Kafkas Univ Vet Fak Derg
17 (1): 25-30, 2011

RESEARCH ARTICLE

Changes in Lipid Peroxidation and Antioxidant Environment of Spinal Fluid with The Use of Bupivacaine for Spinal Anesthesia

Nadide Nabil KAMİLOĞLU * Ebru BEYTUT * Alkan KAMİLOĞLU **

* University of Kafkas, Faculty of Veterinary Medicine, Department of Physiology, TR-36100 Kars - TURKEY
** University of Kafkas, Faculty of Veterinary Medicine, Department of Surgery, TR-36100 Kars - TURKEY

Makale Kodu (Article Code): KVFD-2010-2322

Summary

The objective of this study was to determine if bupivacaine has any effects on the antioxidant defence system and in lipid peroxidation as thiobarbituric acid substance (TBARS) of spinal fluid. The experiment was carried out on 15 male clinically healthy, 3-4 years old, male Tuj ram weighing an average 56±5 kg. Eight ml of 0.5% bupivacaine was used for the induction of spinal anesthesia (SA). To measure the levels of lipid peroxidation (LPO), glutathione (GSH), activity of glutathione peroxidase (GSHPx; EC: 1.11.1.9) and the levels of vitamine E in spinal fluid, samples were taken from the subarachnoid (intrathecal) space before anaesthesia (0. min) and at 5, 15, and 60 min. after anaesthesia. The levels of TBARS slightly increased at 5 min. after spinal injection of bupivacaine and this increase continued trougout anesthesia ($P<0.001$). Vitamin E levels were decreased at 5 min. after anesthesia ($P<0.001$) and this level recovered to control levels at 30 min. after bupivacaine injection. The activity of GSHPx and the levels of GSH in spinal fluid increased continually 5 min. after administration of bupivacain ($P<0.001$). In conclusion, we found that while spinally injection of bupivacaine increased free radical levels in spinal fluid may be supported antioxidant environment of spinal fluid during anesthesia.

Keywords: *Bupivacaine, Spinal anesthesia, Spinal fluid, Antioxidants, Lipid peroxidation, Ram*

Bupivacain ile Yapılan Spinal Anestezi ile Spinal Sıvı Lipid Peroksidasyon Düzeyi ve Antioksidan Sisteminde Meydana Gelen Değişiklikler

Özet

Bu çalışmada, bupivacain ile yapılan spinal anestezinin antioksidan savunma sistemi ve lipid peroksidasyonunun bir indikatörü olan tiyobarbiturik asit substratları (TBARS) düzeyleri, üzerine etkisini belirlemek amaçlanmıştır. Bunun için 15 klinik olarak sağlıklı 3-4 yaşlarında ve ortalama 56±5 kg ağırlığında Tuj koçları kullanılmıştır. Spinal anestezi oluşturmak amacıyla 8 ml %0.5'lik bupivacain kullanıldı. Bupivacain ile spinal anestezi yapılmadan önce (0. dakika) ve anesteziden 5, 30 ve 60 dakika sonra subarachnoid (intrathecal) bölgeden alınan spinal sıvı numuneleri thiobarbiturik asit substans (TBARS), E vitamini ve glutatyon (GSH) düzeyleri ile glutatyon peroxidaz (GSHPx, EC. 1.11.1.9) aktivitelerini belirlemek için kullanıldı. Bu çalışmada, TBARS düzeylerinin anesteziden 5 dak. sonra istatistiksel olarak artmaya başladığı ($P<0.001$) ve bu artışın anestezi boyunca sürdüğü gözlenirken, E vitamini değerlerinin anestezinin 5 dak.'sında düşerken bundan sonraki zaman dilimlerinde başlangıç seviyelerine döndüğü tespit edildi. Eritrosit GSHPx aktivitesi ile GSH düzeylerinin anestezi süresince istatistiksel olarak yükseldiği gözlendi ($P<0.001$). Sonuç olarak, bupivacain ile oluşturulan spinal anestezi ile spinal sıvının serbest radikal miktarı yükselmekle birlikte, bupivacain anestezi süresince spinal sıvının antioksidan ortamını desteklemektedir.

Anahtar sözcükler: *Bupivacain, Spinal anestezi, Spinal sıvı, Antioksidanlar, Lipid peroksidasyonu, Koç*

İletişim (Correspondence)
+90 474 2426807
nkamiloglu@hotmail.com

INTRODUCTION

Because of its simplicity spinal anesthesia is an easy regional technique to use in clinical practice. Providing the spinal anesthesia, among local anesthetics, intrathecal bupivacaine is used extensively to obtain potent and intense motor blockade [1,2]. However, it is showed *in vivo* [3] and *in vitro* [4] that local anesthetics modulate the inflamatory response. Also it is known that bupivacaine have an antioxidant properties aimed against lipid peroxidation [5,6]. Nevertheless, no reports addressed the effects of bupivacaine on the antioxidant system of spinal fluid including antioxidant enzymes as glutathione and glutathione peroxydase and vitamin E. Body has developed an antioxidant defense system which enzymatic, metal chelating and free radical scavenging properties [7]. The radicals formed by autooxidation react with proteins, enzymes and vitamins which then lose their biological function. GSH has a protective mechanism against the cell injury. Earlier findings also suggest that the presence of GSH in cells and tissues are associated to attenuate the oxidative stresss [8]. GSH-P_x is responsible for the neutralization of both organic and inorganic hydroperoxides [9]. Endogen free radicals such as peroxides damage the structure and function of the cells and antioxidant systems of cells such as glutathione and glutathione peroxydase bring about decomposition reactions [10,11]. Thus, it is essential to consider the concentration of antioxidant in the spinal cerebrospinal fluid (CSF) when investigating the relationship between oxidative stress and anesthetics used for spinal anesthesia On the other hand, Pitkanen et al.[12] suggested that the clinical significance of aspirating spinal CSF before and after attempting spinal anesthesia with bupivacaine was found to be small.

While the relation between free radical reaction and defense mechanisms in CSF against CSF related ilness was being in consideration [13-15], there is no report that about the change in lipid peroxidation and antioxidat enzymes levels in spinal CSF with spinal injection of anesthetics in particular bupivacain. So, the purpose of this study is that show the action of spinally injected bupivacaine on LPO and antioxidant enzymes in the spinal CSF.

MATERIAL and METHODS

Animals and Treatments

The experiment was carried out on 15 male clinically healthy Tuj sheep, weighing average approximately 57 kg (50-65) and aged 3-4 years old. The animals were restricted in lateral recumbency on an operating table. For the purpose of preventing possible respiratory and circulatory problems, necessary support was provided so as to hold the animals' neck and thorax in an upward position. The lumbosacral area was prepared for an aseptic injection. Prior to subarachnoid (intrathecal) injection, local infiltrative anaesthesia of the subcutaneous tissues and interspinous ligament was achieved. SA was induced by the administration of 8 ml of 0.5% bupivacaine (Heavy Marcain, Astra Södertälje, Sweden) through the lumbosacral space with an 18-gauge, 1.25×90 mm spinal needle. The spinal needle was inserted into the lumbosacral space and moved slowly forward into the subarachnoidal space. After aspiration of a quantity of cerebro-spinal fluid equivalent to the amount of local anaesthetic agent, bupivacaine was injected slowly. No reaction was noted during subarachnoidal injection in the animals. The desired quality anaesthesia was achieved within 20 to 60 sec. of the injection of the local anaesthetic agent. The duration of the efficacy of the anaesthesia was determined to be between 3.5 and 5 h. Additionally, in order to prevent the occurrence of any respiratory problems due to the expansion of bupivacaine in a cranial direction, the animals were kept inclined at approximately 30°Cduring anaesthesia.

Collection of Samples

To measure the levels of lipid peroxidation (LPO), glutathione (GSH) and activity of glutathione peroxidase (GSHPx; EC: 1.11.1.9) 1,5 ml of spinal CSF samples were taken from the subarachnoid (intrathecal) space before anaesthesia (0. min), and at 5, 30 and 60 minute after anaesthesia. Immediately on removal the spinal fluid samples were centrifuged to remove any contaminates, pipetted into virgin polyethylene tubes and placed in a -20°C freezer until further analysis. Samples were thawed at room temperature and analyzed.

Analytical Procedures

The end product of polyunsaturated fatty acid peroxidation, malondialdehyde (MDA), reacting with thiobarbituric acid (TBA) in samples was determined by Placer et al.[16] method modified by Matkovichs et al.[17]. The values of MDA reactive material were expressed in terms of the malondialdehyde (MDA) content (nmol/ml plasma), which served as a standard of 1,1,3,3-tetraethoxypropane (Sigma,Chemical Company St. Louis, MO, USA).

The GSH levels of spinal fluid were measured spectrophotometrically using Ellman's reagent [18]. GSHPx activity was determined using cumene hydroperoxide and reduced GSH as co-substrates and the loss of GSH following enzymic reaction was measured spectrophotometrically with Ellman's reagent at 37ºC and 412

nm according to Lawrence and Burk [19] and Matkovics et al.[17]. The protein content in the tissue homogenate was measured by the method of Lowry et al.[20] with bovine serum albumin as the Standard.

The vitamin E (α-tocopherol) levels of plasma were determined in the frozen serum samples by the method described by McMurray et al.[21]. The relevant wavelengths for vitamin E detection were 292 and 330 nm. Calibration was performed using a standard solution of α-tocopherol in methanol.

Statistical Analysis

Statistical analysis (Anova Test and Post Hoc. Tukey) was performed using the SPSS software program (16.0). All results were expressed as the mean ± standard deviation (SD). A P value <0.05 was considered to be statistically significant.

RESULTS

Mean values of investigated parameters and differences in spinal fluid between before and after anesthetiation times are presented with Figures.

Effects of spinal injection of bupivacaine on the levels of TBARS in spinal fulid indicated in *Fig. 1*. The levels of TBARS slightly increased at 5 min after spinal injection of bupivacaine and this increase continued trougout anesthesia (P<0.001)

As shown in *Fig. 2* the levels of GSH in spinal fulid regularly increased throughout anesthesia (P<0.001).

Effects of spinal injection of bupivacaine on the activity of GSHPx in spinal fulid introduced in *Fig. 3*. The activity of GSHPx in spinal fluid statistically increased (P<0.001) during anesthetiation times.

Effect of intraspinal bupivacaine injection on vitamin E levels was also exhibited in *Fig. 4*. Vitamin E levels were slightly decreased at 5 min after anesthesia (P<0.001) and this level recovered to control levels at 30 min after bupivacaine injection and approximately remain this levels after that time.

DISSCUSSION

The results of this study indicate that spinally injected bupivacaine has an effect on change the lipid

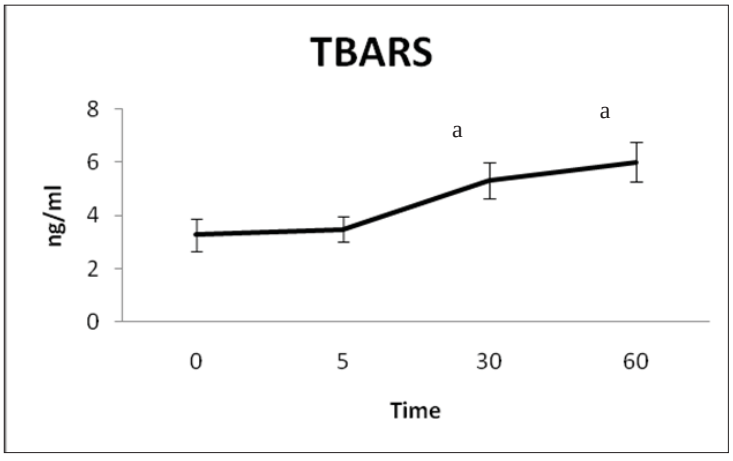

Fig 1. Line graph showing the effects of spinal injection of bupivacaine on the levels of TBARS in spinal fluid. a: P<0.001 in comparision to the 0th point of the time. Values are expressed as mean ±SD

Şekil 1. Grafik intratekal bupivacaine enjeksiyonu ile spinal sıvı TBARS düzeylerinde zamana göre belirlenen değişiklikleri göstermektedir. a: P<0.001 (0. dakikaya göre istatistiksel önemliliği göstermektedir. Değerler Ortalama ± Standart Sapma olarak verilmiştir

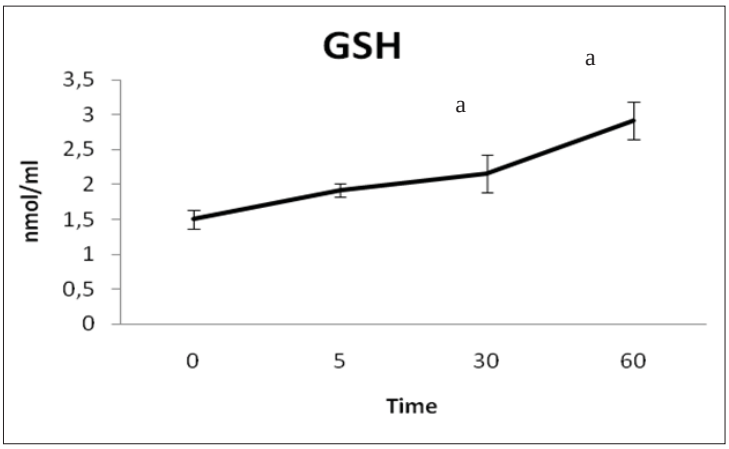

Fig 2. Line graph showing the effects of spinal injection of bupivacaine on the levels of GSH in spinal fulid. a: P<0.001 in comparision to the 0th point of the time. Values are expressed as mean ±SD

Şekil 2. Grafik intratekal bupivacaine enjeksiyonu ile spinal sıvı GSH düzeylerinde zamana göre belirlenen değişiklikleri göstermektedir. a: P<0.001 (0. dakikaya göre istatistiksel önemliliği göstermektedir. Değerler Ortalama ± Standart Sapma olarak verilmiştir)

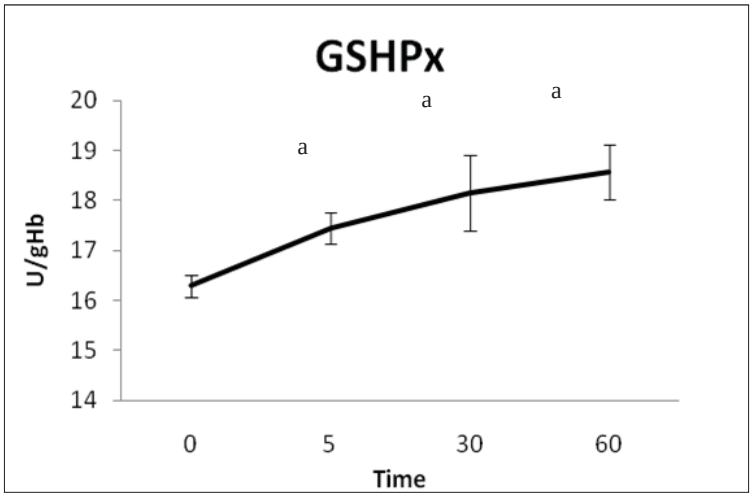

Fig 3. Line graph showing the effects of spinal injection of bupivacaine on the activity of GSHPx in spinal fulid. a: P<0.001 in comparision to the 0th point of the time. Values are expressed as mean ±SD

Şekil 3. Grafik intratekal bupivacaine enjeksiyonu ile spinal sıvı GSHPx aktivitelerinde zamana göre belirlenen değişiklikleri göstermektedir. a: P<0.001 (0. dakikaya göre istatistiksel önemliliği göstermektedir. Değerler Ortalama ± Standart Sapma olarak verilmiştir

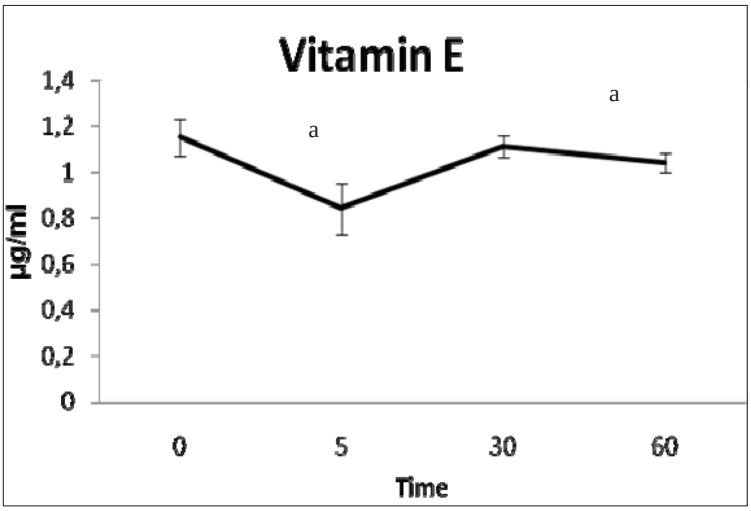

Fig 4. Effects of spinal injection of bupivacaine on the levels of vitamine E in spinal fulid. a: P<0.001 in comparision to the 0th point of the time. Values are expressed as mean ±SD

Şekil 4. Grafik intratekal bupivacaine enjeksiyonu ile spinal sıvı E vitamini düzeylerinde zamana göre belirlenen değişiklikleri göstermektedir. a: P<0.001 (0. dakikaya göre istatistiksel önemliliği göstermektedir. Değerler Ortalama ± Standart Sapma olarak verilmiştir)

peroxidation and antioxidant enzymes in the spinal CSF of rams. LPO is one of the prominant manifestations of oxidative stress. Reactive oxygen species are induced oxidation and peroxidation of membrane phospholipids, thereby causing damage to the phospholipid molecule as well as to other molecules in the cells [7]. Unsaturated phospholipids, glicolipids and cholesterol in cell membranes are particularly susceptible to lipid peroxidation, because they exist in high concentrations and contain on abundant source of peroxidation substrates, particularly Polyunsaturated Fatty Acids (PUFA) [22]. PUFA are found in abundance in mammalian membrane lipids and are the most likely targets of Reactive Oxygen Species (ROS). As the nervous system is enriched with PUFA, it is particularly vulnerable to such stress. Increased levels of lipid hydroxyperoxides and hydroxyphospholipids have been associated with oxidative stress and membrane injury that occur in pathological conditions such as spinal cord injury [23]. The main product of lipid peroxyl radical transformation is lipid hydroperoxide. Lipid peroxides cause membrane instability and degradation and a significant decrease in membrane fluidity [23]. When membrane phospholipids are released from damaged membrane, causing elevation in arachidonic acid metabolism that contribute to inflamation. It is likely that the increased levels of lipid peroxides in the spinal CSF may cause lipid peroxidation. Some disease [24-26] and some drugs used to treat illness [13,27] causes alteration on the free radical defence mechanisms and lipid peroxidation products in CSF. So, it is suggested that the oxidation of CSF lipoproteins play a role in the pathogenesis of neurodegenerative diseases. Nevertheless, Rosen et al.[14] showed that large volume of local anesthetics include bupivacaine, injected for spinal anesthesia, was not neurotoxic when injected in to subaracnoid space of sheep, and also this anesthetic solutions have not been produced significant abnormalities in sheep spinal CSF composition. On the other hand, local anesthetic agents include bupivacaine have membrane stabilizing properties which may induce changes in membrane polarization [28]. This mechanism may account for the protective effect of bupivacaine against oxidative stress. So, Lenfant et al.[29] found that bupivacaine provide a protective effect against induced free radical increase

with 2,2-azobis dihydrochloride. Also, De Iuliis et al.[30] have been showed that bupivacaine have an antioxidant effect on LPO of rat liver microsomes. However, there are no clinical studies that point out the importance of alterations in the lipid peroxidation status of CSF with spinally injected bupivacaine. In the current study we showed that the lipid peroxidation in CSF changed during spinal anesthesia with bupivacaine. This results may be due to bupivacaine induced inflamatory response which cause rise of LPO in spinal fluid.

Free radical-mediated damage occurs as a consequence of GSH depletion, depressed antioxidant enzyme activities and enhanced LPO. On the other hand, marked increase in oxidative stress may have been potentiated by increased antioxidant enzyme activities. Nevertheless, antioxidants can scavenge ROS before they can cause damage top various biomolecules or prevent oxidative damage from spreading out by interrupting the radical chain reaction of LPO. The disappearance of the endogenous antioxidants in plasma was measured in relation the formation of lipid hydroperoxides formed from endogen lipids. GSH is a major non-protein thiol in mammals and is essential for structural and metabolic integrity of cells [8]. GSH play an important role in maintaining the stability of the membrane as a direct free radical scavenger [11] and in the protection of intracellular components such as sulphydryl enzymes against oxidative denaturation. Also, it has been demonstrated to scavenge superacide radical in a dose dependent manner [31]. Further, the key feature of this reaction is that it does not produce H_2O_2 in the system [10]. It is known that H_2O_2 plays a critical role in the regulation and expression of antioxidant enzymes in various cellular systems. Catalase (CAT) and glutathione peroxidase (GSHPx) that two different enzymes present in the cellular system is also neutralized H_2O_2. GSHPx which is GSH dependent antioxidant factor is a cytosolic protein that associates readily with membranes and reduce both organic and inorganic phospholipid hydroperoxides to alchols. GSHPx changes the hydroperoxide group to the much less toxic hydroxy moiety. This enzyme not only allowe the removal of the toxic ROOH moiety but also permits the regeneration of a membrane lipid molecule through reacylation. GSHPx system is particularly important in preventing free radical initiation in membranes since it is a very effective scavenger of H_2O_2 [8,31,32]. H_2O_2 catabolism by GSHPx suppresses hydroperoxide-dependent lipid peroxidation and prevents α-tocopherol oxydation, whereas peroxyl radical scavenging by α-tocopherol prevents radical-induced GSHPx inactivation [33]. The nervous system is vulnerable to the damaging effects of highly reactive free radicals for several reasons. Antioxidant defenses are criticaly important to protect the neural tissues from oxidative damage indeed, numerous pathophysiological conditions have been associated with increased levels of oxidative stress [27]. Intrathecal concentration of antioxidants might be of relevance due to the antioxidant protection against oxidative initiation of degenerative processes. After increase of LPO due to action of many used drugs, a pronounced protective effect of antioxidants was suggestted by researchers [11,13,26,34]. In the present study, we observed relationship between the concentration of free radical reactions as lipid peroxides and the biological defense mechanisms as the activity of GSHPx and the levels of GSH in the spinal fluid. These results showed that increased LPO cause rise in antioxidants levels and activity during anesthesia.

Antioxidant enzymes protect the cellular structures against lipid peroxidation initiated by active species of oxygen and that antioxidant micromolecules such as vitamin E is also responsible for the inactivation of lipid peroxides [32,35]. Vitamin E is a most abundant lipid-soluble antioxidant present in biological membranes and lipoproteins that inhibits peroxidation of polyunsaturated fatty acids (PUFA) by scavenging free radicals [33]. In the nervous system specific mechanisms transfer nutrients and other essential substance from blood into spinal fluid. This mechanisms are also involved in the concentration of the substance within the central nervous system [36]. The neuroprotective effects of vitamin E are wholly mediated by its antioxidant property. Mostly on the basis of symptoms of primary vitamin E deficiency, it has been demonstrated that vitamin E has a central role in maintaining neurological structure and function [34]. So, orally supplemented vitamin E reaches the CSF and brain [37]. In the present study it was observed that intra-spinal injection of bupivacaine has a protective effect on the levels of vitamin E in spinal fluid. Also, low levels of vitamin E in spinal CSF may be due to plasma levels of vitamin E have been present below the reference values. This result may be due to either antioxidant activity of bupivacaine or the antioxidant synergism between α-tocopherol and GSH and GSHPx that mediated via the action of redox cycles.

In conclusion, we found that while spinally injection of bupivacaine increased free radical levels in spinal fluid may be supported antioxidant environment of spinal fluid during anesthesia.

REFERENCES

1. Baran V, Özaydın İ, Beytut E, Kılıç E, Kamiloglu NN, Kamiloğlu A: Cardiovascular effects of lactated ringer's infusion before and during intratrathecal anasthesia induced by bupivacaine in sheep. *Bull Vet Inst Pulawy,* 50, 599-603, 2006.

2. Kuusniemi KS, Pihlajamaki KK, Pitkanen MT: A low dose of plain or hyperbaric bupivacaine for unilateral spinal anesthesia. *Region Anesth Pain Med*, 25 (6): 605-610, 2000.

3. Holman MW, Gross A, Jelacin N, Durieux ME: Local anesthetic effects on priming and activation of human neutrophils. *Anesthesiol*, 95, 113-121,2001.

4. Hönemann CW, Heyse TJ, Möllhof T: The inhibitory effect of bupivacaine on prostoglandin E2 (EP1) receptor functioning: Mechanism of action. *Anast Analg,* 93, 628-634, 2001.

5. Leduc C, Gentili ME, Estebe JP, Le Corre P, Moulinoux JP, Ecoffey C: The effect of local anesthetics and amitriptyline on peroxidation in vivo in an inflamatory rat model: Preliminary report. *Anesth Analg*, 95 (4): 992-996, 2002.

6. Kamiloğlu NN, Beytut E, Kamiloğlu A, Aksakal M, Güven A: Potentiation of the bupivacaine antioxidant effects by lactated ringer's solution infusion during spinal anesthesia in rams. *Rev Med Vet,* 158 (4): 174-177, 2007.

7. Halliwell B, Gutteridge JMC: Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol,* 186, 1-87, 1990.

8. Meister A, Anderson ME: Glutathione. *Annu Rev Biochem,* 52, 711-60, 1983.

9. Halliwell B: Antioxidants in human health and disease. *Ann Review Nutr,* 16, 33-50,1996.

10. Winterbourn CC, Metodiewa D: The reaction of superoxide with reduced glutathione. *Archives Biochem Biophys,* 314, 284-290, 1994.

11. Winterbourn CC: Free radical toxicology and antioxidant defence. *Clin Ex Pharmacol Physiol,* 22, 877-880, 1995.

12. Pitkanen M, Tuominen M, Asantila R, Rosenberg PH: Effect of aspiration of serebrospinal fluid on spinal anaesthesia with isobaric %5 bupivacaine. *Acta Anaesthesiol Scand,* 29 (26): 590-593, 1985.

13. Darii VI: Characteristics of some links of the antioxydant system in patients with complicated cerebral stroke. *Lik Sprava,* 1, 46-49, 2001.

14. Rosen MA, Baysinger CL, Shnider SM, Dailey PA, Norton M, Curtis J, Collins M, Davis RL: Evaluation of neurotoxicity after subarachnoid injection of large volumes of local anesthetic solutions. *Anasth Analg,* 62, 802-808, 1983.

15. Lawes EG: Volume preloading, spinal anesthesia and caesarean section. *Br J Anesth,* 76, 334-335, 1996.

16. Placer ZA, Cushman LL, Johnson BC: Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Anal Biochem,* 16, 359-364, 1966.

17. Matkovics B, Szabo L, Varga IS: Determination of enzyme activities in lipid peroxidation and glutathione patways. *Lab Diagn,* 15, 248-249, 1988 (in Hungarian).

18. Sedlak J, Lindsay RH: Estimation of total protein-bound and non-protein sulfhydryl groups in tissue with ellman's reagent. *Anal Biochem,* 25, 192-205, 1968.

19. Lawrence RA, Burk RF: Glutathione-peroxidase activity in selenium-deficient rat liver. *Biochem Biophys Res Commun,* 71, 952-955, 1976.

20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the folin reagent. *J Biol Chem,* 193, 265-275, 1951.

21. McMurray CH, Blanchflower WJ, Rice DA: Influence of extraction techniquest on the determination of α-tocopherol in animal feedstuffs. *J Assoc Off Anal Chem,* 63, 1258-1261, 1980.

22. Girotti AW: Lipid hydroperoxide generation, turnover and effector action in biological systems. *J Lipid Res,* 39, 1529-1542, 1998.

23. Farooqui AA, Horrocks LA: Lipid peroxides in the free radical pathophsiology of brain diseases. *Cell Mol Neurobiol,* 18, 599-608, 1998.

24. Miketova P, Kaemingk K, Hockenberry M, Pasvogel A, Hutter J, Krull K, Moure I: Oxydative changes in cerebral spinal fluid phosphatidylcoline during treatment for acute lymphoblastic leukemia. *Biol Res Nursing,* 6 (2): 187-195, 2005.

25. Lovell MA, Xie C, Markesbery WR: Decreased glutathione transferase activity in brain and ventricular fluid in Alzheimer's disease. *Neurology*, 51, 1562-1566, 1998.

26. Simpson EP, Henry YK, Henkel JS, Smith RG, Apel SH: İncreased lipid peroxidation in sera of ALS patients: A potential biomarker of disease burden. *Neurology*, 62 (10): 1758-176, 2004.

27. Sen CK, Khanna S, Roy S: Tocotrienol: The natural vitamin E to defend the nervous system? *Ann NY Acad Sci,* 1037, 127-142, 2004.

28. Seeman P: The mem rane action of anesthetics and tranquilizers. *Pharmacol Rev,* 24 (4): 583-655,1972.

29. Lenfant F, Lahet JJ, Volot F, Schafer I, Freysz M, Rochette L: Effects of bupivacaine on human erythrocytes submitted to stresss and evidence for an interaction between bupivacaine and flumazenil. *Br J Clin Pharmacol,* 48, 826-828,1999.

30. De Iuliis A, Zanatta L, Vincenti E, Galzigna L: Differences of ropivacaine and bupivacaine relevant to anti-inflamatory activity platelet aggregation and antioxidant activity in vitro. *Farmaco*, 56 (3): 153-157, 2001.

31. Hussain S, Slikker Jr W, Ali SF: Role of metallothionein and other antioxidants in scavenging superoxide radicals and their possible role in neuroprotection. *Neurochem Int,* 29, 145-152,1996.

32. Sies H: What is Oxidative Stres? **In,** Sies H (Ed): Oxidative Stres: Oxidants and Antioxidants. pp. 1-8, Academic Press, San Diego, 1991.

33. Liebler DC: The role of metabolism in the antioxidant function of vitamine E. *Crit Rev Toxicol,* 23, 147-169, 1993.

34. Muller DP, Goss-Sampson MA: Neurochemical, neurophsiological and neuropathological studies in vitamin E deficiency. Crit Rev Neurobiol, 5, 239-263, 1990.

35. Chow CK: Vitamin E and oxidative stress. *Free Rad Biol Med,* 11, 215-232,1991.

36. Spector R, Eells I: Deoxynucleoside and vitamin transport into the central nervous system. *Fe Proc,* 43 (2): 196-200, 1984.

37. Vatassery GT, Fahn S, Kuskowski MA: alpha-Tocopherol in CSF of subjects taking high-dose vitamin E in the DATATOP study: Parkinson Study Group. *Neurology*, 50, 1900-1902, 1998.