# Effect of deferoxamine on Na$^+$K$^+$ATPase activity after cerebral ischemia in rabbits

*Mehmet Gürbilek,* PhD, *Cemile Topcu,* PhD, *Mehmet Aköz,* MD, *Hülagü Bariskaner,* MD, PhD, *Mehmet E. Ustün,* MD, *Öznur Köylü,* MD.

## ABSTRACT

**Objective:** To study the effects of deferoxamine on tissue sodium-potassium adenosine triphosphatase (Na$^+$-K$^+$ ATPase) activity on cerebral ischemia in rabbits.

**Methods:** We cared for the animals in the Pharmacology Department of the Medical School of Selcuk University in 2004. We used 30, New Zealand, 7-day-old male rabbits in the experiment. We anesthetized all the animals with xylazine hydrochloric acid and ketamine. We divided the rabbits equally into 3 groups. In group 1 (n=10) (sham group), we observed baseline levels, and did not apply ischemia. In group 2 (n=10) (untreated group) we produced cerebral ischemia by clamping the bilateral common carotid arteries for 60 minutes, and in group 3 (n=10), we administered deferoxamine (DFO) 50 mg/kg intravenously immediately after opening the clamps.

**Results:** The Na$^+$-K$^+$ATPase activity increased after DFO treatment ($p$=0.045).

**Conclusion:** We conclude that Na$^+$-K$^+$ATPase activity in cortical brain tissue was higher in DFO-treated rabbits compared with untreated animals after ischemia.

*Neurosciences 2006; Vol. 11 (2): 88-92*

Ischemia is usually considered as a phenomenon resulting from a reduction in blood flow. Hypoxia refers to a condition in which oxygen supplementation for tissue is insufficient. In ischemic hypoxia, metabolites are retained and blood flow decreases, which leads to tissue-oxygen deficiency. Adenosine triphosphate (ATP) consumption increases while blood flow decreases in ischemia. Ischemia results in 2 ultimate events; production of lactic acid caused by anaerobic metabolism stimulation and a decrease in venous oxygen saturation.[1] The production of reactive oxygen radicals in the early reperfusion phase plays a substantial role in this type of brain cell damage. Reactive oxygen radicals such as superoxide and hydrogen peroxide (H$_2$O$_2$) can be converted into the highly reactive hydroxyl radical by transition metals in particular free iron, ultimately leading to lipid peroxidation of the brain cell membrane and cellular damage.[2] Lipid oxidation is one of the more important biological radical processes. Being a chain reaction, only low concentrations of initiator may be a Fenton type reaction. The main problem with this concept is one of solubility. The site of attack is the double-bonded part of the lipids, embedded in the centre of the membrane. Not only are Fe (II) complexes unlikely to be present, but also H$_2$O$_2$ is unlikely to be present in the inner region of the phospholipid double layer. Thus, the probability of there being sufficient of each to initiate autoxidation seems to us to be very low indeed. Nevertheless, catalysis by metal ions does occur. Possibly, membrane proteins are involved in some way.[3] The transmembrane enzyme sodium-potassium adenosine triphosphatase (Na$^+$-K$^+$ATPase) is very susceptible to free radical related

**88**

lipid peroxidation. It has been demonstrated that the activity of $Na^+$-$K^+$ATPase is a useful parameter for evaluating cellular disturbance by ischemia, and can be used to determine the possible therapeutic effect of any agent.[4] Present experimental studies show that chelation of free iron prevented hypoxia-ischemic hypoperfusion and metabolic dearrangement of the brain.[2] In the present study, we investigated whether deferoxamine (DFO) prevented free radical-induced alterations of the brain cell membrane by measuring $Na^+$-$K^+$ATPase activity in the ischemia-reperfusion injury in rabbits.

**Methods.** Animals were cared for in the Pharmacology and Biochemistry Department of the Medical School of Selcuk University in accordance with the 'Guide for the care and use of laboratory animals.' These protocols were approved by the Animal Use Committee of Selcuk University in 2004. Thirty, New Zealand, 7-day-old male rabbits were used in the experiment. All animals were anesthetized with xylazine hydrochloric acid (HCl) 15 mg/kg and ketamine 30 mg/kg intramuscularly. Animals were paralyzed with pancuronium bromide 0.2 mg/kg and mechanically ventilated with oxygen and air (Harvard Apparatus, South Natick, MA). The body temperature was maintained at 37±0.5°C. The trachea was then intubated. For recording systemic arterial blood pressure, the left femoral artery was cannulated and for intravenous injections, the right jugular vein was cannulated with polyethylene catheters. A pressure transducer (Grass PT300) was used for blood pressure, arterial blood gas values were evaluated with a GEM, Premier Plush. A polygraph (Grass79H) was used. Anesthetized rabbits were fixed in the supine position and with a cervical incision, the bilateral common

carotid arteries were exposed. The rabbits were randomly divided into 3 groups. In group 1 (n=10) (sham group), only baseline levels were observed and ischemia was not applied. In groups 2 (untreated group) (n=10) and 3 (DFO-treated group) (n=10), the bilateral common carotid arteries were clamped for 60 minutes. Group 2 received saline, while in group 3, within 5 minutes after the clamps were opened, DFO was administered 50 mg/kg via the jugular vein as a bolus injection. At the end of reperfusion for 60 minutes, approximately 0.25 g of brain sample was resected from both parietal regions (a total of 0.5 g) in all groups. The rabbits were sacrificed after the resections. The samples were stored below -70°C until the homogenization procedure. Frozen cerebral cortices were homogenized in 10 mM Tris-HCl buffer containing 0.32 M sucrose and 0.5 mM EDTA (pH 7.4).[5] After 10 minutes of centrifugation at 3,000 g, +4°C, the supernatant was centrifuged for 90 minutes at 11,000 g and the pellet was resuspended in the 1ml Tris-HCL buffer. The total Na-K ATPase activity was assayed at 37°C in a medium containing 5 mM $MgCl_2$, 140 mM sodium chloride (NaCl), and 14 mM potassium chloride (KCl) in 40 mM Tris-HCL, pH 7.4. The reaction was started by the addition of 3 mM $Na_2ATP$. Inorganic phosphate (Pi), hydrolyzed from the reaction, was measured according to Fiske and Subbarow.[6] The results were expressed as mmol Pi.per mg protein per minute. We used the Statistical Package for Social Sciences Version 11 for data analysis. Results were expressed as the mean ± SD. Two-way ANOVA for repeated measures, and unpaired t-test were used for evaluating arterial blood gas between the groups. One-way analysis of variance and Tuckey-Honestly Significant Difference Test were used for the evaluation of the $Na^+$-$K^+$ATPase results. A value of $p<0.05$ was considered as significant.

**Table 1 -** Physiologic parameters; pH, partial oxygen pressure (pO₂), partial carbon dioxide (pCO₂), temperature, and heart rate in rabbits studied.

| Groups | Mean arterial pressure | pH | pO₂ | pCO₂ | Temperature | Heart rate |
|---|---|---|---|---|---|---|
| *Group 1 (n=10)* | | | | | | |
| Before ischemia | 76 ± 2.8 | 7.47 ± 0.01 | 97.39 ± 2.5 | 27.50 ± 1.1 | 37.80 ± 0.20 | 245 ± 6.7 |
| After 60 min ischemia | 75 ± 2.1 | 7.46 ± 0.02 | 98.30 ± 1.1 | 26.70 ± 1.9 | 37.70 ± 0.30 | 242 ± 6.9 |
| After 120 min ischemia | 79 ± 3.1 | 7.46 ± 0.01 | 97.70 ± 1.0 | 27.40 ± 1.2 | 37.50 ± 0.40 | 247 ± 5.7 |
| | | | | | | |
| *Group 2 (n=10)* | | | | | | |
| Before ischemia | 74 ± 3.1 | 7.45 ± 0.03 | 98.30 ± 2.2 | 25.40 ± 1.8 | 37.70 ± 0.20 | 244 ± 9.8 |
| After 60 min ischemia | 79 ±1.2 | 7.43 ± 0.01 | 96.20 ± 1.4 | 26.36 ± 1.2 | 37.50 ± 0.20 | 268 ± 8.0* |
| After 120 min ischemia | 76 ± 2.6 | 7.43 ± 0.02 | 96.45 ± 1.1 | 26.32 ± 1.3 | 37.30 ± 0.30 | 264 ± 8.8* |
| | | | | | | |
| *Group 3 (n=10)* | | | | | | |
| Before ischemia | 74 ± 1.8 | 7.46 ± 0.04 | 96.35 ± 1.7 | 28.25 ± 2.0 | 37.10 ± 0.30 | 243 ± 6.0 |
| After 60 min ischemia | 78 ± 1.9 | 7.43 ± 0.02 | 95.62 ± 1.8 | 27.50 ± 2.8 | 37.30 ± 0.60 | 265 ± 9.1* |
| After 120 min ischemia | 75 ± 2.3 | 7.44 ± 0.01 | 95.47 ± 2.0 | 28.10 ± 1.0 | 37.50 ± 0.20 | 246 ± 8.9 |

* Compared to Group 1, $p=0.045$, values are expressed as mean ± SD, min - minutes
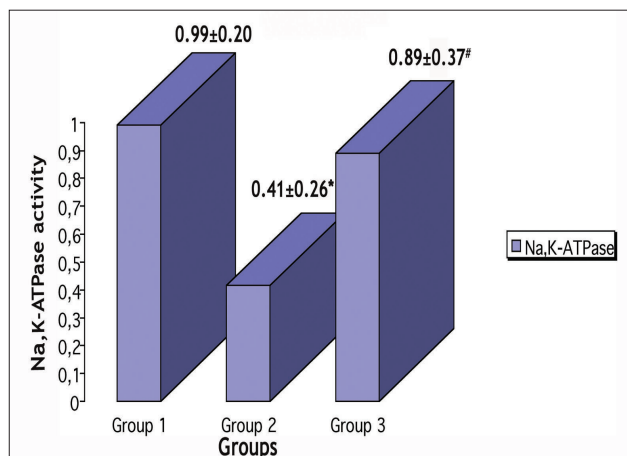
**Figure 1 -** Values of $Na^+K^+ATPase$ activity of cortical brain tissue one hour after hypoxia-ischemia in group 1 (sham group), group 2 (untreated group), and group 3 (DFO-treated group). *$p$=0.045, compared to group one, #$p$=0.045 compared to group 2.

**Results.** **Table 1** shows mean values of aortic blood pressure, pH, and blood gases before, and 60 minutes after ischemia. The mean arterial pressure, pH, blood gases, body temperature, and heart rate were similar in all groups before ischemia. A significant increase in heart rate was found in group 2 in comparison with group 1 only after 120 minutes of ischemia ($p$=0.045). **Figure 1** shows the $Na^+$-$K^+ATPase$ activity values in the cortical brain tissue at 60 minutes after ischemia in both study groups, and in the sham animals. The $Na^+$-$K^+ATPase$ activity was significantly higher in the sham group (group 1) compared with untreated rabbits (group 2) (mean ± SD: 0.99±0.20 versus 0.41±0.26 mmol Pi.mgprt⁻¹10min⁻¹; $p$=0.045). The $Na^+$-$K^+ATPase$ activity was significantly higher in the DFO-treated rabbits compared with the untreated rabbits (mean ± SD: 0.89±0.37 versus 0.41±0.26 mmol Pi.mgprt⁻¹min⁻¹; $p$=0.045).

**Discussion.** In recent years, the idea that tissue damage occurs more during reperfusion rather than the ischemic period has been accepted more because the main cause of damage is reoxygenation.[7] Free oxygen radicals, which have a role in reperfusion damage can be formed by the xanthine-oxidase system,[8] activated neutrophils,[9] electron transport cascades, and the arachidonic acid pathway in mitochondria,[10] oxidation of catecholamines and other compounds.[1] Jennings and Ganote[11] reported that reperfusion causes over accumulation of calcium in mitochondria by means of excess calcium uptake to the cytosol and destroys the mitochondria's ATP formation ability. Rapid decreasing of energy sources brings out a

loss in activity in $Ca^{++}$ and $Na^+$-$K^+$ special ATPase and then an insufficiency of the $Na^+$-$Ca^{++}$ exchange system occurs.[12] Lipid peroxidation is responsible for the changes in $Na^+$-$K^+ATPase$ activity observed after hypoxia-ischemia and reperfusion. In addition to lipid peroxidation, hypoxia-induced activation of protein kinase C may reduce $Na^+$-$K^+ATPase$ activity through phosphorylation of the catalytic subunit.[2]

The brain, with its high lipid content, is most susceptible to peroxidative damage, and the degree of peroxidation correlates directly with regional iron concentration.[13] Deferoxamine is an iron chelator in clinical use that has a very high affinity for ferric iron. Gutteridge et al[14] have shown that DFO binds ferric iron tightly and inhibits hydroxyl radical formation. The DFO can prevent the inhibition of $Na^+$-$K^+ATPase$ caused by intracerebral injection of iron-rich hemoglobin. The DFO is isolated as the iron chelate from *Streptomyces pilosus* and is treated chemically to obtain the metal-free ligand. The DFO has the desirable properties of a remarkably high affinity for ferric iron ($K_a$=$10^{31}$) coupled with a very low affinity for calcium ($K_a$=$10^2$). Studies in vitro have shown that it removes iron from hemosiderin and ferritin and, to a lesser extent, from transferrin. Iron in hemoglobin or cytochromes is not removed by DFO.[15] In the present study, we investigated whether DFO prevented free radical-induced alterations in the brain cell membrane after global hypoxia and ischemia by measuring brain cell membrane $Na^+$-$K^+ATPase$ activity in the rabbit. We found significantly higher $Na^+$-$K^+ATPase$ activity in the brain tissue in the iron chelator DFO-treated group than in the control (untreated) group after a severe ischemia/reperfusion injury. This result suggests that free iron chelation has a beneficial effect on the ATP-dependent $Na^+$/$K^+$ brain cell membrane in the ischemia-reperfusion period.

Investigators have shown that DFO treatment reduced the malondialdehyde content and induced the recovery of $Na^+$-$K^+ATPase$ activity, exerting a brain protective role against the detrimental effect of the hemorrhage.[16] Deferoxamine has been used in animal models of vasospasm, brain edema, cerebral ischemia, and head injury. It has a low molecular weight and passes freely through the blood-brain barrier and cell membrane. Keberle[17] showed that the brain has the highest glutathione reductase levels that were decreased due to injury and lowered the increased malondialdehyde levels. This results from the effect of DFO that has a remarkably high affinity for ferric ion and prevents the initiation and propagation of lipid peroxidation. The effects of DFO are related to iron chelation. Iron may potentiate reperfusion injury by catalyzing the generation of more damaging free radicals from superoxide and nitric oxide.[18]

Both iron(II) and iron(III) are effective catalysts for hydroperoxide degradation, but the former is more so. These include complexes of iron salts with low molecular weight chelates, non-hem iron proteins, free hem, hemoglobin, and myoglobin. Reduced metal complexes react with lipid hydroperoxides to give alkoxyl radicals. Oxidized iron complexes react more slowly to produce alkoxyl and peroxyl radicals and, under certain conditions, ferryl complexes. Alkoxyl and peroxyl radicals can initiate new rounds of lipid peroxidation and propagate further radical chain reactions thus amplifying the initial damage.[3] Deferoxamine binds ferric iron tightly and inhibits hydroxyl radical formation. It can prevent the lipid peroxidation in the brain cell membrane and inhibit Na$^+$-K$^+$ATPase activity. In this study, lipid peroxidation was not measured, as there is sample evidence that Na$^+$-K$^+$ATPase activity is affected by ischemia/reperfusion-induced lipid peroxidation of the brain. Alternately, Na$^+$-K$^+$ATPase is an important enzyme in membrane. This enzyme is very susceptible to free radical-related lipid peroxidation. An assessment of ischemia-reperfusion periods concludes that the intracellular enzyme brings in escape from many tissues. Calcium influx and cellular membrane damage occur in tissues. Excess calcium uptake to the cytosol destroys the mitochondria's ATP formation ability. Rapid decreasing of the energy sources brings out a loss in activity in Ca$^{++}$ and Na$^+$-K$^+$ATPase. In addition to lipid peroxidation, activation of protein kinase C may reduce Na$^+$-K$^+$ATPase activity through phosphorylation of the catalytic subunit.[2]

A study in newborn piglets by DiGiacomo et al[19] using $^{31}$P-magnetic resonance spectroscopy, revealed that the severity of decreases in Na$^+$-K$^+$ATPase activity are dependent on decreases in phosphocreatine. Also, they found a strong correlation between Na$^+$-K$^+$ATPase activity and electrocortical brain activity and stated that the electrical stability of the cortical brain cell membrane is directly related to electrical brain activity. Endogenous ET-1 (endothelin-1), released during a spreading neuronal/astroglial depolarization wave, seemed to play minor role in the vasoconstrictor process.[20] This may suggest that the mechanism itself that triggers spreading neuronal/astroglial depolarization waves is important in the pathogenesis of cortical spreading ischemia. This mechanism may be related to a decrease in Na$^+$-K$^+$ATPase activity, an enzyme that accounts for approximately half of the cerebral energy consumption.[21] Consistently, Petzold et al,[20] showed that ET-1 reduced Na$^+$-K$^+$ATPase activity, particularly the activity of the astroglial and neuronal enzyme subunits. Both ET-1-mediated inhibition and stimulation of the Na$^+$-K$^+$ATPase have been described, depending on the predominant ET receptor subtype in the examined tissue.[22,23] The ET$_B$ receptor mediated inhibition in cultured human epithelium, whereas ET$_A$ receptor activation stimulated Na$^+$-K$^+$ATPase in cultured rat brain capillary endothelium. The predominant subtype in the neuronal/astroglial network is the ET$_B$ receptor, thereby leading to ATPase inhibition.[24] The ET-1 has been reported to inhibit Na$^+$-K$^+$ATPase from cerebral cortex preparations in vitro. Furthermore, despite ET$_A$ receptor-mediated Na$^+$-K$^+$ATPase activation, the action of ET-1 on vascular ET$_A$ receptors in vivo may still indirectly lead to a decreased ATPase activity in the cerebral tissue, as it has also been shown that hemoglobin decreased Na$^+$-K$^+$ATPase activity.[25] It is not entirely clear how down-regulation of Na$^+$-K$^+$ATPase activity contributes to the mechanism underlying cortical ischemia. As suggested by Petzold et al,[20] possibly, when the repolarization process becomes prolonged by a decrease in Na$^+$-K$^+$ATPase activity, the increase in [K$^+$] and thus the [K$^+$] induced vasoconstriction are also prolonged. The longer the vasoconstriction lasts, the more severe the energy compromise becomes. Energy compromise by itself inhibits neuronal repolarization, so that a vicious circle of K$^+$ induced vasoconstriction and vasoconstriction inhibiting K$^+$ reuptake is initiated. The Na$^+$-K$^+$ATPase activity down-regulation is important for the mechanism of cortical spreading ischemia.

In this study, there was significantly higher Na$^+$-K$^+$ATPase activity in the DFO-treated rabbit. It suggests that free iron chelation prevents failure of the ATP-dependent Na$^+$/K$^+$-pump of the brain cell membrane. Britton et al[26] reported that in patients with beta thalassemia who undergo regular transfusions, DFO treatment has been shown to be effective in preventing iron-induced tissue injury and in prolonging life expectancy. Recently, it has been shown that erythropoietin (EPO) is also expressed in the central nervous system and that it exerts a potent neuroprotective effect. Although, this may be explained by insufficient binding of EPO by the scavenger, it appears more likely that there are also other protective signaling cascades involved in hypoxia-induced tolerance in the brain that are independent of EPO.[27] Also, Prass et al[28] reported that hypoxia-induced stroke tolerance in the mouse is mediated by EPO. This is strong experimental evidence for an essential functional role of endogenous EPO in ischemic preconditioning in vivo.

We conclude that Na$^+$-K$^+$ATPase activity in cortical brain tissue was higher in DFO treated rabbits compared with untreated animals after severe ischemia-reperfusion.

## References

1. Gürbilek M, Dilsiz A, Kaymakçı A, Gündogan AH, Çeri A. Biochemical changes due to liver injury caused by ischemia-reperfusion in rats. *Turkish Journal of Veterinary & Animal Sciences* 1995; 19: 93-96.

2. Groenendeaal F, Shadid M, McGowan JE, Mishra OP, Van Bel F. Effects of deferoxamine a chelator of free iron, on Na⁺,K⁺-ATPase activity of cortical brain cell membrane during early reperfusion after hypoxia-ischemia in newborn lambs. *Pediatr Res* 2000; 48: 560-564.

3. Evans CA, Diplock AT, Symons MCR. Techniques in free radical research. In: Burdon RH, Van Knippenberg PH editors. Laboratory techniques in biochemistry and molecular biology. New York (NY): Elsevier; 1991. p. 40-42.

4. Baykal S, Ceylan S, Aktürk F, Usul H, Efe H, Aliyazıcıoglu Y, et al. Effects of a Calcium channel-blocking agent, Nimodipine, on injured-spinal cord Na⁺,K⁺-ATPase activity. *Turkish Neurosurgery* 1995; 5: 9-11.

5. Harik SI, Douli GH, Dick APK. Specific oubain binding to brain microvessels and choroid plexus. *J Cereb Blood Flow Metab* 1985; 5: 156-160.

6. Fiske C, Subbarow Y. The colorimetric determination of phosphorus. *J Biol Chem* 1925; 66: 375-400.

7. Gurbilek M, Vatansev H, Gultekin F, Dilsiz A. Vatansev C, Akoz M. Prevention of testicular damage by free radical scavangers after acute experimental torsion. *Biomed Res* 2000; 11: 315-319.

8. Ward PA. Mechanism of endothelial cell injury. *J Lab Clin Med* 1991; 118: 421-426.

9. Kloner RA, Giacomelli F, Alker KJ. Influx of neutrophils into the walls of large epicardial coronary artery in response to ischemia/reperfusion resolve and unresolved issues. *Circulation* 1989; 80: 1115-1127.

10. Halliwel B, Gutteridge JMC. Free Radicals in biology and medicine. Oxford: Clarendon Press; 1989.

11. Jennings RB, Ganote C. Mitochondrial structure and function in acute myocardial ischemic injury. *Circ Res* 1976; 38 (Suppl 1): 80-191.

12. Iles RA, Poole-Wilson PA. Ischemia, hypoxia and reperfusion. In: Cohen RD, editor. New York (NY): Bailliere Tindall; 1990. p. 332-337.

13. Palmer C, Roberts RL, Bero C. Deferoxamine posttreatment reduces ischemic brain injury in neonatal rats. *Stroke* 1994; 21: 1039-1045.

14. Gutteridge JMC, Richmond R, Halliwel B. Inhibition of iron catalysed formation of hydroxyl radicals from superoxide and of lipid peroxidation by desferrioxamine. *Biochem J* 1979; 184: 469-472.

15. Hardman JG, Limbird LE. Goodman & Gilman's the Pharmacological basis of therapeutics. 10th ed. In: Klaassen CD, editor. Heavy Metals and Heavy Metals Antagonists. New York (NY): McGraw Hill; p. 1871-1872.

16. Bilgihan A, Turkozkan N, Aricioglu A, Aykol S, Cevic C, Göksel M. The effect of deferoxamine on brain lipid peroxide levels and Na⁺,K⁺-ATPase activity following experimental subarachnoid hemorrhage. *Gen Pharmacol* 1994; 25: 495-497.

17. Keberle H. The biochemistry of desferrioxamine and its relation to iron metabolism. *Ann N Y Acad Sci* 1964; 119: 758-768.

18. Suzer T, Coskun E, Demir S, Tahta K. Lipid peroxidation and glutathione levels after cortical injection of ferric chloride in rats: Effect of trimetazidine and deferoxamine. *Res Exp Med* 2000; 199: 223-229.

19. DiGiacomo JE, Pane CR, Gwiazdowski S, Mıshra OP, Delivoria-Papadopoulos M. Effect of graded hypoxia on brain cell membrane injury in newborn piglets. *Biol Neonate* 1992; 61: 25-32.

20. Petzold GC, Einhäupl KM, Dirnagl U, Dreier JP. Ischemia triggered by spreading neuronal activation is induced by endothelin-1 and hemoglobin in the subarachnoid space. *Ann Neurol* 2003; 54: 591-598.

21. Dreier JP, Petzold G, Tille K, Lindauer U, Amold G, Heinemann U et al. Ischemia triggered by spreading neuronal activation is inhibited by vasodilators in rats. *J Physiol* 2001; 531: 515-526.

22. Prasanna G, Dibas A, Hulet C, Yorio T. Inhibition of Na⁺,K⁺-ATPase by endothelin-1 human nonpigmented ciliary epithelial cells. *J Pharmacol Exp Ther* 2001; 296: 966-971.

23. Kawai N, Yamamoto T, Yamamoto H, McCarron RM, Spatz M. Endothelin-1 stimulates Na⁺K⁺-ATPase and Na⁺K⁺-Cl- cotransport through ETA receptors and protein kinase C-dependent pathway in cerebral capillary endothelium. *J Neurochem* 1995; 65: 1588-1596.

24. Chow M, Dumont AS, Kassel NF. Endothelin receptor antagonists and cerebral vasospasm: an update. *Neurosurgery* 2002; 51: 1331-1341.

25. Sadrzadeh SM, Anderson DK, Panter SS, Hallaway PE, Eaton SW. Hemoglobin potentiates central nervous system damage. *J Clin Invest* 1987; 79: 662-664.

26. Britton RS, Leicester KL, Bacon BR. Iron toxicity and chelation therapy. *Int J Hematol* 2002; 76: 219-228.

27. Ruscher K, Freyer D, Karsch M, Isaev N, Megow D, Sawitzki B, et al. Erythropoietin is a paracrine mediator of ischemic tolerance in the brain: evidence from an in vitro model. *J Neurosci* 2002: 22: 10291-10301.

28. Prass K, Ruscher K, Karsch M, Isaew N, Megow D, Sawitzki B, et al. Desferrioxamine induces delayed tolerance against cerebral ischemia i vivo and in vitro. *J Cereb Blood Flow Metab* 2002; 22: 520-525.