CAFFEIC ACID PHENETHYL ESTER DECREASES THE LEVEL OF S-100B PROTEIN AFTER MIDDLE CEREBRAL AFTER OCCLUSION IN RABBITS

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ABSTRACT

Effects of caffeic acid phenethyl ester (CAPE) on the serum S-100B levels were studied as an index for brain damage after permanent middle cerebral artery (MCA) occlusion in rabbits. Twenty rabbits were divided into four groups (n=5): control, sham, non-treatment and CAPE. The right MCA was occluded using a microsurgical procedure with bipolar coagulation and was then transected in non-treatment and CAPE groups. The rabbits in the sham group underwent a surgical procedure but the MCA was not occluded. No surgery was performed in the control group. CAPE was administered after MCA occlusion at the dose of 10µg/kg, once a day intraperitoneally for 7 days in the CAPE group. Serum S-100B levels were determined on days 1, 2, 4 and 7. Serum S-100B level was significantly increased following permanent MCA occlusion. Posttreatment of CAPE significantly reduced the serum S-100B level. This study demonstrated that CAPE is capable of attenuating increased serum S-100B level induced by MCA occlusion in rabbits. CAPE may be useful as a neuroprotective agent.

Keywords: Brain, caffeic acid phenethyl ester, CAPE, experimental stroke, middle cerebral artery occlusion, MCA, S100B.

INTRODUCTION

As a result of brain injury, neurobiochemical markers such as S-100B are released into the cerebrospinal fluid (CSF). After passage via an impaired blood-brain barrier the molecules can be found in increased concentrations in the peripheral blood. Because of availability and detectability of these markers in CSF and serum, they have been used to determine the extent of dysfunction and damage in the central nervous system. S-100 protein is an acidic Ca-binding protein of 21 kD molecular weight. The protein consists of two subunits (α and β), and there are three possible variants (S-100 ββ = S-100B, S-100 αβ = S-100A and S100αα = S-100A0) (Donato, 1991; Shirasaki *et al*., 2004). S-100B is abundant in the nervous system where it is located essentially in astrocytes and Schwann cells (Buttner *et al*., 1997; Donato, 1991; Martens *et al*., 1998). Concentrations of S-100B in CSF and serum are highly correlated with the extent of neuronal injury after acute brain damage such as head trauma, cerebral hypoxia, cerebral bleeding and ischemic stroke (Buttner *et al*., 1997;. Lamers *et al*., 2003; Raabe *et al*., 1998). Previous studies have suggested that determination of this protein in CSF and serum can be helpful to determine severity of brain damage and to monitor treatment effects (Shirasaki *et al*., 2004; Lamers *et al*., 2003).

Caffeic acid phenetyl ester (CAPE) is an active component of honeybee propolis (Irmak *et al*., 2003). CAPE has antiinflammatory (Khayyal *et al*., 1993; Orban *et al*., 2000), immunomodulatory (Natarajan *et al*., 1996) and antioxidant effects (Pascual *et al*., 1994), and suppresses lipid peroxidation induced by brain injury (Irmak *et al*., 2003). Recently, it has also been found that CAPE has a neuroprotective potential (Tsai *et al*., 2006; Altuğ *et al*., 2008). However, effects of CAPE on serum S-100B levels in focal cerebral ischemia has not been investigated to date. Therefore, the present study was conducted to investigate the effects of CAPE on serum S-100B levels, as an index for brain damage, in rabbits after middle cerebral artery (MCA) occlusion.

MATERIAL AND METHODS

Experimental protocol and groups

Experiments were performed on New Zealand white male rabbits weighing 2.5-3 kg. Animal protocols were approved by the Ethics Commitee for Care and Use of Laboratory Animals of Mustafa Kemal University. Housing and handling of the animals were in strict accordance with the "Guide for the Care and Use of

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Laboratory Animals" (NIH Publication No.85-23, revised, New York, 1996).

Twenty animals were divided into four groups (n=5): control, sham, non-treatment and CAPE. The rabbits were housed in a room kept at 24 ˚C. The surgical procedures carried out under aseptic conditions. The animals were anesthetized intramuscularly with 1 mg/kg atropine sulphate (Atropan®, Vetas, Turkey), 5 mg/kg xylazine hydrochloride (Rompun®, Bayer, Turkey) and 50 mg/kg ketamine hydrochloride (Alfazyne®, Egevet, Turkey). The animals were restrained in the left lateral position with spontaneous respiration. Occlusion of the MCA was carried out with a technique first performed by Yamamoto *et al*. (1985). A skin incision was made at the dorsal orbital ridge, and then the eyeball was retracted ventrally. A craniectomy was carried out around the optic foramen using a dental drill and a surgical microscope (Olympos S261, Tokyo, Japan). The dura was incised and the MCA was carefully exposed at the level of the olfactory tract. The MCA was occluded with bipolar coagulation and was then transected to prevent recanalization, during which continuous saline irrigation was performed. The craniectomy defect was covered with absorbable gelatin sponge and the incision was closed using 5-0 nylon sutures. The rabbits in the sham group underwent a surgical procedure similar to the treatment and non-treatment group but the MCA was not occluded. No surgery was performed in the control group. CAPE (Sigma) was administered after MCA occlusion at the dose of 10μg/kg in 1 ml saline, once a day intraperitoneally for 7 days in the treatment group. The rabbits in control group received only 1 ml saline via intraperitoneal injection. The dosage of CAPE was selected (10 μmol/kg/day) on the basis of previous experimental reports, which have demonstrated its potent antioxidant properties at that dose (Irmak *et al*., 2003; Tsai *et al*., 2006; Serarslan *et al*., 2007).

On the 1st, 2nd, 4th and 7th days, 5 ml of blood were drawn from auricular veins of the animals into a test tube with no additive. Blood samples were centrifuged at 1500 g for 15 minutes, and serum was stored at -70ºC until biochemical analysis. The ELISA was used for the measurement of S-100B in serum. For the quantitative measurement of S-100B protein in plasma samples, a horseradish peroxidase (HRP) labelled antibody based, Biovendor sandwich enzyme immunoassay kit (Cat. No.RD 192090100, Czech Republic) were used. For assay of serum S-100B in ELISA, calibrators, controls and samples were incubated with polyclonal anti-cow S-100B antibody coated in microtitration wells. After 90 minutes of incubation and then washing, horseradish peroxidase (HRP) labelled antibody was added to the wells and incubated with captured S-100B. After 90 minutes incubation and another washing step, the remaining conjugate was allowed to react with substrate tetramethylbenzidine and H_2O_2. The reaction was stopped by addition of acidic solution and absorbance of the resulting yellow colour product was measured spectrophotometrically at 450 nm. A standart curve was constructed by plotting absorbance values versus S-100B concentrations of calibrators and concentrations of unknown samples were determined using the standart curve (Hauschild *et al*., 1999).

STATISTICAL ANALYSIS

All data were expressed as mean±standard error of the mean (SEM). Statistical analysis were accomplished with the use of SPSS computur programme (version 13.0). The results were compared between baseline and subsequent treatments within each group using a repeated measured analysis of variance, with Bonferroni correction for *post hoc* comparisons. The differences between groups at each time point were analyzed using a one-way ANOVA, with Bonferroni correction for multiple comparisons. *P* values less than 0.05 were considered significant.

RESULTS

Results of the present study indicate that CAPE administration significantly lowered the increased serum levels of S-100B induced by MCA occlusion in rabbit. The levels of S-100B in all groups are summarized in table 1. The S-100B levels were the highest in the non-treatment group. The level of S-100B in the CAPE group was statistically less than that in non-treatment group (table 1).

Table 1: Serum S-100B levels (pg/ml) at the different time points in rabbits

	1st day	2nd day	4th day	7th day
Non-treatment	1109±148,2	1244±163,02	1425±66,7	1377±178,5
Sham	1086±144,2	1154±170,4	1213±121,8	1177±62,2
CAPE	1047±150,7	1048±131,7	882±88,5**	792±96,4*
Control	712±61,8	708±58,2	697±67,1***, †	694±54,6**,†

Data were expressed as mean ± SEM.
*p<0.05 versus non-treatment group; ** p<0.005 versus non-treatment group; *** p<0.001 versus non-treatment group; † p<0.05 versus sham group.

DISCUSSION

In our experimental study we observed a statistically significant higher serum S-100B levels in non-treatment and sham groups compared to the CAPE and control groups on the 4th and 7th days (table 1). The study provided that CAPE treatment attenuates the increase in serum S-100B levels induced by MCA occlusion.

Shirasaki *et al*. (2004) used a rat embolic model produced by injection of microspheres to examine whether serum S100-B is a useful biochemical marker for ischemic brain injury. They reported that the decrease in serum S-100B levels is largely mediated by neuroprotection, suggesting that serum S-100B serves as a useful biomarker for the assesment of neuroprotectants. In the present study we used a rabbit ischemic model induced by permanent occlusion of the MCA. Consistently, we observed that serum S-100B levels were ameliorated by CAPE when administered after MCA occlusion at the dose of 10µg/kg.

Hardemark *et al*. (1989) observed that a peak concentration of S100 occured after about 2 to 4 days in the CSF, after which the values declined toward normal in rats with MCA occlusion. Our results were compatible with the finding of Hardemark *et al*. In the current study we observed that a distinct peak concentrations of S-100B occured on the 4th day in the non-treatment group. In contrast to the finding of Hardemark *et al*, the second peak level was not observed after MCA occlusion. A possible explanation for this difference could be that nerve cells may continue to succumb for at least several days after the production of ischemia, or secondary brain damage however seems not to be reflected in the serum. In the present study, the peak of S-100B levels was not seen on the 4th day in the CAPE group.

Irmak *et al*., (2003) showed that prophylactic administration of CAPE suppressed ischemia-reperfusion-induced cerebral lipid peroxidation and reperfusion injury in the brain. Tsai *et al*., (2006) demonstrated that CAPE pretreatment reduced infarct volume and increased plasma nitric oxide content in rats subjected to focal cerebral ischemia. The authors suggested that neuroprotective effect of CAPE may be due to the antioxidant activity in biological system. Altuğ *et al*., (2008) investigated the effect of CAPE on brain injury after focal permanent MCA occlusion. Their findings suggest that CAPE provides neuroprotection against cerebral ischemia injury due to attenuation of lipid peroxidation and its antioxidant activity. In recent years there are acumulating evidence that CAPE possesses neuroprotective properties in focal cerebral ischemia injury in rats possibly through its antioxidant effect and/or via the upregulation of nitric oxide production (Tsai *et al*., 2006; Altuğ *et al*., 2008; Bizzozero *et al*., 2007; Ma *et al*., 2006). Our results also demonstrated that posttreatment of CAPE at the dose of 10µg/kg significantly reduced serum S-100B levels in rabbits with MCA occlusion. In consistent with that report, the present study demonstrated that CAPE appears to have a neuroprotective effect against focal cerebral ischemia in postischemic period after MCA occlusion.

In conclusion, the report presents the first evidence that CAPE can decrease the level of serum S-100B following permanent MCA occlusion. Therefore, CAPE could be used as a neuroprotective agent.

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