Effect of the Spinal Drug Tramadol on the Fatty Acid Compositions of Rabbit Spinal Cord and Brain

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Tramadol is an important spinal drug which produces analgesia following intrathecal injection. It is well known that fatty acids (FAs) play an important role in membrane fluidity of the blood-brain barrier (BBB) tissue, which blocks and/or controls the transportation of toxic substances into the brain. The aim of this study was to investigate the effect of a spinal drug (tramadol) on the concentrations and compositions of fatty acid in BBB tissues of New Zealand male rabbits. The total cellular fatty acid profiles of the tissues in three spinal cord sections (cervical, thoracic, and lumbar) and in the brain of rabbits with or without drug administration were determined by gas chromatography using Sherlock Microbial Identification System (MIS) software (Microbial ID, Newark, DE, U.S.A.) with a database of FAME profiles for eukary. The relative percentage of the fatty acid methyl ester (FAME), 24:1 αc nervonic and 17:1 αc, did not change with tramadol treatments. However, there was an increase in the concentration of the FA 16:0, 18:1 ω7c DMA, 18:1 ω9c, sum in future 4, sum in future 8, sum in future 9, 18:0, 20:0: 4 ω6c, sum in future 14, 22:0 4 ω6c, in contrast to a decrease in the percentages of the following FAMEs; 20:0, 20:1 9c7c. In the brain, there was an increase in the concentration of the FA 18: 1 ω9c, sum in future 8 and 18: 0, in contrast to a decrease in the percentages of two FAMEs, 16:0, 20:0: 4 ω6c and 22:6 6 ω3c. The number of fatty acids were 20 in the spinal cord sections and 8 in the brain tissues of control animals compared to 15—18 fatty acids in the spinal cord section and 7 in the brain tissues of drug administered animals. The overall changes in the concentrations and numbers of FAs suggest that the spinal drug tested in this study has a side effect of disrupting of membrane fluidity of the BBB, which may cause neurotoxicity.

Key wordstramadol; blood-brain barrier (BBB); rabbit; fatty acid methyl ester (FAME); microbial identification system (MIS)

Spinal drugs used in anesthesia and pain management have led to intense interest in understanding the neuropharmacology of spinal cord processing of noxious input. Specific pain pathways can be potentially impeded by the direct application of receptor-specific therapeutics at the spinal cord, which limit systemic side effects, but this practice also carries the inherent risk of injury to the central nervous system or behavioral/clinical derangement. Physiologic neurotoxicity of spinal drugs can be detected based on changes in spinal cord blood flow, disruption of the blood-brain barrier (BBB), and changes in the electrophysiology of impulse conduction. Thus, the neurotoxicity of spinal drugs has become a central safety issue. After exposure to a spinal drug, spinal cord or nerve root toxicity may manifest itself as histologic and/or physiologic. Therefore, effects of spinal drugs need to be tested in terms of histological, physiologic and behavioral changes in several animal species that will be followed by safety trials in humans before widespread use.

Tramadol is an atypical, centrally acting analgesic agent, effective in the treatment of moderate to moderately severe pain with a relatively low addiction potential. A striking feature of the pharmacology of tramadol is its relatively weak opioid receptor affinity in comparison with its antinociceptive efficacy, indicative of the involvement of another mechanism. Interestingly, its antinociceptive action in some animal models is only partially antagonized by naloxone, strongly suggesting a non-opioid component to its analgesic potency. Tramadol has been administered spinally, although this route is unsupported by toxicological data and it has not been registered to be used as analgesic.

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. Fatty acids also have nutritional importance in the mammalian diet in preventing many disorders. It is well documented that the concentration and composition (profiles) of fatty acids in certain tissues from closely related organisms are similar and remain constant as long as they live or grow under standard conditions. However, changes in environmental parameters, diet, age and drug treatments may cause differences in the fatty acid profiles of the tissues. Thus, it is expected that the determination of FA composition in BBB tissues may give significant information about the effects of spinal drugs administered for anesthesia and pain management in humans. FA’s changes in biological tissues can easily be detected by an automated gas chromatography system with a computer interface and software, originally developed for microbial identification, but recently used for the characterization of FA profiles of higher organisms, too.

The aim of this study was to investigate the side effects of a spinal drug (Tramadol) based on FA profiles in BBB tissues of New Zealand male rabbits, in addition to physiological parameters including hemodynamic changes, heart rate, respiratory rate and arterial blood gas.

MATERIALS AND METHODS

Preparation of Animals and Drug Injection After obtaining approval from the Medical Faculty Ethics Committee, a total of 12 New Zealand albino male rabbits, weighing 0.9—1.2 kg, were used in this study. 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 6: group C (control) and group T (tramadol; Grunenthal,
Germany. The animals were fasted the day before the study. Under local anesthesia, a femoral artery and vein were cannulated to provide 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 a lateral position and the head flexed. The subarachnoid position of the needle was confirmed by aspiration of cerebrospinal fluid (CSF). Then, 0.20 ml of 0.9% saline (pH 7) and 200 μg tramadol (pH 6.5) were injected into the intracisternal subarachnoid space of the animals in the control and tramadol groups, respectively. The needles were withdrawn immediately after injection.

**Observation and Perfusion of Animals** In all animals, arterial blood pressure and an ECG for heart rate (HR), arterial blood gases and respiratory rate (RR) were continuously monitored, then noted at three different times: after vascular cannula; 3 min after spinal injection; and 1 h after spinal injection, as described by Malinovsky et al.

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 the spinal injection.

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

**Analysis and Extraction of FAMEs** Small pieces from each of the brain and three spinal cord segments (cervical, thoracic and lumbar) were excised and used for fatty acid extraction and analysis, as described in the manufacturing manual of the Microbial Identification System (Sherlock Microbial Identification system version 4.0) with the eukary data-base of FAME profiles for eukaryotic cells (Sherlock Microbial Identification System, version 4.0).

In brief, approximately 40 mg of tissue from each segment was collected, then added to 1 ml 1.2 M NaOH in 50% aqueous methanol with 5 glass beads (3 mm dia) in a screw cap tube, and finally 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% 6N HCl in 46% aqueous methanol, followed by incubation at 80 °C for 10 min in a water bath. After rapid cooling, methylated fatty acids 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, the top phase was removed for analysis. Following the base wash step, the extract (FAMEs) was 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, U.S.A.) with a fused-silica capillary column (25 m by 0.2 mm) with cross-linked 5% phenylmethyl silicone (HP 19091B-102). The operating parameters for the study were set and controlled automatically by a 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 a calibration standard mix, Microbial ID 1201-A. Cellular fatty acids were identified on the basis of equivalent chain length data. FAME profiles were identified by comparing the commercial eukary database with the MIS software package.

**Statistical Analysis** All statistical analysis was performed using Statistica software release 6.0. Analysis of variance was performed and means were separated using Fisher’s protected least significant differences (LSD) test at p≤0.05.

**RESULTS AND DISCUSSION**

Our study showed no sensory or motor blockade with 0.9% saline serum-injected animals (control). None of the animals receiving tramadol induced spinal anesthesia with respiratory depression. In none of animals were hypoxemia or hypercapnia observed in our study. 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, or heart rate (data not shown). Therefore, the results of this study showed that the spinal drug tested does not have any neurotoxic effects in animals on the basis of physiologic parameters. Similar findings in the literature confirmed our data.

The results of FAME analysis summarized in Table 1 showed that there were no significant differences between spinal segments of the controls in terms of fatty acid concentration. Unique fatty acid profiles, composed of 20 different FAs in the thoracic, lumbar and cervical segments, were observed in the BBB tissues of control animals. The fatty acid with the highest concentration was 18:1ω9c (30%) The following FAs and their average concentrations were approximately 18:0 (17%), 16:0 (16%), Sum in future 8 (7%) and 20:1ω9c (7%). Also, FAs and their concentrations in the brain were 16:0 (28%), 18:0 (27%), 18:1ω9c (19%), 20:4ω6c (9.9%), 22:6ω3c (6.1%) (Table 2). All of the remaining FAs have a concentration of less than 5% (Tables 1, 2). This is the first study to determine the fatty acid profiles of BBB tissue in rabbit. In the case of animals treated with tramadol, FA profiles of spinal cord segments in BBB tissues were similar (not identical) to each other, but quite different than those of the control. There were quantitative differences in the FA profiles of treated animals compared to the controls (Tables 1, 2). 2—7 different FAs disappeared in the cervical, thoracic and lumbar profiles of drug treated animals, while only one FA (22:4 ω6c) disappeared in the brain. The relative percentage of FAMEs 24:1ω9c nervonic and 17:1ω8c did not change with tramadol treatments. However, there was an increase in the concentration of the FA 16:0, 18:1ω7c DMA, 18:1ω9c, sum in future 4, sum in future 8, sum in future 9, 18:0, 20:4ω6c, sum in future 14, 22:4ω6c, in
contrast to a decrease in the percentages of the following FAMEs: 20:0, 20:1 \( \omega 9c \). In the brain, there was an increase in the concentration of the FA 18:1 \( \omega 9c \), sum in future 8 and 18:0 in contrast to a decrease in the percentages of following FAMEs, 16:0, 20:4 \( \omega 6c \) and 22:6 \( \omega 3c \). The numbers of fatty acids were 20 and 8 in the spinal cord sections and the brain tissues of control animals compared to 15—18 and 7 fatty acids in the spinal cord section and the brain tissues of drug administered animals, respectively.

Previous studies have demonstrated that neural membranes are composed of phospholipids, glycolipids, cholesterol and proteins. The phospholipids include glycerophospholipids and sphingomyelin.\(^5\) The glycerophospholipids of neural membranes contain a large number of long chain polyunsaturated fatty acids.\(^9\) The loss of unsaturation in the lipid bilayer as a result of lipid peroxidation has been reported to decrease membrane fluidity.\(^10,11\) Decreased membrane fluidity leads to structural and functional alterations in membrane-related events and plays a significant role in neurotoxicity.\(^12\)

Our results suggested that at least four polyunsaturated FAs (18:2 \( \omega 6c \), 20:3 \( \omega 6c \), 22:6 \( \omega 3c \), 22:1 \( \omega 9c \)) in spinal cord segments, and three of those (20:4 \( \omega 6c \), 22:6 \( \omega 3c \), 22:4 \( \omega 6c \)) in the brain were either completely lost or their amount was significantly decreased after drug administration. Therefore, it is possible to speculate that the spinal drug (tramadol) tested in the present study decreased membrane fluidity of the BBB which may cause neurotoxicity due to the loss of unsaturation and serious changes in the concentrations and numbers of FAs. In addition, the data imply that the physiological parameters may not provide sufficient evidence for proving the neurotoxic effects of spinal drugs. Thus, it is necessary to have molecular data, like FAME analysis, in order to demonstrate neurotoxic and/or additional side effects of the drugs commonly used for human disease treatments. This is the first attempt to study the relationship between fatty acid content and neurotoxicity in animals due to drug administration using MIS.

REFERENCES

5) Sherlock Microbial Identification System (version 4.0), MIS Operating Manual, 145 pp., MIDI, Inc., Newark, DE, U.S.A.


