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ORIGINAL PAPER

Spinal Morphine Administration Reduces the Fatty Acid Contents in Spinal Cord and Brain by Increasing Oxidative Stress

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Abstract It is well known that oxidative stress damages bimolecules such as DNA and lipids. No study is available on the morphine-induced oxidative damage and fatty acids changes in brain and spinal tissues. The aim of this work was to determine the effects of morphine on the concentrations and compositions of fatty acid in spinal cord segments and brain tissues in rabbits as well as lipid peroxidation (LP) and glutathione (GSH) levels in cortex brain.

Twelve New Zealand albino rabbits were used and they were randomly assigned to two groups of 6 rabbits each. First group used as control although morphine administrated to rats in second group. Cortex brain and (cervical, thoracic, lumbar) samples were taken.

The fatty acids between n:18.0 and 21.0 were present in spinal cord sections and n:10 fatty acids in control animals were present in the brain tissues. Compared to n:20.0–24.0 fatty acids in spinal cord sections and 8.0 fatty acids in the brain tissues of drug administered animals. The concentration and composition of the fatty acid methyl esters in spinal cord and brain tissues was decreased by morphine treatments. LP levels in the cortex brain were increased although GSH levels were decreased by the morphine administration.

In conclusion, unsaturated fatty acids contents in brain and spinal cord sections and GSH were reduced by administrating spinal morphine although oxidative stress as LP increased. The inhibition oxidative damage may be a useful strategy for the development of a new protection for morphine administration as well as opiate abuse.

Keywords Morphine · Fatty · acids · Brain · Lipid peroxidation · Spinal cord · Glutathione

Abbreviations

BBB	Blood-brain barrier
FAs	Fatty acids
FAMEs	Fatty acid methyl esters
ROS	Reactive oxygen species
GSH	Reduced glutathione
PUPAs	Polyunsaturated fatty acids
HR	Heart rate

Introduction

Reactive oxygen substances (ROS) and lipid peroxides are produced by a free radical chain reaction, which can also be initiated by ROS [1]. The ROS, i.e. singlet

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oxygen, superoxide anion radical, perhydroxyl radical and hydroxyl radical, contribute to tissue damage [2]. ROS also cause injury by reacting with biomolecules such as lipids and nucleic acids as well as by depleting enzymatic and/or nonenzymatic antioxidants in the brain [3]. ROS and/or free radicals may be involved in the morphine and opiate abuse [4–6].

Oxidative stress is associated with significantly lower omega 3 polyunsaturated fatty acids (PUFAs) fractions [2] and glutathione system [3]. The brain consumes the highest (about 60%) amount of oxygen in the human body. Although most of the oxygen used in brain tissues is converted to CO_2 and water, small amounts of oxygen forms ROS. The existences of PUFAs which are targets of the ROS in the brain and spinal cord, make this organs more sensitive to oxidative damage [2, 3]. There are various antioxidant mechanisms in the brain that neutralize the harmful effects of ROS; however, with depression, the loss of efficiency of antioxidants mechanisms and the alterations in the Na^+, K^+-ATPase system or dopamine levels result in increases in the free radical formation due to toxic effect of the morphine [4].

Fatty acids (FAs) are the major constituents of lipids in biological membranes as phospholipids, glycolipids and/or lipopolysaccharides, influencing membrane properties such as fluidity, integrity, permeability, and the activities of membrane-bound enzymes [7]. Fatty acids have also nutritional importance in mammalian diet in preventing many disorders [8]. There are direct link between FA composition of brain and oxidative stress [9]. However, changes in the environmental parameters, diet, age, and drug treatments may cause differences in the FA profiles of the tissues. Thus, it is expected the determination of FA compositions in tissues related with blood-brain barrier (BBB) may give significant information about the effects of spinal drugs administrated for anesthesia and pain management in humans. FAs changes in biological tissues can easily detected by an automated gas chromatography system with a computer interface and software [10] to be developed for identification of FA profiles of microbial and higher organisms.

Morphine as opioid analgesics is commonly used to produce analgesia following intrathecal administration in animals [11, 12]. However, neurotoxicity studies are required before this agent can be considered safe for clinical use. Intrathecally or epidurally administered opioids produce selective spinal analgesia [13]. Morphine has been used intrathecally and epidurally at the chronic and acute pain treatment for a long time [14]. It is well recognized that the use the morphine is associated with degeneration in the brain cells [4–6]. To our knowledge, so far there has been no attempt to study FAs changes and oxidative stress in tissues related to BBB in animals.

The objective of this study was to investigate oxidative damages of lipids as well as the indexes related antioxidative defence such as glutathione and PUFA changes based on FA profiles in spinal cord segments and brain tissues in rabbits.

Experimental procedure

Twelve adult male New Zealand albino rabbits were used in the study. At the start of the experiment the rabbits weighed 900–1200 g and three of months. These rabbits were kept in spacious cages, two rabbits per cage, with free access to food and water. They were randomly assigned to two groups of six rabbits each. Twelve New Zealand albino rabbits were used and they were randomly assigned to two groups of six rabbits each. First group used as control although morphine (Biosel, Istanbul, Turkey) as a single intra spinal dose of 6 mg kg^{-1} administrated to rats in second group. The animals were fasted a day before the study. Under local anesthesia, a femoral arter and vein were cannulated to measure arterial blood pressure monitoring, arterial blood gas samples, and a route for fluid administration. Percutaneous puncture of the intracisternal subarachnoid space through the atlantooccipital membrane was performed using a 22-G needle with the conscious animal in lateral position and the head flexed. The subarachnoid position of the needle was confirmed by aspiration of cerebrospinal fluid. Then 0.20 ml of 0.9% saline (pH 7) and 200 μg morphine (pH 5.5) were injected to the intracisternal subarachnoid space of animals in the groups of control and morphine, respectively. The needles were withdrawn immediately after injection. The latest dose was administrated 12 h before. Protocol of study was approved Ethical Committee of Ataturk University.

Observation and persecution of Animals

In all animals, arterial blood pressure and an ECG for heart rate (HR), arterial blood gases and respiratory rate were continuously monitored, and then noted at three different times; after vascular cannulation; 3 min after spinal injection; and in 60th min after spinal injection [12].

Animals were maintained in the erect position for 5 min. Ringer's lactate (Baxter) was infused intravenously when systolic blood pressure dropped to less than 90 mmHg. If respiratory depression occurred, the

lungs were manually ventilated via a face mask. Arterial and venous catheters were withdrawn after recovery from spinal injection.

Animal's scarification and specimen collection

The animals were euthanized by thiopental overdose on day 8. Craniectomy and laminectomy was performed within 30 min after death. The brain and spinal cord with dura was carefully and quickly removed. The hippocampus structure extracted from the brain, and then the spinal cord divided into the segments from three zones: midcervical, midthoracic, midlumbar segments. Tissues were excised, rinsed with ice-cold physiological saline solution, weighed and then immediately used for FA analysis extraction.

Extraction and analysis of FAMEs

A small pieces from each of the four spinal cord segments (cervical, thoracic, lumbar and brain) were excised and used for FA extraction and analysis as described in manufacturing manual of Sherlock (Sherlock 4.0 MIDI, Inc., Newark, DE) with the eukary database of FA methyl ester (FAME) profiles for eukaryotic cells [10].

As it was described previously [15], approximately 40 mg of tissue from each segment was collected, and added to 1 ml 1.2 M NaOH in 50% aqueous methanol with five glass beads (3 mm dia) in a screw cap tube, then incubated at 100°C for 30 min in a water bath. After the saponified samples were cooled at room temperature for 25 min, they were acidified and methylated by adding 2 ml 54% 6 N HCl in 46% aqueous methanol and incubated at 80°C for 10 min in a water bath. After rapid cooling, methylated FAs were extracted with 1.25 ml 50% methyl-tert butyl ether (MTBE) in hexane. Each sample was mixed for 10 min and the bottom phase was removed with a Pasteur pipette. The top phase was washed with 3 ml 0.3 M NaOH. After mixing for 5 min then the top phase was removed for analysis. Following the base wash step, the extract (FAMEs) is cleaned in anhydrous sodium sulfate and then transferred into a GC sample vial for analysis.

FAMEs were separated by gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA) with a fused-silica capillary column (25 m by 0.2 mm) with cross-linked 5% phenylmethyl silicone. The operating parameters for the study were set and controlled automatically by computer program. The chromatograms with peak retention times and areas were produced on the recording integrator and were electronically transferred to the computer for analysis, storage and report generation. Peak naming and column performance was achieved through the use of calibration standard mix, Microbial ID 1201-A.Cellular FAs were identified on the basis of equivalent chain length data. FAME profiles were identified by comparing the commercial eukary database with the Sherlock software package.

Lipid peroxidation (LP) determinations

Lipid peroxidation levels in brain (cortex) were measured with the thiobarbituric-acid reaction by the method of Placer et al. [16] as described in previous studies [7]. The quantification of thiobarbituric acid reactive substances was determined by comparing the absorption to the standard curve of malondialdehyde equivalents generated by acid catalyzed hydrolysis of 1,1,3,3 tetramethoxypropane. The values of LP were expressed as μmol g^{-1} protein. In the current study we used the thiobarbituric-acid reaction method for determination of LP. Although the method is not specific for LP, measurement of thiobarbituric-acid reaction is an easy and reliable method, which is used as an indicator of LP and ROS activity in biological samples.

Reduced glutathione (GSH) and protein assay

The GSH content of the cortex brain homogenate was measured at 412 nm using the method of Sedlak and Lindsay [17] as described own studies [3]. The samples were precipitated with 50% trichloracetic acid and then centrifuged at 1000 g for 5 min. The reaction mixture contained 0.5 ml of supernatant, 2.0 ml of Tris–EDTA buffer (0.2 M; pH 8.9) and 0.1 ml of 0.01 M 5,5′-dithio-bis-2-nitrobenzoic acid. The solution was kept at room temperature for 5 min, and then read at 412 nm on the spectrophotometer. The protein content in the brain and spinal cord was measured by method of Lowry et al. [18] with bovine serum albumin as the standard.

Statistical analysis

The collected values from groups were reported as mean ± SE. Statistical analysis was performed using Statistica software release 6.0. The variance analysis (ANOVA) and LSD test were used for comparison between groups. Statistical significance at $p \leq 0.05$ was considered significant.

Results

Fatty acids results

The results of the present study showed no sensory or motor blockade with 0.9% saline-injected animals (control). None of the animals receiving morphine induced spinal anesthesia with respiratory depression. Therefore, no hypoxemia or hypercapnia has been observed in our study as reported by some other studies [14]. Between intrathecal injection and death, none of the animals presented obvious neurologic impairment or behavioral disturbances. There were no significant differences between treatment groups based on the data of hemodynamic changes, arterial blood gases, and HR.

The results of FAME analysis summarized in Tables 1, 2 and they showed that there were no significant differences between three spinal segments (thoracal, lumbar and cervical) of controls in terms of FAs concentration.

A unique FA profiles, composed of 21 different FAs in thoracic and lumbar, and 18 FAs in cervical segment, were observed in spinal cord segments and brain tissues of control animals. Fatty acid with highest concentration was 18:1 ω9c (26.7%) The following FAs and their average concentrations were 18:0 (15%), 16:0 (13.8%), Sum in future 8 (6.4%) and 18:1 ω7c DMA (6.9%). The remaining FAs have the concentration less than 5% (Table 1). On the other hand, a total ten different FAs were found in the brain tissues of control animals. The profile of the FAMEs in brain tissues was composed of nine different FAs that were 16:0 (27%) at highest concentration, followed by 20:4 ω6c (17%), 18:0 (14%), 18:1 ω9c (16.3%), 22:6 ω3c (8.9%), 22:4 ω6c, Sum in future 4 and 8 (<5%) (Table 2). This is the crucial study of determination of FA profiles in spinal cord segments and brain tissues in rabbit. In the case of treated animals with morphine, FA profiles in those tissues were similar (not identical) to each other, but quite different than those of control. There were both

Table 1 Fatty acids methyl ester (FAME) contents of spinal cord segments in control and morphine administrated rabbits (n = 6, mean ± SE)

Fatty acids*	FAME percentage in three different segments of spinal cord tissues					
	Cervical		Thoracic		Lumbar	
	Control	Morphine	Control	Morphine	Control	Morphine
14:0	ND	1.1 ± 0.3	ND	0.9 ± 0.05	ND	0.9 ± 0.1
14:0 aldehyde	ND	0.9 ± 0.1	ND	1.6 ± 0.12	ND	1.8 ± 0.2
16:0	13.8 ± 0.5	13.9 ± 0.3	14.0 ± 0.9	14.0 ± 1.0	11.2 ± 1.0	11.2 ± 0.9
16:1 ω9c	ND	ND	0.4 ± 0.09	ND	0.3 ± 0.5	ND
17:1 ω8c	1.3 ± 0.2	1.4 ± 0.5	1.4 ± 0.3	1.4 ± 0.3	1.2 ± 0.1	1.3 ± 0.5
17:1 ω11c	0.9 ± 0.05	ND	0.8 ± 0.01	ND	0.4 ± 0.2	ND
Sum in future 4	3.1 ± 0.3	5.9 ± 0.9[a]	3.5 ± 0.2	4.7 ± 0.3[a]	4.3 ± 0.1	5.8 ± 0.1[a]
18:2 ω6c	2.1 ± 0.5	1.0 ± 0.1[a]	2.2 ± 0.2	1.1 ± 0.8[a]	2.8 ± 0.2	1.3 ± 0.8[a]
18:1 ω9c	26.7 ± 0.7	24.0 ± 0.2[a]	26.5 ± 0.5	23.0 ± 0.7[a]	26.1 ± 1.1	22.3 ± 0.5[a]
Sum in future 8	6.4 ± 0.4	6.3 ± 0.2	8.0 ± 0.09	8.0 ± 0.3[a]	6.8 ± 0.9	6.8 ± 0.3
18:0	15.4 ± 0.3	16.5 ± 0.2[a]	14.1 ± 1.0	15.1 ± 0.4[a]	14.4 ± 0.6	15.7 ± 0.6[a]
18:1 ω7c DMA	6.9 ± 0.7	6.8 ± 0.2	6.7 ± 0.03	6.8 ± 0.4	6.6 ± 0.6	6.6 ± 0.9
20:4 ω6c	3.8 ± 0.3	3.2 ± 0.1[a]	2.7 ± 0.03	2.4 ± 0.1[a]	3.5 ± 0.7	2.5 ± 0.3[a]
20:3 ω6c	ND	ND	1.1 ± 0.02	0.9 ± 0.3[a]	2.0 ± 0.1	0.8 ± 0.5[a]
20:2 ω6c	ND	ND	1.1 ± 0.05	0.8 ± 0.3[a]	2.2 ± 0.2	1.0 ± 0.1
20:1 ω9c	5.8 ± 0.8	4.5 ± 0.5[a]	5.5 ± 0.02	4.6 ± 0.9[a]	5.7 ± 0.4	4.6 ± 1.0[a]
Sum in future 14	2.9 ± 0.9	2.9 ± 0.2	3.1 ± 0.08	3.0 ± 0.8	3.6 ± 0.1	3.7 ± 0.1
20:0	1.9 ± 0.1	2.9 ± 0.3[a]	1.3 ± 0.06	2.3 ± 0.3[a]	1.3 ± 0.1	2.8 ± 0.2[a]
22:6 ω3c	2.9 ± 0.4	1.5 ± 0.3[a]	1.5 ± 0.3	1.2 ± 0.05[a]	2.7 ± 0.1	1.7 ± 0.1[a]
22:4 ω6c	2.1 ± 0.6	1.3 ± 0.1[a]	2.2 ± 0.07	1.1 ± 0.05[a]	2.1 ± 0.2	1.4 ± 0.1[a]
22:1 w9c	0.9 ± 0.07	ND	0.6 ± 0.08	ND	0.6 ± 0.03	ND
24:1 ω9c nervonic	1.6 ± 0.2	1.1 ± 0.4[a]	2.1 ± 0.03	1.8 ± 0.08[a]	1.9 ± 0.1	1.8 ± 0.2[a]
24:0	1.5 ± 0.2	2.8 ± 0.3[a]	1.2 ± 0.02	2.2 ± 0.03[a]	1.3 ± 0.2	2.3 ± 0.2[a]
Unknown 16.975	ND	1.2 ± 0.1	ND	0.5 ± 0.05	ND	0.8 ± 0.2
22:1 ω7c/22:3 ω3c	ND	ND	ND	0.9 ± 0.17	ND	0.4 ± 0.01
24:5 ω 3)	ND	ND	ND	0.8 ± 0.01	ND	1.3 ± 0.01
19:1 (ω8) alcohol	ND	0.8 ± 0.01	ND	0.9 ± 0.03	ND	1.2 ± 0.02

[a] Statistically important at $p \leq 0.05$

* Name of FAs = Sum in future (imperfect peak mach) 4:17:1 ISO I/ANTEI B (10-Hexadecenoic acid, 15-methyl/3-Hexadecenoic-acid, 14-methyl); Sum in future 8:18:1 ω9t; Sum in future 14:20:1 ω9t/20:1 ω7c; unknowns: FAs are not identified; ND: not detected

Table 2 Fatty acids methyl ester (FAME) contents of brain in control and morphine administrated rabbits (n = 6, mean ± SE)

Fatty acids*	FAME percentage in brain tissue	
	Control	Morphine
16:0	27.0 ± 1.2	24.5 ± 0.3[a]
Sum in future 4	2.4 ± 0.3	6.7 ± 0.1[a]
18:1 ω9c	16.3 ± 0.2	14.7 ± 0.3[a]
Sum in future 8	4.4 ± 0.2	7.6 ± 0.2[a]
18:0	14.0 ± 0.9	20.7 ± 0.9[a]
18:2 ω6c	1.8 ± 0.17	ND
18:1 ω7c DMA	1.5 ± 0.1	ND
20:4 ω6c	17.0 ± 1.2	14.8 ± 0.7[a]
22:6 ω3c	12.5 ± 0.9	9.5 ± 0.7[a]
22:4 ω6c	3.1 ± 0.3	1.5 ± 0.1

[a] Statistically important at $p \leq 0.05$

* Name of FAs = Sum in future (imperfect peak mach) 4:17:1 ISO I/ANTEI B (10-Hexadecenoic acid. 15-methyl/3-Hexadecenoicacid. 14-methyl); Sum in future 8:18:1 ω9t; unknowns: FAs are not identified; ND: not detected

quantitative and quantitative differences in FA profiles of drug treated animals compared to control (Table 1). Approximately, 24 different FAs were detected in the profiles of drug treated animals. The relative percentage of the FA methyl ester (FAME) 17:1 ω8c, 16:0, Sum in future 8 and 14, 18:1 ω7c DMA did not change with morphine treatments. However, there was an increase in the concentration of the FA Sum in future 4, 18:0, 20:0, 24:0 in contrast to a decrease in the percentages of the following FAMEs; 18:2 ω6c, 18:1 ω9c, 20:4 ω6c, 20:3 ω6c, 20:2 ω 6c, 20:1 ω9c, 22:6 ω3c, 22:4 ω6c, 24:1 ω9c nervonic,. On the other hand, the existence of FAMEs 16:1 ω9c, 17:1 ω11c, 22:1 ω9c, were only observed in the FA profiles of all tissues obtained from control rabbits. The FAMEs profiles in the brain tissue of rabbits treated with morphine were different than those of control and other spinal segments with or without treatment (Table 2). Totally nine different FAs were observed in the brain profiles of morphine treated animals as compared 10 FAs in the control. 18:2 ω6c, 18:1 ω7c DMA were only observed in the brain tissues of control animals.

Lipid peroxidation (LP) and GSH results

The mean LP values in the two groups of brain are shown in Fig. 1. The mean values in control and morphine administrated group were found 650.02 and 856.15 as μmol g^{-1} protein, respectively. The results showed that the levels of LP level of brain in morphine administrated group was significantly ($p < 0.001$) higher than in the control group.

The mean GSH values in the three groups are shown in Fig. 2. The mean values in the morphine administrated control groups were 38.17 and 19.92 as μmol g^{-1} protein, respectively. GSH level in the brain was decreased in the morphine administrated group and its value was significantly ($p < 0.001$) lower in morphine administrated group than in control group.

Discussion

Oxidative stress in lipid metabolism is involved in a variety of biological phenomena, such as carcinogenesis, diabetes and neurodegenerative disease. Detailed mechanisms of involvement of ROS in the pathology of disease are now available. In this sense, monitoring oxidative stress is quite important in the current morphine study. In the present study, we found that increases of oxidative damage of FAs as well as decreases of GSH as antioxidant because by the end of the injection period, the amounts of almost all FAs in the brain had decreased in the administered group as compared with those of the control group. In addition, the numbers of decreased several FAs in the spinal segments were higher than in the brain due to side effects of morphine.

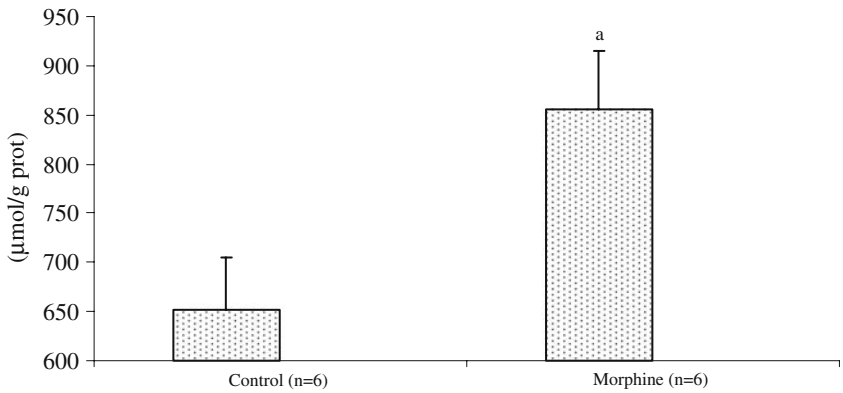

Fig. 1 The effects of morphine on lipid peroxidation levels in the rat cortex brain. (mean ± SD). [a] $p < 0.05$ vs control according to the ANOVA test

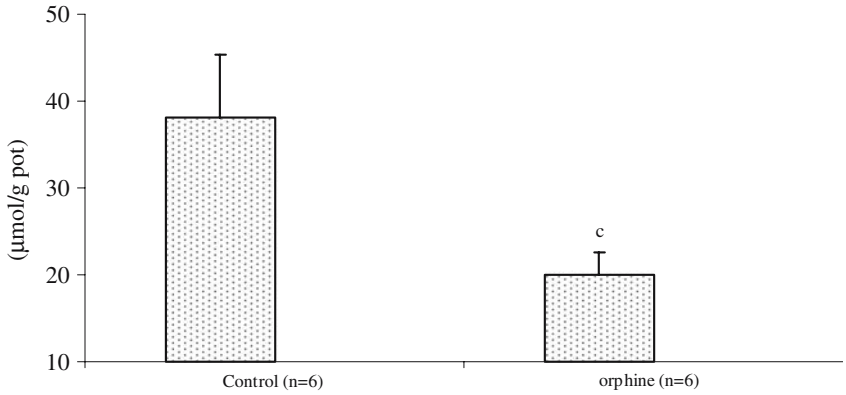

Fig. 2 The effects of morphine on glutathione levels in the rat cortex brain. (mean ± SD). [c] $p < 0.001$ vs control according to the ANOVA test

In the present study, we found that LP levels in the brain were higher in morphine administrated animals than in the control. Some data in different tissues reported by other researchers supported our results. Although there are no direct evidence for production of ROS in morphine administration, morphine has been shown to elevate synaptic dopamine [19], which can be oxidized by monoamino oxidase- B in man, giving rise to dihydroxyphenylacetic acid and hydrogen peroxide [20]. The hydrogen peroxide can interact with transition metal ions and produce the high toxic hydroxyl radical via the Fenton reaction. Morphine is able to induce oxidative cell injury in neuronal cells and neurodegeneration [5, 19]. Further, a metabolite of morphine directly affects the formation of superoxide radicals in glomerular mesangial cells [21]. Another research showed that single morphine administration increased dopamine and xanthine oxidative metabolism with a consequent increase in ROS production [6]. In addition, morphine could also be metabolized into free radicals [22] and the over production of ROS could lead to oxidative damage.

GSH levels of the current study in the brain were lower in morphine administered animals than in the control. The decrease in this compound in the brain suggest that morphine un protected the brain from oxidative stress. These results may be confirmed by Goudas et al. [23], whom observed acute decreases of GSH levels in cerebrospinal fluid samples taken from patients after intracerobroventricular doses of morphine for intractable cancer pain. Such doses may by depleting antioxidant GSH, render the central nervous system vulnerable to damage from oxidative stress.

In a previous study it has been demonstrated that neural membranes are composed of phospholipids, glycolipids, cholesterol, and proteins although he phospholipids include glycerophospholipids and sphingomyelin [24]. The glycerophospholipids of neural membranes are containing a large number of long chain PUFAs [9]. The loss and decrease of unsaturation in the lipid bilayer as a result of lipid peroxidation has been reported to decrease membrane fluidity [25]. Decreased membrane fluidity lead to structural and functional alteration in membrane-related events and play a significant role in the neurotoxicity [26].

In the present study, amount of ten PUFAs (16:1 ω9c, 18:2 ω6c, 18:1 ω9c, 20:4 ω6c, 20:3 ω6c, 20:2 ω6c, 20:1 ω9c, 22:6 ω3c, 22:4 ω6c and 24:1 ω9c nervonic) in spinal cord segments, and amount of six of those (22:6 ω3c, 22:4 ω6c, 20:4 ω6c, 18:2 ω6c, 18:1 ω9c, and 18:1 ω7c DMA) in the brain were either completely lost or their amount was significantly decreased after drug administration. The concentrations of saturated FAs (18:0, 20:0 and 24:0) were also found to be decreased significantly in spinal cord segments.

The brain as well as spinal segments uses massive amounts of oxygen, generating large quantities of ROS. Because of its lipid composition, rich in unsaturated FAs, the brain is especially vulnerable to ROS. Furthermore, oxidative damage in the brain is often associated with morphine, which has pro-oxidative properties [4]. Morphine-mediated oxidative damage in the brain is compounded by the fact that brain ROS distribution is non-uniform, being particularly high unsaturated FA contents in areas sensitive to neurodegeneration [5, 6]. ROS cause decrease in FAs of tissues and Yilmaz et al. [8] and Celik et al. [9] reported that the level of saturated FAs were decreased in liver, muscle and brain of rats and lambs, but the levels of unsaturated FAs were increased intraperitoneal or dietary vitamin E and selenium administration. In a recent study [27], we observed similar results that concentrations of saturated FAs increased in the spinal cord and brain of spinal drug Tramadol injected rabbit. Decrease in the FAs of brain and spinal segment may be caused due to increase in

production of ROS. Hence our results were confirmed by the results of Yilmaz et al. [8], Celik et al. [9] and Alici et al. [27].

In the current study, there were no significant differences on the hemodynamic changes, arterial blood gases, and HR between treatment and control groups. The results were similar to previous reports [13, 14].

In conclusion, the level of most FAs in spinal segments and brain tissues and GSH levels in brain were reduced by administrating morphine although lipid peroxidation increased. Our results indicated that there was seriously oxidative stress and FA damage in the tissues. Therefore, strategies of blocking oxidative stress may be useful in the development of therapy of morphine degeneration as well as morphine abuse.

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