Reduced Blood-Brain Barrier Permeability After Cardiac Arrest by Conjugated Superoxide Dismutase and Catalase in Piglets

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Background and Purpose Cardiac arrest and resuscitation in immature piglets result in a delayed increase in blood-brain barrier permeability. We tested the hypothesis that pretreatment with oxygen radical scavengers reduces postischemic permeability.

Methods Permeability was assessed by measuring the plasma-to-brain transfer coefficient of the small amino acid, α-aminoisobutyric acid, in 2- to 3-week-old anesthetized piglets. Three groups were studied: (1) a nonischemic time control group (n=5), (2) an ischemia group (n=8) pretreated with 5 mL of polyethylene glycol vehicle, and (3) an ischemia group (n=8) pretreated with polyethylene glycol conjugated to superoxide dismutase (10 000 U/kg) and to catalase (20 000 U/kg). The ischemia protocol consisted of 8 minutes of ventricular fibrillation, 6 minutes of cardiopulmonary resuscitation, defibrillation, and 4 hours of spontaneous circulation.

Conclusions Pretreatment with oxygen radical scavengers reduces postischemic blood-brain barrier permeability by a small amino acid. These data are consistent with oxygen radical-mediated dysfunction of cerebral endothelium in a pediatric model of cardiopulmonary resuscitation.

Key Words • blood-brain barrier • cardiopulmonary resuscitation • free radicals • superoxide dismutase • pigs

Materials and Methods Piglets aged 2 to 3 weeks (4.5 to 5.5 kg) were anesthetized with pentobarbital (35 to 40 mg/kg IP) and ventilated with a volume-cycled ventilator through an endotracheal tube secured by a tracheostomy. Fractional inspired concentration of O2 was 0.3 to 0.45. End-tidal CO2 was monitored to maintain PCO2 at 35 to 40 mm Hg. Additional pentobarbital (13 to 20 mg/kg) was administered intravenously to maintain a depth of anesthesia adequate for surgical preparation. Saline-filled catheters were advanced through a femoral artery and vein into the thoracic aorta and thoracic inferior vena cava, respectively. Catheters were also advanced through the axillary artery and vein into the subclavian artery and vein. At the conclusion of surgery, the animals were paralyzed with pancuronium (0.2 mg/kg), and heparin (1000 U) was administered.
Ventricular fibrillation was induced by passing a 60-Hz current through a 4F pacing wire that was advanced into the right ventricle through a femoral vein. Ventilation was stopped at that time. After 8 minutes of cardiac arrest, CPR was begun with external chest compressions over the lower sternum with a pneumatic chest compressor (Thumper, Michigan Instruments). The chest compressor and ventilator were synchronized by a microprocessor. Chest compressions were performed at a rate of 100 per minute, with a compression duration of 30% of the total cycle time. Compression force was set at 40 to 50 N to produce a cyclic sternal displacement equivalent to approximately 20% of the anteroposterior chest diameter. Ventilation was provided with 100% oxygen after every fifth chest compression at an airway pressure of 30 to 35 cm H2O.

We studied three groups of piglets in which AIB was allowed to circulate for 10 minutes before stopping brain perfusion. In a time control group, group 1 (n=5), measurements were made during spontaneous circulation, and AIB was injected 6 hours after induction of anesthesia. In groups 2 (n=8) and 3 (n=8), ventricular fibrillation was produced 2 hours after induction of anesthesia. Fibrillation, documented by the aortic pressure tracing, lasted 8 minutes before CPR was begun. CPR was performed for 6 minutes, followed by defibrillation within four attempts. Four hours after defibrillation, AIB was injected.

In group 2, PEG (5 mL) alone was given as a bolus injection through the axillary vein catheter. In group 3, PEG-SOD (10,000 U/kg) and PEG-catalase (20,000 U/kg) (Sigma Chemical Co) were given as a bolus injection through the axillary vein catheter. In both groups 2 and 3, the injection occurred at the beginning of the surgical preparation, 2 hours before ventricular fibrillation was induced. Nonischemic piglets in group 1 received neither PEG alone nor PEG-SOD plus PEG-catalase.

Epinephrine was given as an intravenous bolus of 10 µg/kg into the right atrium at the onset of CPR in piglets in groups 2 and 3. An infusion of 4 µg/kg per minute diluted in saline was administered into the subclavian vein at a volumetric rate of 1.9 mL/min for the duration of CPR. This dose and volume maintain cerebral perfusion pressure at levels sufficient for near-normal cerebral blood flow for 20 minutes of CPR in piglets.16 After defibrillation was accomplished, epinephrine infusion rate was decreased in approximately half-logarithmic decrements at 2-minute intervals when the systolic aortic blood pressure was greater than 90 mm Hg. With this criterion, the infusion was stopped at 30 minutes in all piglets. In addition, after defibrillation, volume infusions of lactated Ringer's solution (5 mL/kg) were administered every 30 minutes. We recorded vascular pressures from the thoracic aorta and right atrium throughout the experiment as well as arterial blood gas values and pH, rectal temperature, and glucose concentration.

We assessed permeability of the blood-brain barrier using a low-molecular-weight tracer [14C]AIB (molecular weight, 104) (Dupont-New England Nuclear Products) as described previously.17 A dose of 150 µCi of AIB was injected into the right atrium, and timed arterial samples were obtained over a 10-minute circulating period. To correct for AIB in the plasma space, [3H]inulin was injected 8 minutes after AIB. The piglets were killed 10 minutes after AIB administration by injection of potassium chloride. The brain was immediately dissected, and tissue and plasma samples were prepared for double-label β scintillation counting. The Ki of AIB was calculated as previously described.13,17

Because the distribution of Ki values was skewed, the nonparametric Mann-Whitney rank U test was used to test the hypothesis that Ki in the ischemic group treated with PEG was not different from Ki in the ischemic group treated with PEG-SOD plus PEG-catalase. Repeated measurements of physiological variables were analyzed by split-plot, two-way ANOVA. If there was a significant effect of time, values were compared with the prearrest value by the paired t test with the Bonferroni correction. Values are presented as mean±SEM.

Results

The values of Ki averaged for all cortical regions were 1.54±0.37, 2.04±0.26, and 1.29±0.25 µL/g per minute in the nonischemic time control group (group 1), the postischemic group receiving PEG (group 2), and the postischemic group receiving PEG-SOD and PEG-catalase (group 3), respectively. Values in groups 2 and 3 were significantly different from each other. Individually, Ki was lower in group 3 than in group 2 in cervical spinal cord, cerebellum, medulla, anterior cerebral artery territory, middle cerebral artery territory, and posterior-middle border territory (Figs 1 and 2).
In groups 2 and 3, chest compression CPR was associated with increased central venous pressure and arterial acidemia (Table). Arterial pressure during chest relaxation in between chest compressions (28±3 and 36±4 mm Hg in groups 2 and 3, respectively) was adequate for successful defibrillation. After the return of spontaneous circulation, arterial pressure briefly increased. The maximum systolic pressures attained on return of spontaneous circulation were 205±5 mm Hg in group 2 and 197±11 mm Hg in group 3. Arterial samples were obtained within 10 minutes of resuscitation so that ventilation adjustments to maintain normocapnia could be made. These samples indicated that arterial glucose concentrations transiently increased, but the levels were similar in group 2 (220±18 mg/dL) and group 3 (214±27 mg/dL). Glucose concentration gradually recovered thereafter (Table). Rectal temperature was maintained during early resuscitation and increased slightly 2 hours after resuscitation (Table). However, there were no differences between groups 2 and 3 in vascular pressures, arterial blood values, or temperature. In group 1, there was no significant change in vascular pressures, arterial blood gas and glucose values, or in temperature over time (data not shown), and the values were similar to the prearrest values obtained in groups 2 and 3.

**Discussion**

We have shown previously that 8 minutes of cardiac arrest followed by 6 minutes of CPR and return of spontaneous circulation in 2- to 3-week-old piglets result in an increase in blood-brain barrier permeability to AIB. The increase in permeability was significant at 4 hours of reperfusion but not during the brief hypertensive period immediately after the return of spontaneous circulation. These findings suggest that increased permeability was not attributable to the immediate mechanical effects of increased hydrostatic transmural pressure. We postulated that there is a delayed endothelial injury related to the generation of oxygen radicals. The results of the present study are consistent with this hypothesis in demonstrating that treatment with the oxygen radical scavengers PEG-SOD and PEG-catalase reduces the Ki of AIB 4 hours after resuscitation in the same experimental model as in our previous study. There are several lines of evidence that support a role of oxygen radicals in vascular injury after cerebral ischemia. First, exogenous generation of oxygen radicals causes increased blood-brain barrier permeability, decreased oxygen consumption of cerebral arterioles, abnormal arteriolar reactivity, and altered transport and other functions of endothelium in brain and other tissues. Thus, oxygen radicals are capable of causing...
vascular damage. Second, asphyxia in the piglet and complete cerebral ischemia in the piglet and cat result in the generation of superoxide anion during early reperfusion on the cortical surface as assessed by the nitroblue tetrazolium technique. Cytochemical localization suggests that the primary source of superoxide is cerebral vessels. Thus, oxygen radicals are capable of being generated in the perivascular space during reperfusion. Third, in some cases oxygen radical scavengers improve vascular function. For example, treatment with SOD alone or in combination with other scavengers reduces delayed hyperperfusion after ischemia in dogs and asphyxia in lambs, partially restores hypercapnic responsivity after ischemia in piglets, and improves blood flow during focal ischemia in cats, which may partially account for the observed reductions in infarction size. Protein extravasation associated with severe hypertension is ameliorated by systemic administration of SOD, and local extravasation after cerebral ischemia in the cat is ameliorated by topical application of SOD and catalase. Changes in membrane fluidity of cerebral endothelial cells have been detected after ischemia and reperfusion, and these changes are blunted by treatment with liposomal-entrapped SOD. Therefore, a significant amount of evidence indicates that oxygen radicals contribute to postischemic alterations in cerebrovascular function.

In newborn piglets subjected to 20 minutes of complete cerebral ischemia by elevation of intracranial pressure, Armstead et al observed that increased brain uptake of urea at 2 hours of reperfusion is reduced by systemic administration of PEG-SOD plus PEG-catalase. Our results showing that systemic administration of PEG-SOD plus PEG-catalase reduces permeability of AIB are consistent with those of Armstead and colleagues. Moreover, our findings are extended by results demonstrating oxygen radical involvement (1) after a shorter duration of complete ischemia (8 versus 20 minutes), (2) after whole-body ischemia associated with cardiac arrest and CPR, and (3) in somewhat older piglets (2 to 3 weeks versus 1 to 5 days of age). In addition, we used AIB as a tracer because it is avidly taken up by the A1 amino acid transporter and is presumably subject to less back-diffusion than urea. Nevertheless, despite these differences in methodology and experimental procedures for producing ischemia, both of these studies support involvement of oxygen radicals in postischemic blood-brain barrier dysfunction in immature pigs.

PEG-conjugated SOD and catalase were used in this study to lengthen the naturally short plasma half-life of the unconjugated forms of these two drugs. No increase in the brain level of SOD has been seen after the administration of PEG-SOD in normotensive rats or in immature pigs under normal conditions and after ischemia. However, PEG-SOD and PEG-catalase are taken up by cultured endothelial cells over a period of several hours. Because superoxide appears in the extracellular space in close proximity to cerebral vessels and its appearance is inhibited by anion channel blockers, superoxide may be derived from within endothelial cells. In the present study, we administered PEG-SOD and PEG-catalase 2 hours before cardiac arrest to enhance the likelihood of endothelial uptake. Another possibility not tested in the present study is that superoxide is derived from leukocytes in the vascular space, in which case delayed treatment after resuscitation would be expected to be effective.

Other physiological parameters may play a role in blood-brain barrier dysfunction. There were no differences in vascular pressures, arterial blood gas and glucose levels, or rectal temperatures between groups that could account for the lower AIB Kᵢ with scavenger treatment. However, resuscitation with epinephrine was associated with a brief period of arterial hypertension. We previously found that permeability to AIB was not increased during the first 10 minutes of spontaneous circulation when the brief period of hypertension occurred. These data suggest that there was not a gross, mechanically induced opening of the blood-brain barrier. However, severe arterial hypertension can generate superoxide production and lead to permeability changes that are also reduced by SOD treatment. Therefore, we cannot exclude the possibility that the increase in permeability 4 hours after resuscitation was due to superoxide generation at the start of recirculation and that early transient hypertension may have amplified oxygen radical generation. However, we believe that the experimental findings remain relevant to the clinical setting of CPR where epinephrine is commonly used and brief hypertension after recirculation may occur.

In our previous study, Ki was significantly elevated in most brain regions 4 hours after resuscitation. In the present study, Ki in the ischemic group treated with PEG was significantly higher than that in the nonischemic time control group in caudate nucleus only. However, the fact that Ki was numerically higher in all 14 regions suggests that the effect of ischemia on Ki is real and that the lack of attaining a probability value of .05 is due to less statistical power in this study than in the previous study. In addition, differences between the ischemia groups treated with PEG versus PEG-SOD plus PEG-catalase did not attain statistical significance in every brain region. Perhaps quantitative autoradiography would have revealed more specific sites of increased permeability. It is possible that with a more prolonged period of ischemia, as used by Armstead et al, there would have been more widespread permeability changes. Furthermore, we did not attempt to determine at what time point during reperfusion maximal dysfunction occurred in this experimental protocol. It is possible that more consistent changes in permeability occur at other time points.

In conclusion, the use of PEG-SOD and PEG-catalase reduced blood-brain barrier permeability to AIB in cerebrum, cerebellum, and selected brain stem regions after 8 minutes of cardiac arrest and 4 hours of reperfusion in immature piglets. Thus, oxygen radicals appear to play a role in blood-brain barrier dysfunction after cardiac arrest in an experimental model of pediatric CPR.

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Second, the radical-mediated damage described in the radical-mediated processes may evolve after insult to catalase. This straightforward communication is of interest from several perspectives. First, it suggests that an amino acid is blunted through pretreatment with the amino acid while demonstrating that the passage of this citation in immature piglets result in a delayed increase in blood-brain barrier permeability to a small neutral amino acid. The present communication appears to involve rather subtle cellular damage reflected in the altered blood-to-brain transport of a small neutral amino acid. This is in contrast to previous observations that have linked oxygen radical-mediated events to more overt endothelial changes such as increased permeability of the brain capillary endothelium in vivo. Am J Physiol. 1992;263:H1234-H1242.


Editorial Comment

In this article, Dr Schleien and colleagues confirm their previous observation that cardiac arrest and resuscitation in immature piglets result in a delayed increase in blood-brain barrier permeability to a small neutral amino acid while demonstrating that the passage of this amino acid is blunted through pretreatment with the oxygen radical scavengers, superoxide dismutase and catalase. This straightforward communication is of interest from several perspectives. First, it suggests that radical-mediated processes may evolve after insult to exert their maximal effects several hours after injury. Second, the radical-mediated damage described in the present communication appears to involve rather subtle cellular damage reflected in the altered blood-to-brain transfer of a small neutral amino acid. This is in contrast to previous observations that have linked oxygen radical-mediated events to more overt endothelial changes such as altered permeability to macromolecules or destructive lesions of the luminal endothelial membrane. While these observations are important, they become even more significant when their overall therapeutic implications are considered. The fact that this paper implies a delayed radical-mediated increase in blood-brain barrier permeability suggests that a therapeutic window of
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