

Effect of Caffeic Acid Phenethyl Ester on Malondialdehyde Levels in Spinal Cord Injury in Rats

Kafeik Asit Fenetil Esterin Ratlarda Spinal Kord Yaralanmasında MDA Düzeylerine Etkisi

Havva Şahin Kavaklı¹, Cemile Koca², Özlem Alıcı³, Yunsur Çevik¹

¹ Department of Emergency, Ankara Atatürk Training and Research Hospital, Ankara, Turkey

² Department of Biochemistry, Fatih University, Faculty of Medicine, Ankara, Turkey

³ Department of Infectious Diseases and Clinical Bacteriology, Medical Park Hospital, Istanbul, Turkey

Abstract

Objective: The aim of this study was to investigate the effects of caffeic acid phenethyl ester on prevention of secondary damage in spinal cord injury through determination of malondialdehyde levels in rats.

Materials and Methods: Thirty adult Wistar albino rats were randomized into three groups. Spinal cord injury was performed by the weight-drop model. Group 1 (control) underwent laminectomy followed by spinal cord injury and received no medication. Group 2 underwent laminectomy followed by spinal cord injury and received caffeic acid phenethyl ester (10 micromol/kg). Group 3 underwent laminectomy followed by spinal cord injury and received methylprednisolone (30 mg/kg) intraperitoneally. Twenty four hours later, blood samples were obtained, then serum malondialdehyde levels were determined and results were compared.

Results: Malondialdehyde levels in the control group was higher than the caffeic acid phenethyl ester and methylprednisolone groups (respectively Group 1 and 2 $p < 0.003$ Group 1 and 3 $p < 0.001$). Furthermore, malondialdehyde levels in the methylprednisolone group were lower than the caffeic acid phenethyl ester group (Grup 2 ve 3 $p < 0.036$).

Conclusion: In our study, caffeic acid phenethyl ester decreased MDA levels compared to the control group, however the lowest MDA levels were determined in the methylprednisolone group. Based on these results, , caffeic acid phenethyl ester may be another neuroprotective agent in spinal cord injury in addition to methylprednisolone. (*JAEM 2010; 9: 121-3*)

Key words: Caffeic acid phenethyl ester, spinal cord injury, malondialdehyde

Received: 23.01.2010

Accepted: 24.02.2010

Özet

Amacı: Bu çalışmanın amacı ratlarda kafeik asit fenetil esterinin serum malondialdehit düzeylerini belirleme yoluyla spinal kord yaralanmasında sekonder hasarı önleme üzerine etkilerini araştırmaktır.

Gereç ve Yöntemler: Otuz adet Wistar albino rat randomize olarak üç gruba ayrıldı. Ağırılık düşürme modeliyle spinal kord yaralanması oluşturuldu. Grup 1 (kontrol) laminektomi ve spinal kord yaralanması yapıldı, ancak tedavi verilmedi. Grup 2 laminektomi ve spinal kord yaralanması yapıldı, kafeik asit fenetil ester (10 micromol/kg) verildi. Grup 3 laminektomi ve spinal kord yaralanması yapıldı, methylprednisolone (30 mg/kg) verildi. Yirmi dört saat sonra ratlardan kan örnekleri alınarak serum malondialdehit düzeyleri belirlendi ve sonuçlar karşılaştırıldı.

Bulgular: kontrol grubunda malondialdehit düzeyleri kafeik asit fenetil ester ve metilprednizolon grubundan daha yüksekti (sırasıyla Grup 1 ve 2 $p < 0.036$).

Sonuç: Bizim çalışmamızda kafeik asit fenetil ester, kontrol grubuyla karşılaştırıldığında MDA düzeylerini azalttı, ancak en düşük MDA düzeyleri metilprednizolon grubunda belirlendi. Bu bulgulara dayanarak, metilprednizolona ilaveten kafeik asit fenetil esterinin spinal kord yaralanmasında diğer bir nöroprotektif ajan olabilir. (*JAEM 2010; 9: 121-3*)

Anahtar kelimeler: Kafeik asit fenetil ester, spinal kord yaralanması, malondialdehit

Alındığı Tarih: 23.01.2010

Kabul Tarihi: 24.02.2010

Introduction

The pathophysiology of acute spinal cord injury (SCI) involves a complex cascade of secondary neurodegenerative events, including oxidative stress, which are initiated by the primary injury (1, 2). Secondary injury processes not only exacerbate the pathology at the primary injury site, but also result in the spreading of injuries to the otherwise healthy adjacent tissue (3). Lipid peroxidation is the main cause of the further secondary damage, which starts after mechani-

cal destruction of tissues (4, 5). A wide variety of end products are released after decomposition of peroxidized lipids (6). MDA is a well-known secondary product of lipid peroxidation in spinal myelin, glial and neural membranes (7). Caffeic acid phenethyl ester (CAPE), one of the major components of honeybee propolis, was recently found to be a potent free radical scavenger antioxidant and is used in folk medicine (8, 9). In this study, we aimed to investigate the effects of caffeic acid phenethyl ester on the prevention of secondary damage in spinal cord injury via determination of malondialdehyde levels in rats.

Methods

Thirty male Wistar albino rats (250-300 g) were used in the study. All the protocols were approved by the institutional animal ethics committee. The rats in each group were kept in separate cages in rooms with controlled light and temperature. They were fed on standard chow and water ad libidum. The animals were anesthetized by an intraperitoneal injection of 10 mg/kg xylazine (Rompun, Bayer Turk Kimya Sanayii Limited Sirketi, Istanbul, Turkey) and 50 mg/kg ketamine hydrochloride (Ketalar, Pfizer Ilaclari Limited Sirketi, Istanbul, Turkey). Rats were positioned on a thermistor-controlled heating pad in the prone position and a rectal probe was inserted. Surgical procedures were performed under sterile conditions with the assistance of a surgical microscope. Following a T5-12 midline skin incision and paravertebral muscle dissection, the spinous processes and laminae arcs of T7-10 were removed. The dura was left intact. Weight-drop model was performed for SCI (1). The animals were subjected to an impact of 50 g/cm to the dorsal surface of the spinal cord. The force was applied via a stainless steel rod (3-mm diameter tip, weighing 5g) which was rounded at the surface. The rod was dropped vertically through a 10-cm guide tube positioned perpendicular to the center of the spinal cord. Afterwards, the muscles and incision were sutured with 6-0 vicryl (Vicryl, Ethicon, Johnson& Johnson Intl, Lanneke Marelaan, Belgium).

The rats were randomized into three groups each having 10 rats. Group 1 underwent laminectomy followed by SCI and received no medication. Group 2 underwent laminectomy followed by spinal cord injury and received CAPE (10 micromol/kg) intraperitoneally. Group 3 underwent laminectomy followed by SCI and immediately followed by methylprednisolone (Prednol-L, Mustafa Nevzat Ilac Sanayi Anonim Sirketi, Istanbul, Turkey) intraperitoneally in a single dose of 30 mg/kg. Following the surgical procedure, the rats were placed in a warming chamber and their body temperatures were maintained at approximately 37 °C until they were completely awake. In the early postoperative period, the rats received 3 ml of saline intraperitoneally to compensate for the blood loss during the surgical procedure, while the water intake was limited.

Biochemical analysis

Twenty four hours later blood samples were obtained from all rats. The blood samples were immediately frozen and stored in a -20°C freezer for assays of MDA levels.

The levels of the end product of lipid peroxidation, serum MDA, were determined by thiobarbituric acid (TBA) test in which 1, 1, 3, 3-tetraethoxypropane was used as standard. The TBA test is based upon the principle of calorimetrically measured concentration of the pink product at 535 nm wavelength, which is formed as a result of the reaction of TBA with lipid peroxides (MDA), called TBA reactive substances. The results were expressed as nmol MDA/ml (10).

Statistical analysis

For statistical evaluation, we used the software package SPSS 15.0 and a probability value of less than 0.05 was accepted as statistically significant. Statistical analysis was performed using analysis of variance (ANOVA) followed by Tukey test when comparing groups. The results are given as the mean ± Standard deviation of the mean (SD).

Results

MDA levels in the control group were higher than the CAPE and methylprednisolone group (respectively Group 1 and 2 $p < 0.003$; Group 1 and 3 $p < 0.001$). On the other hand, MDA levels in the methylprednisolone group were lower than the CAPE group (Group 2 and 3 $p < 0.036$).

For all groups; MDA levels are given as mean±SD in Table 1. Differences of MDA levels in all groups are shown graphically in Figure 1.

Discussion

In our study, serum MDA levels were found to be significantly increased in the control group when compared to the CAPE and methylprednisolone groups.

Spinal cord injury (SCI) resulting from trauma mainly occurs by two mechanisms; primary and secondary injury (1, 2, 11). Secondary injury after the primary impact includes different pathophysiological and biochemical events, such as lipid peroxidation.

Oxidative stress can result from increased reactive oxygen species (ROS) production, and/or from decreased ROS scavenging capability. Prime targets of ROS attack are the polyunsaturated fatty acids (PUFA) in the membrane lipids causing lipid peroxidation, which may lead to disorganization of cell structure and function. Lipid peroxidation products increase immediately after SCI and the peak concen-

Table 1. Serum MDA levels (mean±SD)

	MDA (nmol MDA/ml)
Group 1 (control)	7.71±1.16
Group 2 (CAPE)	5.86±0.38
Group 3 (methylprednisolone)	4.60±0.58

P values:
Group 1 and 2 $p < 0.003$ Group 1 and 3 $p < 0.001$ Group 2 and 3 $p < 0.036$

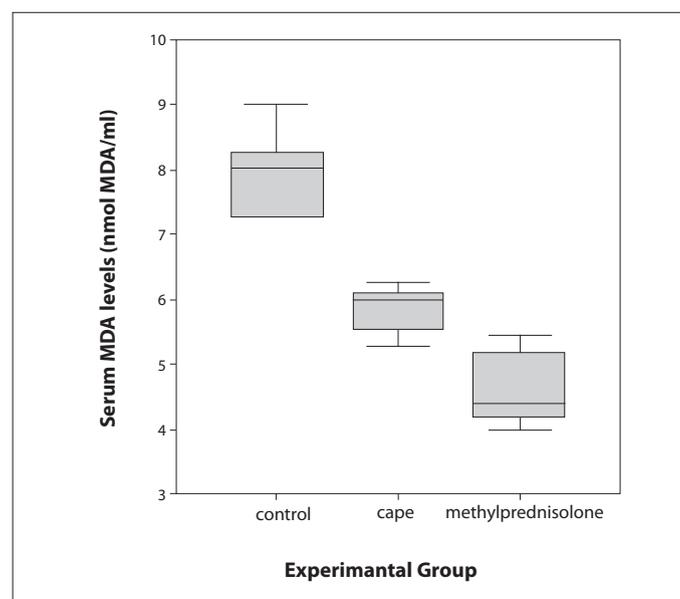


Figure 1. Differences of MDA in groups

trations of reactive oxygen and nitrogen species occur within the first 24 hours (1). In this study, we determined MDA concentration since it is the end product of lipid peroxidation. MDA is the breakdown product of the major chain reactions leading to the oxidation of PUFA, and thus serves as a reliable marker of oxidative stress-mediated lipid peroxidation (12). Although it is difficult to limit the primary injury, there is increasing evidence regarding the possibility of lowering the impact of the secondary injury by using pharmacological strategies (8, 13, 14). Antioxidant treatments decrease damage of SCI by reducing oxidative stress (1). At a concentration of 10 μ M, CAPE completely blocks production of reactive oxygen species (ROS) in human neutrophils and the xanthine/xanthine oxidase system. Previous studies have demonstrated that CAPE also exhibits antioxidant properties as well as anti-inflammatory, cytostatic, antiviral, antibacterial and antifungal properties (6, 9, 15). In one of these studies especially, CAPE ameliorates spinal cord injury by decreasing MDA levels, which are signs of oxidative stress (8). Various studies have clearly pointed out that methylprednisolone enhances functional recovery and induces regenerative responses after SCI in humans and experimental animals (16). Our study demonstrated that CAPE decreases MDA levels compared to the control group.

Our results suggest that, potentially CAPE, like methylprednisolone, may be able to reduce the secondary damage in spinal cord injury in rats.

Limitations

We think it is preferable to carry out a histopathological examination in addition to biochemical analysis in the blunt spinal cord injury model. Unfortunately, during the period of this study the technical facilities of the laboratory were not sufficient. We were therefore unable to perform a histopathological examination.

Conflict of Interest

No conflict of interest is declared by the authors.

References

1. Yucel N, Cayli SR, Ates O, Karadag N, Firat S, Turkoz Y. Evaluation of the neuroprotective effects of citicoline after experimental spinal cord injury: improved behavioral and neuroanatomical recovery. *Neurochem Res* 2006; 31: 767-75.
2. Hall ED, Springer JE. Neuroprotection and acute spinal cord injury: a reappraisal. *NeuroRx* 2004; 1: 80-100.
3. Hamann K, Durkes A, Ouyang H, Uchida K, Pond A, Shi R. Critical role of acrolein in secondary injury following ex vivo spinal cord trauma. *J Neurochem* 2008; 107: 712-21.
4. Michael-Titus AT. Omega-3 fatty acids and neurological injury. *Prostaglandins Leukot Essent Fatty Acids*. 2007; 77: 295-300.
5. Golding JD, Rigley MacDonald ST, Juurlink BH, Rosser BW. The effect of glutamine on locomotor performance and skeletal muscle myosins following spinal cord injury in rats. *J Appl Physiol* 2006; 101: 1045-52.
6. Ilhan A, Koltuksuz U, Uz E, Ciralik H, Akyol O. The effects of caffeic acid phenethyl ester (CAPE) on spinal cord ischemia/reperfusion injury in rabbits. *Eur J Cardiothorac Surg* 1999; 16: 458-63.
7. Rahman A, Ustundag B, Burma Ozercan IH, Erol FS. Neuroprotective effect of regional carnitine on spinal cord ischemia-reperfusion injury. *Eur J Cardiothorac Surg* 2001; 20: 65-70.
8. Bilen BT, Kilinc H, Alaybeyoglu N, Celik M, Iraz M, Sezgin N, Gültek A. Effect of caffeic acid phenethyl ester on survival of axial pattern flaps in rats with ischaemia-reperfusion injuries. *Scand J Plast Reconstr Surg Hand Surg* 2006; 40: 73-8.
9. Fidan H, Sahin O, Yavuz Y, Kilbas A, Cetinkaya Z, Ela Y, et al. Caffeic acid phenethyl ester reduces mortality and sepsis-induced lung injury in rats. *Crit Care Med* 2007; 35: 12: 2822-9.
10. Yoshioka, T., Kawada, K., Shimada, T, Mori M. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. *Am J Obstet Gynecol* 1979; 135: 372-6.
11. Guimaraes JS, Freire MA, Lima RR, Souza-Rodrigues RD, Costa AM, dos Santos CD, et al. Mechanisms of secondary degeneration in the central nervous system during acute neural disorders and white matter damage. *Rev Neurol* 2009; 48: 304-10.
12. Balci M, Namuslu M, Devrim E, Durak I. Effects of computer monitor-emitted radiation on oxidant/antioxidant balance in cornea and lens from rats. *Mol Vis* 2009; 15: 2521-5.
13. Hall ED. Pharmacological treatment of acute spinal cord injury: how do we build on past success? *J Spinal Cord Med* 2001; 24: 142-6.
14. Faden AI, Stoica B. Neuroprotection: challenges and opportunities. *Arch Neurol*. 2007; 64: 794-800.
15. Armutcu F, Gurel A, Hosnuter M, Pabucu O, Altinyazar C. Caffeic Acid Phenethyl Ester Improves Oxidative Erythrocyte Damage in a Rat Model of Thermal Injury. *J Burn Care Rehabil* 2004; 25: 171-8.
16. Xu WB, Lv G, Wang YF, Lu XH, Huang T, Zhu Y, et al. Combination of dexamethasone and aminoguanidine reduces secondary damage in compression spinal cord injury. *Cell Mol Neurobiol*. 2009; 29: 683-9.