ELSEVIER

Prophylactic effects of magnesium and vitamin E in rat spinal cord radiation damage: evaluation based on lipid peroxidation levels

Selçuk Peker[a],*, Ufuk Abacioglu[b], Ibrahim Sun[a], Deniz Konya[a],
Meral Yüksel[c], Necmettin M. Pamir[a]

[a] *Department of Neurosurgery, Marmara University School of Medicine, Istanbul 34662, Turkey*
[b] *Department of Radiation Oncology, Marmara University School of Medicine, Istanbul 34662, Turkey*
[c] *Department of Biochemistry, Marmara University School of Medicine, Istanbul 34662, Turkey*

Abstract

This study investigated the neuroprotective effects of magnesium sulfate prophylaxis and vitamin E prophylaxis in a rat model of spinal cord radiation injury. Groups were subjected to different treatment conditions for 5 days prior to irradiation, and outcomes were evaluated on the basis of lipid peroxidation levels in cord tissue. Four groups of rats were investigated: no radiation/treatment (n = 4), intraperitoneal (i.p.) saline 1 ml/day (n = 6), i.p. vitamin E 100 mg/kg/day (n = 6), and i.p. magnesium sulfate 600 mg/kg/day (n = 6). The thoracic cord of each non-control rat was exposed to 20 Gy radiation in a LINAC system using 6 MV x-rays, and malondialdehyde (MDA) levels (reflecting lipid peroxidation level) were determined 24 hours post-irradiation. The MDA levels in thoracic cord segments from the control rats were used to determine baseline lipid peroxidation. The mean levels in the control, saline-only, vitamin E, and magnesium sulfate groups were 12.12 ± 0.63, 27.0 ± 2.81, 17.71 ± 0.44, and 14.40 ± 0.47 nmol/mg tissue, respectively. The MDA levels in the saline-only group were significantly higher than baseline, and the levels in the vitamin E group were significantly lower than those in the saline group ($P < 0.05$ for both). The levels in the magnesium sulfate group were dramatically lower than those in the saline group ($P < 0.001$). The results indicate that i.p. magnesium sulfate has a marked neuroprotective effect against radiation-induced oxidative stress in the rat spinal cord.
© 2004 Elsevier Inc. All rights reserved.

Keywords: Lipid peroxidation; Magnesium; Myelopathy; Prophylaxis; Radiation; Spinal cord; Vitamin E

* Corresponding author. Tel.: +1-90-216-3264559; fax: +1-90-216-3057961.
E-mail address: peker@atlas.net.tr (S. Peker).

Introduction

Radiation myelopathy is a serious complication of spinal cord radiotherapy. Such damage can result in sensory changes, incomplete or complete paralysis, and ataxia. A number of agents have been investigated as methods of protecting the cord from this form of injury. Magnesium is an N-methyl-D-aspartate (NMDA) receptor antagonist that is widely used in obstetrical and cardiovascular practice (McLean, 1994). The neuroprotective effects of magnesium have been demonstrated in traumatic and ischemic injury to the spinal cord and brain (Suzer et al., 1999; Kaptanoglu et al., 2003; Lang-Lazdunski et al., 2000; Ustun et al., 2001); however, no study has yet assessed the specific radioprotective effects of magnesium in the spinal cord.

Vitamin E is a fat-soluble vitamin that acts as an antioxidant, protecting cell membranes and other fat-soluble elements in the body. In addition to its antioxidant functions, it is now known that vitamin E acts through other mechanisms, including direct effects on inflammation, blood cell regulation, connective tissue growth, and genetic control of cell division. Previous research has investigated the radioprotective effects of vitamin E in various types of tissue including spinal cord (Yanardag et al., 2001; El-Nahas et al., 1993).

The purpose of this study was to compare levels of protection against oxidative stress after pre-treatment with magnesium and with vitamin E in a rat model of spinal cord radiation injury.

Materials and methods

Animals and tissue collection

The experimental protocol was approved by the Animal Care and Use Committee at Marmara University School of Medicine. Twenty-two female Sprague-Dawley rats weighing approximately 250-300 g were housed four animals per cage at the Marmara University Neurological Sciences Institute. They were fed a standard rodent chow diet and water ad libitum, and were kept at a constant temperature (22 °C) on 12 h cycles of light and dark. The rats were randomly allocated to one of four groups:

Group I   Controls ($n = 4$): None of the animals in this group was irradiated or received any treatment. Each rat was euthanized and 2 cm of thoracic spinal cord was removed. The mean lipid peroxidation level in these cord tissues was recorded as baseline for the study.

Group II   Saline-only ($n = 6$): For the 5 days prior to irradiation (see details for irradiation method below), each rat received a daily intraperitoneal (i.p.) injection of 1 ml physiologic saline.

Group III Vitamin E treatment (n = 6): For the 5 days prior to irradiation, each animal received a daily i.p. injection of 100 mg/kg vitamin E (dI-alpha tocopherol acetate, Aksu Farma, Istanbul, Turkey).

Group IV Magnesium sulfate (MgSO$_4$) treatment ($n = 6$): For the 5 days prior to irradiation, each rat received a daily i.p. injection of 600 mg/kg MgSO$_4$ (Biofarma, Istanbul, Turkey).

Irradiation was carried out as described below, and all rats in Groups II, III and IV were euthanized 24 hours later. A 2 cm length of thoracic spinal cord was removed from each animal, and send for biochemical analysis.

Irradiation

The animals were irradiated using a LINAC system (Saturne 42, GE, France) and 6 MV x-rays. In each case, the rat was anesthetized with an i.p. injection of ketamine (50 mg/kg) and xylazine (9 mg/kg) and then placed on a board in prone position. One centimeter-thick bolus material was used to shift the build-up region and irradiate the thoracic spinal cord homogeneously. This region of the cord received 20 Gy irradiation at a dose rate of 2 Gy/min, with collimation and blocking to 2×3 cm single anterior fields.

Measurement of lipid peroxidation

The level of malondialdehyde (MDA) in each spinal cord specimen was taken to reflect the extent of lipid peroxidation in the tissue. Levels of MDA were determined using the method described by Yagi (1984). Briefly, the cord tissue was homogenized in ice-cold TCA (10% v/v) and then centrifuged. The supernatant was transferred to a test tube containing an equal volume of TBA (0.67% w/v), and this mixture was then heated to 90 °C and maintained at that temperature for 15 minutes. The MDA concentration for each specimen was determined in a spectrophotometer based on the level of absorbance at 532 nm, and was expressed as nmol/g tissue.

Statistical analysis

All data were evaluated in blinded fashion and expressed as mean $\pm$ SD. Group data were statistically compared using one-way analysis of variance (ANOVA). If a statistical difference was
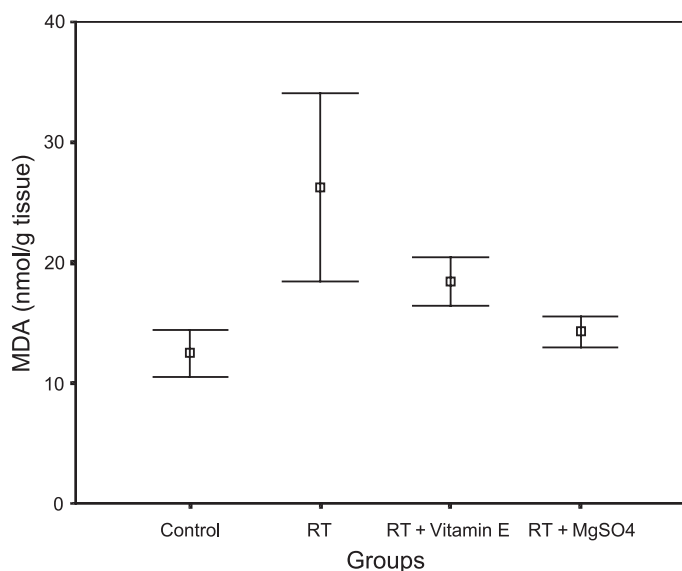

Fig. 1. Comprison of the malonyldialdehyde levels in the four study groups.

identified, ANOVA was followed by Tukey's multiple comparison test. Probability values < 0.05 were considered to indicate significant difference.

Results

The respective mean MDA levels in the control, saline, vitamin E, and $MgSO_4$ groups were 12.12 ± 0.63, 27.01 ± 2.81, 17.71 ± 0.44, and 14.40 ± 0.47 nmol/mg tissue. The mean level in the saline group was significantly higher than that in the control group ($P < 0.05$). The mean level in the vitamin E group was significantly lower than that in the saline group ($P < 0.05$). The mean MDA level in the $MgSO_4$ group was also lower than the level in the saline group, and this difference was even more significant ($P < 0.001$). Fig. 1 shows a comparison of the groups' mean MDA values.

Discussion

Ionizing radiation is a form of therapy that involves indirect production of oxygen-derived free radicals. Radiation penetrates the body well and is able to reach and damage targets; however, it also causes unintended harm to other tissues. It is estimated that approximately two-thirds of the biological damage from x-rays is indirect tissue injury (Hall, 2000). The physical effect of irradiation is free-radical production, a process that begins 10^{-18} seconds after tissue is exposed to radiation. Free radicals have a variety of effects, including peroxidation of lipids in membranes, inactivation of enzymes, depolymerization of polysaccharides, and disruption of nucleic acids (Choi, 1993). The unsaturated bonds of cholesterol and fatty acids in membranes readily react with free radicals and undergo peroxidation. Each lipid peroxide molecule that forms is also a free radical. Therefore, once it starts, the process of peroxidation can become autocatalytic, with each lipid peroxide attacking a neighboring fatty acid to yield additional lipid peroxide products (Bast and Goris, 1989).

In the brain, unsaturated fatty acids in membrane lipids account for more than 20% of the total fatty acids present (Floyed et al., 1984). Although the brain has high levels of unsaturated fatty acids, it has relatively low levels of potentially protective enzymes and free-radical scavengers. Consequently, neural tissue is prone to oxidative damage. Free radicals are known to play neurodestructive roles in various conditions and states, including ischemia, infarction, seizure disorders, aging, Alzheimer's disease and Parkinson's disease (Choi, 1993).

Spinal cord radiation

Radiation myelopathy is a serious complication of spinal cord irradiation. This type of treatment is commonly used in patients with tumors that have metastasized to the spine, or in cases of subtotally excised intramedullary glial tumors. It is also administered prophylactically to patients with tumors that are prone to spinal cord seeding, such as medulloblastomas and germinomas. Neck and nasopharyngeal cancers are also often treated with radiotherapy, and these patients are at risk for radiation myelopathy as well, because the spinal cord is located close to the target site. For many

types of tumors, the proximity of the spinal cord frequently limits what dose of radiation therapy can safely be administered (Stewart et al., 1995).

Histopathological examination of tissue with radiation injury reveals demyelination and vascular changes (Lo et al., 1991; Hopewell et al., 1987). Van der Kogel (1979) reported that early lesions predominantly feature demyelination, whereas late lesions are mainly characterized by blood vessel injury.

Most previously published experimental studies of tolerance to spinal cord radiation have assessed effects in rats. Concerning radiation dosages in rat models, researchers have identified 20 Gy as the effective dose that causes paraplegia in 50% of animals (ED50) due to white matter necrosis (Hopewell et al., 1987; Van der Kogel, 1979). This was our basis for administering 20 Gy in our experiment.

Protection against radiation myelopathy

In vitro and in vivo experiments have demonstrated that antioxidants can be radioprotective; however, there is some controversy about the effect of vitamin E in this regard. One study by Konings and Drijer (1979) indicated that vitamin E-deficient mice are more sensitive to radiation than mice with normal vitamin E levels. However, other research has shown that dietary vitamin E supplementation does not increase survival time in irradiated mice (Haley et al., 1954; Ershoff and Steers, 1960). Gutin et al. (1992) found that rats fed a vitamin E-supplemented diet and rats fed a vitamin E-deficient diet exhibited similar severities of spinal cord radiation injury after 18.5–21.5 Gy cervical cord irradiation.

Hornsey et al. (1990) irradiated the cervical spinal cords of rats and then treated with desferrioxamine and a low-iron diet. One week after radiation exposure until death, the animals were investigated for ataxia. Blood-spinal cord barrier disruption was investigated at 8 months 2 weeks after radiation exposure. The authors found that this regimen was radioprotective, and concluded that the effect was due to prevention of lipid peroxidation induced by free radicals. In other experimental work in which rat spinal cords were subjected to gamma irradiation, Kim et al. (2003) demonstrated that nimodipine treatment resulted in less apoptosis in the irradiated tissue. Research on another rat model of spinal cord radiation revealed that injection of amifostine (WR-2721), a commercially available radioprotectant, into the lateral ventricles is radioprotective (Spence et al., 1986).

Vitamin E

Vitamin E is located at various sites in cell membranes, and in close proximity to membrane-bound enzymes that can generate free radicals (Kelly, 1988). This substance acts as a free-radical scavenger, helping to protect the cell against lipid peroxidation (Yanardag et al., 2001; Packer, 1991; McCay, 1985; Ura et al., 1987). Vitamin E blocks the chain reaction that propagates the pre-oxidation cascade along a membrane (Choi, 1993).

The impact of vitamin E in radiation myelopathy has been studied previously, and the general effects that administration of this substance has on radiation injury are well known. Research has shown that vitamin E reduces radiation injury through its antioxidant effects (Sarma and Kesaven, 1993). Erol et al. (2004) examined MDA levels and histopathological features in rats' brains subjected

to gamma irradiation, and found that treatment after irradiation with vitamin E prevented neural tissue necrosis in radiation-damaged brain tissue. Yanardag et al. (2001) observed that pre-treatment with vitamin E plus selenium had protective effects in the livers of gamma-irradiated rats. Further, Konopacka et al. (1998) found that vitamin E had a radioprotective effect against DNA damage due to gamma irradiation in bone marrow polychromatic erythrocytes and exfoliated bladder cells of rats. Other work has revealed that a water-soluble derivative of vitamin E, alpha-TMG, is very effective at preventing radiation-induced aberrant metaphase in whole-body-irradiated adult mice (Satyamitra et al., 2001).

In our study of rats subjected to 20 Gy thoracic spinal cord x-ray irradiation, we found that the MDA levels in the group pre-treated with vitamin E were significantly lower than the levels in the saline-only group.

Magnesium

Magnesium provides neuroprotection through a number of mechanisms, namely, dilatation of cerebrovascular arteries, blockage of NMDA receptors, and blockage of voltage-gated calcium channels. In addition, this element may decrease the severity of endothelial and neuronal reperfusion injury by directly inhibiting lipid peroxidation, and by preventing depletion of glutathione (Dickens et al., 1992; Regan et al., 1998). Magnesium is believed to indirectly reduce levels of lipid peroxidation by-products, possibly by acting as a glutamate antagonist (Dumont et al., 2001; Lang-Lazdunski et al., 2000). In one study of rats with experimental head injury, prophylactic treatment with $MgSO_4$ prevented the decrease in intracellular free magnesium ion that usually occur post-trauma, and was associated with significantly better acute neurological outcome (Vink et al., 1996). Previous authors have also investigated the protective effect of $MgSO_4$ on tissue damage related to spinal cord trauma (Suzer et al., 1999; Kaptanoglu et al., 2003). Suzer et al. (1999) found that cord tissue from spinal cord-injured rats treated with $MgSO_4$ exhibited lower levels of lipid peroxidation than control animals. These authors found that treatment with 600 mg/kg $MgSO_4$ was more effective than 300 mg/kg, and this was our rationale for using 600 mg/kg daily doses in our study.

Magnesium has important actions in living tissue. Hypomagnesemia has been documented in patients with seizures, head trauma, subarachnoid hemorrhage, and cerebral infarction (Chernow et al., 1989; Muir and Lees, 1995; Polderman et al., 2000; Verive et al., 2000; Van der Bergh et al., 2003). In research on patients with subarachnoid hemorrhage, Van der Bergh et al. (2003) found that hypomagnesemia was associated with both delayed cerebral ischemia and poor prognosis.

To our knowledge, no previous study has investigated the potential protective effects of magnesium in spinal cord radiation injury. In our investigation, the rats pre-treated with $MgSO_4$ had dramatically lower MDA levels than the saline-only group. The neuroprotective effect of magnesium in this type of cord injury in rats is very significant, and is more marked than the neuroprotection offered by vitamin E prophylaxis.

Conclusion

The cord tissue lipid peroxidation findings in this study show that prophylactic i.p. injection of $MgSO_4$ has significant protective effects in rat spinal cord tissue subjected to radiation. We also found

that prophylactic i.p. injection of vitamin E was radioprotective, but the effects were less marked than those observed with $MgSO_4$. These two agents are readily available and easy to administer. Magnesium is available in forms appropriate for clinical use, and is used to treat a number of clinical conditions in humans. We believe that magnesium supplementation may be of significant benefit for patients who are scheduled to receive radiotherapy for spinal cord lesions or targets close to the cord. The protection offered by magnesium might even allow dose limits to be increased in some cases, leading to more effective treatment of the lesion. However, the radioprotectant properties of magnesium require further investigation. Another study focused on the effects of magnesium on late-stage tissue damage of the spinal cord is currently underway at our institution.

References

Bast, A., Goris, R.J., 1989. Oxidative stress, biochemistry and human disease. Pharmaceutisch weekblad. Scientific edition 11, 199–206.

Chernow, B., Bamberger, S., Stoiko, M., Vadnais, M., Mills, S., Hoellerich, V., Warshaw, A.L., 1989. Hypomagnesemia in patients in postoperative intensive care. Chest 95, 391–397.

Choi, B.H., 1993. Oxygen, antioxidants and brain dysfunction. Yonsei Medical Journal 34, 1–10.

Dickens, B.F., Weglicki, W.B., Li, Y.-S., Mak, I.T., 1992. Magnesium deficiency in vitro enhances free radical-induced intracellular oxidation and cytotoxicity in endothelial cells. FEBS Letters 311, 187–191.

Dumont, R.J., Verma, S., Okonkwo, D.O., Hurlbert, R.J., Boulos, P.T., Ellegala, D.B., Dumont, A.S., 2001. Acute spinal cord injury, part II: contemporary pharmacotherapy. Clinical Neuropharmacology 24, 265–279.

El-Nahas, S.M., Mattar, F.E., Mohamed, A.A., 1993. Radioprotective effect of vitamin C and E. Mutation Research 301, 143–147.

Erol, F.S., Topsakal, C., Ozveren, M.F., Kaplan, M., Ilhan, N., Ozercan, I.H., Yildiz, O.G., 2004. Protective effects of melatonin and vitamin E in brain damage due to gamma radiation. Neurosurgical Review 27, 65–69.

Ershoff, B.H., Steers, C.W., 1960. Antioxidants and survival time of mice exposed to multiple sublethal doses of x-irradiation. Proceedings of the Society for Experimental Biology and Medicine 104, 274–276.

Floyed, R.A., Zaleska, M.M., Harmon, H., 1984. Possible involvement of iron and oxygen free radicals in aspects of aging in brain. In: Armstrong, R. (Ed.), Free Radicals in Molecular Biology, Aging and Disease. Raven Press, New York, pp. 143–161.

Gutin, P.H., Levin, K.J., McDermott, M.W., Smith, M.T., Cashman, J.R., Chan, P.H., Ross, G.Y., Phillips, T.L., Levin, V.A., Davis, R.L., 1992. Lipid peroxidation does not appear to be a factor in late radiation injury of the cervical spinal cord of rats. International Journal of Radiation Oncology Biology Physics 25, 67–72.

Haley, T.J., McCulloh, E.F., McCormick, W.G., 1954. Influence of water-soluble vitamin E on survival time in irradiated mice. Science 119, 126–127.

Hall, E.J., 2000. Radiobiology for the radiologist, fifth ed. Lippincott Williams and Wilkins, Philadelphia.

Hopewell, J.W., Morris, A.D., Dixon-Brown, A., 1987. The influence of field size on the late tolerance of the rat spinal cord to single doses of X rays. British Journal of Radiology 60, 1099–1108.

Hornsey, S., Myers, R., Jenkinson, T., 1990. The reduction of radiation damage to the spinal cord by post-irradiation administration of vasoactive drugs. International Journal of Radiation Oncology Biology Physics 18, 1437–1442.

Kaptanoglu, E., Beskonakli, E., Okutan, O., Surucu, S., Taskin, Y., 2003. Effect of magnesium sulphate in experimental spinal cord injury: evaluation with ultrastructural findings and early clinical results. Journal of Clinical Neurosciences 10, 329–334.

Kelly, M.J., 1988. The pharmacology of vitamin E. Progress in Medicinal Chemistry 25, 249–290.

Kim, S.H., Cho, T.H., Lim, D.J., Park, J.Y., Chung, Y.G., Suh, J.K., 2003. The effects of nimodipine on irradiation-induced apaptosis in the rat spinal cord. Journal of Korean Neurosurgical Society 33, 576–581.

Konings, A.W.T., Drijer, E.B., 1979. Radiation effects on membranes: I. Vitamin E deficiency and lipid peroxidation. Radiation Research 80, 494–501.

Konopacka, M., Widel, M., Rzeszowska, W.R., 1998. Modifying effect of vitamins C, E and beta-carotene against gamma-ray-induced DNA damage in mouse cells. Mutation Research 417, 85–94.

Lang-Lazdunski, L., Heurteaux, C., Dupont, H., Widmann, C., Lazdunski, M., 2000. Prevention of ischemic spinal cord injury: comparative effects of magnesium sulfate and riluzole. Journal of Vascular Surgery 32, 179–189.

Lo, Y.C., McBride, W.H., Withers, H.R., 1991. The effect of single doses of radiation on mouse spinal cord. International Journal of Radiation Oncology Biology Physics 22, 57–63.

McCay, P.B., 1985. Vitamin E: interactions with free radicals and ascorbate. Annual Review of Nutrition 5, 323–340.

McLean, R.M., 1994. Magnesium and its therapeutical uses: a review. American Journal of Medicine 96, 63–76.

Muir, K.W., Lees, K.R., 1995. A randomized, double-blind, placebo-controlled pilot trial of intravenous magnesium sulfate in acute stroke. Stroke 26, 1183–1188.

Packer, L., 1991. Protective role of vitamin E in biological systems. American Journal of Clinical Nutrition 53, 1050–1055.

Polderman, K.H., Bloemers, F.W., Peerdeman, S.M., Girbes, A.R., 2000. Hypomagnesemia and hypophosphatemia at admission in patients with severe head injury. Critical Care Medicine 28, 2022–2025.

Regan, R.F., Jasper, E., Guo, Y., Scott, P.S., 1998. The effect of magnesium on oxidative neuronal injury in vitro. Journal of Neurochemistry 70, 77–85.

Sarma, L., Kesaven, P.C., 1993. Protective effects of vitamins C and E against gamma ray induced chromosomal damage in mouse. International Journal of Radiation Biology 63, 759–764.

Satyamitra, M., Devi, P.U., Murase, H., Kagiya, V.T., 2001. In vivo radioprotection by alpha-TMG: preliminary studies. Mutation Research 479, 53–61.

Spence, A.M., Krohn, K.A., Edmondson, S.W., Steele, J.E., Rasey, J.S., 1986. Radioprotection in rat spinal cord with WR-2721 following cerebral lateral intraventricular injection. International Journal of Radiation Oncology Biology Physics 12, 1479–1482.

Stewart, P.A., Vinters, H.V., Wong, C.S., 1995. Blood-spinal cord barrier function and morphometry after single doses of X-rays in rat spinal cord. International Journal of Radiation Oncology Biology Physics 32, 703–711.

Suzer, T., Coskun, E., Islekel, H., Tahta, K., 1999. Neuroprotective effect of magnesium on lipid peroxidation and axonal function after experimental spinal cord injury. Spinal Cord 37, 480–484.

Ura, H., Denda, A., Yokose, Y., Tsutsumi, M., Konishi, Y., 1987. Effect of vitamin E on the induction and evolution of enzyme-altered foci in the liver of rats treated with diethyl-nitrosamine. Carcinogenesis 8, 1595–1600.

Ustun, M.E., Gurbilek, M., Ak, A., Vatansev, H., Duman, A., 2001. Effects of magnesium sulphate on tissue lactate and malondialdehyde levels in experimental head trauma. Intensive Care Medicine 27, 264–268.

Van der Bergh, W.M., Algra, A., van der Sprenkel, J.W.B., Tulleken, C.A.F., Rinkel, G.J.E., 2003. Hypomagnesemia after aneurysmal subarachnoid hemorrhage. Neurosurgery 52, 276–282.

Van der Kogel, A.J., 1979. Late effects of radiation on the spinal cord: dose-effect relationships and pathogenesis. Thesis, TNO, Rijswijk

Verive, M.J., Irazuzta, J., Steinhart, C.M., Orlowski, J.P., Jaimovich, D.G., 2000. Evaluating the frequency rate of hypomagnesemia in critically ill pediatric patients by using multiple regression analysis and a computer-based neural network. Critical Care Medicine 28, 3534–3539.

Vink, R., Health, D.L., McIntosh, T.K., 1996. Acute and prolonged alterations in brain free magnesium following fluid percussion-induced brain trauma in rats. Journal of Neurochemistry 66, 2477–2483.

Yagi, K., 1984. Assay for blood plasma or serum. Methods in Enzymology 105, 328–331.

Yanardag, R., Bolkent, S., Kizir, A., 2001. Protective effects of DL-alpha-tocopherol acetate and sodium selenate on the liver of rats exposed to gamma radiation. Biological Trace Element Research 83, 263–273.