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Research Report

# Caffeic acid phenethyl ester protects rabbit brains against permanent focal ischemia by antioxidant action: A biochemical and planimetric study

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ABSTRACT

The present study was conducted to investigate whether caffeic acid phenethyl ester (CAPE), an active component of propolis extract, has a protective effect on brain injury after focal permanent cerebral ischemia, and to determine the possible antioxidant mechanisms. Cerebral infarction in adult male New Zealand rabbits was induced by microsurgical procedures producing right focal permanent middle cerebral artery occlusion (pMCAO). CAPE was administered to the treatment group after pMCAO at a dose of 10 μmol $kg^{-1}$ once a day intraperitoneally for 7 days. Neurological deficits were evaluated, using a modified six-point scale. Spectrophotometric assay was used to determine the contents of malondialdehyde (MDA), glutathione (GSH), catalase (CAT), nitric oxide (NO) and xanthine oxidase (XO). In the ipsilateral hemisphere, the infarct volume of the brain was assessed in brain slices stained with heamatoxylen and eosin. The results showed that treatment with CAPE significantly reduced the percentage of infarction in the ipsilateral hemisphere compared with the ischemia group. CAPE treatment significantly attenuated the elevation of plasma MDA, CAT and XO content ($p<0.05$), whereas it significantly increased the levels of plasma GSH and NO ($p<0.05$). Therefore, subacute CAPE administration plays a protective role in focal pMCAO due to attenuation of lipid peroxidation and its antioxidant activity. All of these findings suggest that CAPE provides neuroprotection against cerebral ischemia injury through its antioxidant action.

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*Abbreviations:* ANOVA, one-way analysis of variance; CAPE, caffeic acid phenethyl ester; CAT, catalase; DTNB, dinitro2,2-dithiobenzoic acid; GSH, reduced glutathione; MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; MDA, malondialdehyde; NO, nitric oxide; pMCAO, permanent middle cerebral artery occlusion; ROS, reactive oxygen species; XO, xanthine oxidase

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## 1. Introduction

In focal or global cerebral ischemia, cerebral blood flow is reduced in brain regions that are supplied with oxygen by the occluded vessels (Irmak et al., 2003). Therefore, reactive oxygen species (ROS) play an important role in the pathophysiology of cerebral ischemia (Gringo, 1997; Nakashima et al., 1999). This process is followed by numerous enzymic oxidation reactions. Despite many existing methodologies that allow investigators to quantify various oxygen radicals in ischemic tissue and in neurons that are under oxidative stress, the causative role of ROS in ischemic brain injury remains elusive to stroke researchers (Irmak et al., 2003).

Caffeic acid phenethyl ester (CAPE), an antioxidant flavonoid, is the active component of the propolis purified from the hives of honeybees. CAPE is a small, lipid-soluble compound that is currently being tested for its ability to prevent the formation of ROS, malondialdehyde (MDA), and peroxynitrite in many in vivo studies (Ilhan et al., 1999; Russo et al., 2002; Song et al., 2002). CAPE inhibits 5-lipooxygenase-catalyzed oxygenation of linoleic acid and arachidonic acid in the micromolar concentration range. At a concentration of 10 μmol, it completely blocks production of ROS in human neutrophils and suppresses the xanthine/xanthine oxidase system (Sud'ina et al., 1993). Previous studies have demonstrated that CAPE also exhibits an antioxidant property as well as anti-inflammatory, cytostatic, antiviral, antibacterial and antifungal properties (Dobrowolski et al., 1991; Pascual et al., 1994). It has been demonstrated that CAPE potentially and specifically inhibits lipid peroxidation (Sud'ina et al., 1993) and lipoxygenase activities (Laranjinha et al., 1995). In addition, CAPE also prevents inactivation of nitric oxide (NO) and other radicals (Aladag et al., 2006). Most recently, it has been demonstrated that CAPE is able to block ischemia- and low-potassium-induced neuronal death (Amodio et al., 2003; Irmak et al., 2003; Wei et al., 2004), and that it increases plasma NO content in rats subjected to focal cerebral ischemia (Tsai et al., 2006). Although the antioxidant effects of CAPE in cerebral ischemia–reperfusion injury has been investigated in a few recent studies, the effect of subsequent CAPE in focal permanent cerebral ischemia has not been investigated to date. Therefore, the objective of our study was to investigate the effects of CAPE on infarct volume, antioxidant status, lipid peroxidation, and neurological deficit in the in vivo rabbit stroke model of focal permanent middle cerebral artery occlusion (pMCAO).

## 2. Results

### 2.1. Effects of CAPE on physiological parameters

Body temperatures of the animals before and during operation, recovery from anaesthesia and the following days were measured as given in Table 1. The effects of CAPE on the mean arterial blood pressure and levels of blood gases, pH, $PaO_2$, and $PaCO_2$, before ischemia and after unilateral pMCAO, were given in Table 2. CAPE administration (10 μmol $kg^{-1}$) did not produce any significant changes in blood gases (pH, $PaO_2$ and $PaCO_2$) or mean arterial blood pressure in the rabbits studied.

### 2.2. The analysis of oxidant/antioxidant stress markers

The effects of subacute CAPE (10 μmol $kg^{-1}$) administration on plasma MDA, XO, NO, GSH contents, and erythrocyte CAT activity after the focal permanent middle cerebral artery occlusion (pMCAO), were shown in Table 3. We observed that CAPE produced significant changes in NO, GSH, CAT, XO, and MDA levels of the rabbits after the focal pMCAO (Table 3).

Compared to the first day, CAPE treatment significantly decreased MDA on the 7th day and CAT and XO activities on the 4th and 7th days ($p<0.05$), whereas it significantly increased the level of NO on the 2nd and 4th days ($p<0.05$) and GSH on the 2nd, 4th and 7th days ($p<0.05$).

The plasma NO level was significantly higher in the CAPE group in comparison with ischemia group on the 2nd and 4th days ($p<0.05$). In addition, in the CAPE group, XO activity was non-significantly lower on the 4th and 7th days, and CAT activity was significantly lower on the 2nd ($p<0.01$), 4th ($p<0.01$) and 7th days ($p<0.05$); GSH activity in the CAPE group was significantly higher on the 4th ($p<0.05$) and 7th days ($p<0.01$) when compared with the sham group.

### 2.3. Neurological evaluation

In none of the study groups, no animal died. The neurological deficit score was non-significantly lower in the CAPE group compared to the ischemia group on the 7th day after the focal pMCAO (Table 4; $p>0.05$). We found no evidence of brain injury in sham-operated animals on the seventh day after the focal pMCAO.

### 2.4. Effects of CAPE on infarct volume

Seven days after the permanent occlusion of the right MCA, the rabbits were sacrificed under anaesthesia. In terms of the total brain volumes, no significant differences were detected between the ischemia, sham and CAPE groups (mean±SD: 11.91±2.92 $cm^3$, 12.77±2.59 $cm^3$ and 11.22±0.94 $cm^3$, respectively, $p>0.05$), as can be seen in Fig. 1. Seven days after the permanent occlusion of the right MCA, the infarct volume and infarct volume / total brain volume (% of tissue loss in the ipsilateral brain hemisphere) were significantly lower in the

**Table 1 – Body temperatures before, during and after the experimental procedure in rabbits**

| Group | Baseline | During op | Recovery | First day | Second day | Fourth day | Seventh day |
|---|---|---|---|---|---|---|---|
| Ischemia | 37.3±0.2 | 36.6±0.4 | 36.5±0.4 | 36.6±0.4 | 36.8±0.3 | 37.0±0.3 | 37.0±0.2 |
| Sham | 37.4±0.2 | 37.0±0.4 | 37.1±0.4 | 37.2±0.4 | 37.3±0.3 | 37.4±0.2 | 37.3±0.3 |
| CAPE | 37.3±0.1 | 36.9±0.3 | 36.8±0.3 | 37.0±0.3 | 37.0±0.2 | 37.1±0.2 | 37.1±0.2 |

Table 1 summarizes the body temperatures of rabbits (as mean±SD). There were no statistically significant differences between the groups ($p>0.05$).

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