereas others (bcl-2, ced-9) were for antiapoptotic pathways, and the balance of these genes in individual neurones ultimately results in either cell survival or apoptosis (21).

In this study we observed that the apoptosis gene Bcl-2 and Bax both increased after HCA. However, the expression of the antiapoptotic Bcl-2 mRNA and protein in the HCArcp group was higher than that in the HCA group, while the expression of the proapoptotic Bax mRNAs and protein was lower in the HCArcp group than that in the HCA group, which indicated that the neuroprotective effect of the retrograde cerebral perfusion of oxygenated, compacted RBC might be due to its antiapoptotic effect.

Many studies have proved that maintaining a higher Hct has a brain protective effect during

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### Table 3

Mean score of the mitochondrion and wet/dry ratio.

<table>
<thead>
<tr>
<th></th>
<th>Sham operation</th>
<th>HCA</th>
<th>HCArcp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrion score</td>
<td>0</td>
<td>29.3 ± 0.68</td>
<td>19.35 ± 1.24**</td>
</tr>
<tr>
<td>Wet/dry ratio</td>
<td>2.93 ± 0.29</td>
<td>3.91 ± 0.24*</td>
<td>4.09 ± 0.34*</td>
</tr>
</tbody>
</table>

Mean ± SEM.

*P < 0.05, compared with the sham operation group, ANOVA; **P < 0.05, compared with the hypothermia circulation arrest (HCA) group, t-test.

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![Fig. 6. Changes of C-fos, Bcl-2 and Bax mRNA in the hippocampus CA1 after hypothermia circulation arrest (HCA). Lane M: maker; lane 1: sham op. group; lane 2: HCA group; and lane 3: HCArcp group. Both in the HCA group and in the HCArcp group, the expression of the c-fos, Bcl-2 and the Bax mRNAs in the hippocampus increased compared with the sham op. group (P < 0.05). The levels of the C-fos and the Bax mRNA in the HCA group was higher than in the HCArcp group (P < 0.05); however, the expression of Bcl-2 mRNA in the HCArcp group was higher than in the HCA group (P < 0.05).](image-url)
HCA (8–10). However, a higher Hct would increase the blood viscosity and more donor blood must be primed into the circulation bypass. Blood transfusion might transmit many diseases such as hepatitis or AIDS. Retrograde cerebral perfusion of the oxygenated, compacted RBC reduced the quantity of donor blood needed to prime the CPB circulation, and minimized a possible disadvantage caused by higher systemic HCT.

Unlike the continuous retrograde cerebral perfusion that might result in a higher venous pressure leading to cerebral oedema (24), retrograde cerebral perfusion of the oxygenated, compacted RBC was conducted only once at the beginning of the circulation arrest and no more oedema was found in comparison with the HCA group. Therefore it proves to be a simple and safe method to protect the brain during HCA.

### Conclusion

We established a new rat model of HCA comparable to the clinical setting. Retrograde cerebral perfusion of oxygenated, compacted RBC is a simple, effective, and safe method to protect the brain during HCA. However, the present study remains an initial one. Further researches are needed in the future.

### Acknowledgement

These studies were funded by the Health Bureau, Shanghai, China.

### References


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