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Green Tea Polyphenol (−)-Epigallocatechin Gallate Prevents Oxidative Damage on Periventricular White Matter of Infantile Rats with Hydrocephalus

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Department of Neurosurgery, Kocaeli University Faculty of Medicine, Kocaeli, Turkey, [1]*Center of Experimental Studies,* [2]*Department of Biochemistry, Istanbul University Cerrahpasa Faculty of Medicine, Istanbul, Turkey*

ETUS, V., ALTUG, T., BELCE, A and CEYLAN, S. *Green Tea Polyphenol (−)-Epigallocatechin Gallate Prevents Oxidative Damage on Periventricular White Matter of Infantile Rats with Hydrocephalus* Tohoku J. Exp. Med., 2003, **200** (4), 203–209 —— Hydrocephalus causes damage to periventricular white matter at least in part through chronic ischemia. Emphasizing the periventricular ischemia/hypoxia in hydrocephalus, various authors indicated the secondary biochemical impairment and oxidative damage in experimentally induced and congenital hydrocephalic rat brain. (−)-Epigallocatechin gallate (EGCG), the main constituent of green tea polyphenols, has been shown to be of some protective value in various models of neurological injury as a free oxygen radical scavenger. In the present study the effects of EGCG were examined on the periventricular oxidative damage in experimental childhood-onset hydrocephalus. Hydrocephalus was induced in 3 weeks-old rat pups by kaolin injection into the cisterna magna. A single daily dose of 50 mg/kg of EGCG injected into the peritoneum of the rats for 15 days significantly reduced periventricular white matter malondialdehyde levels when compared to non-treated hydrocephalic animals. Our results indicate that EGCG may have a protective effect against periventricular white matter oxidative damage in hydrocephalus induced infantile rats. ———— (−)-epigallocatechin gallate; green tea; hydrocephalus; periventricular white matter; lipid peroxidation

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It was suggested that oxidative brain damage might represent an important factor resulting from or contributing to the pathogenesis of hydrocephalus (Caner et al. 1993; Socci et al. 1999). Even if the blockage of cerebrospinal fluid does not cause intracranial hypertension, it diminishes both cerebral blood flow and compliance of the brain. If ventricles continue to

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dilate, the ependyme ruptures. Damages in the microvascular structures and ischemia-induced axonal impairment lead to progressive neural tissue damage (Gonzales-Darder et al. 1984; Nakada et al. 1992). Diminution of cerebral blood flow also causes ischemia induced metabolic changes. In hydrocephalus cerebral blood flow was shown to be significantly diminished in periventricular white matter (Higashi et al. 1986). Ischemia and hypoxia increase lipid peroxidation reactions and free oxygen radicals that lead to secondary neural tissue damage. Periventricular white matter seems to be the primary site where oxidative biochemical reactions are triggered by the ischemic changes due to mechanical damage done by the enlarging ventricles (Rosenberg et al. 1983; Higashi et al. 1986). Emphasizing the periventricular ischemia/hypoxia in hydrocephalus, some authors indicated the secondary biochemical neuronal injury mechanisms in experimentally induced and congenital hydrocephalic rat brain (Caner et al. 1993; Socci et al. 1999).

In the recent years various agents were being used in order to minimize the secondary brain damage following central nervous system ischemia and trauma. The effects of such agents are not well evaluated in hydrocephalus-induced secondary brain insult yet. Using in vitro and in vivo models, numerous studies suggested the protective effects of green tea polyphenols on neuronal damage induced by free radical attack (Matsuoka et al. 1995; Inanami et al. 1998; Lin et al. 1998; Hong et al. 2000; Lee et al. 2000; Livites et al. 2001; Chan et al. 2002; Skrzydlewska et al. 2002). Being the main constituent of green tea polyphenols, (−)-epigallocatechin gallate (EGCG) was indicated to be the key polyphenolic antioxidant responsible for neuroprotection (Valcic et al. 1999).

Kaolin induction of hydrocephalus in the rat is one of the most studied animal models of hydrocephalus in the literature. Up to date numerous studies have focused on histochemical, neurophysiological and neurochemical changes associated with kaolin-induced hydrocephalus.

In the present study, the effect of EGCG on lipid peroxidation levels of periventricular white matter in kaolin-induced hydrocephalic infantile rats was examined. We hypothesized that EGCG would ameliorate the oxidative damage in experimental childhood-onset hydrocephalus.

Materials and Methods

Animals and hydrocephalus induction

Thirty-two Sprague-Dawley rat pups at age 3 weeks (weight 42–63 g) were used in the study. The rats were provided by the Center of Reproduction of Animals for Experimental Studies of the University of Istanbul, Cerrahpasa Medical School. The experiment was approved by the local animal use committee. All efforts were made to minimize suffering and the number of animals used. Rats were divided into 3 groups. Group 1 and Group 2 consisted of eleven rats and Group 3 (control group) consisted of ten rats. Anesthesia was induced in the rats by intramuscular injection of ketamine (10 mg/kg). Each rat's neck was shaved and a median cranio-cervical incision was done. Under binocular loupe magnification superficial and deep cervical muscles were separated off the midline and the atlanto-occipital membrane was identified. The membrane was penetrated at a right angle by a sterile insulin injector of 27 G introduced into the cisterna magna. The cerebrospinal fluid outflow from the injection site was observed. Then 0.05 ml of sterile kaolin (Kaolin hydrated Aluminum Silicate, Sigma Chemical Co., St. Louis, MO, USA) suspension (250 mg/ml in 0.9% saline) was slowly injected into the cisterna magna of the rats in Group 1 and Group 2. The animals in Group 3 received a sham injection consisting of needle insertion only. Cervical muscles and skin were closed with separate sutures. Pups were housed in standard cages (four animals/cage) and provided with a normal 12-hour day/night lighting schedule with

free access to water and pelleted food. After 24 hours, a single daily dose of 50 mg/kg of EGCG (Sigma Chemical Co.) solution prepared with 0.9% saline was injected into the peritoneum of the rats in Group 1. The second and the third group were injected same volume of 0.9% saline solution. Injections were made once daily for 15 days. The rats were decapitated at the end of the 15th day and their brains were removed. All the brains were transected at the bulbomedullar junction and then periventricular white matter were separated.

Biochemical analysiss

Assay for lipid peroxidation was performed by determining malondialdehyde (MDA) levels. The measurement of lipid peroxidation has been favored as the indicator of free radical activity in many ex vivo and in vitro studies of oxidative stress, in particular by the measurement of MDA one of the end products of lipid peroxidation reactions. In various experimental studies neural tissue concentrations of MDA, were assayed as an index of membrane oxidative damage (Koudelova and Mourek 1994; Socci et al. 1999). MDA was analyzed in the periventricular tissue by modified Angel method (Angel et al. 1988). Firstly, tissue samples were weighed with the use of electronic scales (Shimadzu Libror AEU-210), then homogenized in electronic homogenizer (Heidolph RZR 2021) at 4°C and a 1800 cycle/minute speed in order to form a 20% suspension with Tris KCL buffer (pH=7.4). This tissue homogenate was incubated for 15 minutes at 37°C, and then centrifuged at 2000 rpm speed for 5 minutes. Five hundred microliter (μl) tiobarbituric acid (0.75%)+334 μl trichlorasetic acid (30%)+134 μl 5M HCl were added to a 500 μl sample taken from this supernatant. The tubes were left for 15 minutes in boiling water and centrifuged in Eppendorph centrifuge (Hettich μl) for 5 minutes at 10 000 g. The supernatant was read at 535 nm in a spectrophotometer. MDA values were obtained by the multiplication of the extension coefficient of the absorbance values (1.56×10^5 M^{-1} cm^{-1}) and the dilution coefficient (100/20) and were expressed by nanomol MDA/gram wet tissue weight.

Statistical analysis

Statistical analysis was performed by using the computer software program SPSS for Windows (release 11. 0. 1., standard version, SPSS Inc.). MDA values of the groups were analyzed by analysis of variance (ANOVA) with post hoc Bonferroni calculations for intergroup comparisons. All p-values less than 0.05 were considered to be statistically significant. The data of this study were expressed as a mean value±standard error of mean (S.E.M.).

RESULTS

All the rat pups in the first and the second groups experienced gross enlargement of the cerebral ventricles. Periventricular white matter MDA values (mean±S.E.M.) for the groups were as follows: Group 1 (EGCG treated hydrocephalic rats) 2668.4±96.7 nanomol/gram wet tissue, Group 2 (non-treated hydrocephalic rats) 3218.7±180.0 nanomol/gram wet tissue, and Group 3 (control) 2152.4±68.3 nmol/g wet tissue.

The MDA values of Group 2 were significantly higher compared to Group 3 ($p<0.001$). Likeliwise the values of the Group 1 were significantly higher than the values of the Group 3 ($p<0.05$). Importantly, the MDA values of Group 1 were significantly lower than those of Group 2 ($p<0.05$). Mean MDA values of the groups are summarized in Fig. 1.

DISCUSSION

Because of the brain's high consumption of oxygen, high concentration of polyunsaturated fatty acid and transition metals, and low concentration of antioxidants, the brain is vulnerable to ischemia -induced reactive oxygen species, which cause oxidative damage to lipids and brain DNA.

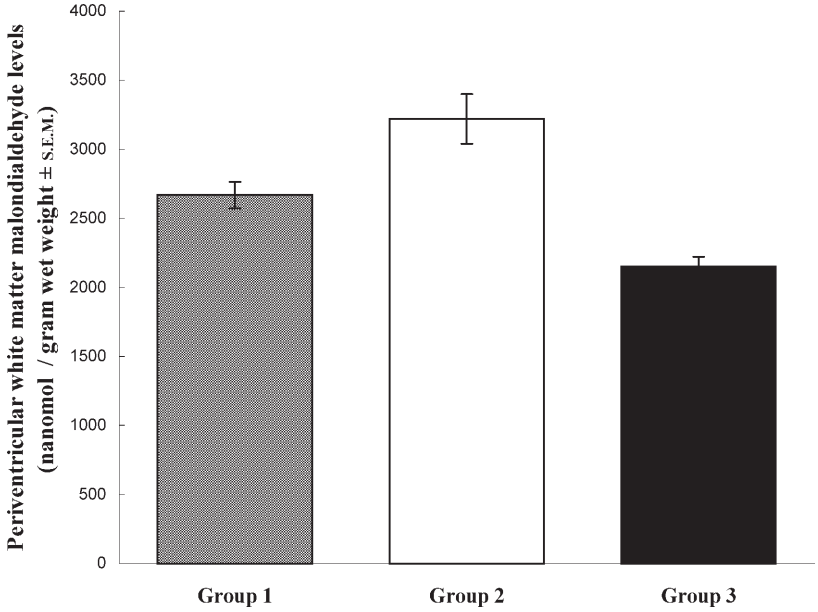


Fig. 1. Bar graph showing periventricular white matter malondialdehyde (MDA) levels (mean±S.E.M.) of the groups. Group 1 ($n=11$), 2668.4±96.7 nmol MDA/g wet tissue weight; Group 2 ($n=11$), 3218.7±180.0 nmol MDA/g wet tissue weight; Group 3 ($n=10$), 2152.4±68.3 nmol MDA/g wet tissue weight. MDA values of the groups were analyzed by analysis of variance (ANOVA) with post hoc Bonferroni calculations for intergroup comparisons.
Group 1–Group 2: $p<0.05$.
Group 2–Group 3: $p<0.001$.
Group 1–Group 3: $p<0.05$.

The mechanism of brain damage in hydrocephalus is known to bear some similarities to those associated with trauma and stroke. Hydrocephalus causes damage to periventricular white matter at least in part through chronic ischemia. It was suggested that oxidative brain damage might represent an important factor resulting from or contributing to the pathogenesis of hydrocephalus (Caner et al. 1993; Socci et al. 1999). In the study of Socci et al. (1999), MDA levels were reported to be significantly higher in the hippocampus and basal ganglia of 25-day-old hydrocephalic H-Tx rats. Caner et al. (1993) suggested that vascular changes observed in hydrocephalic rats might be due to the high level of lipid peroxidation, which in turn might be the consequence of ischemia caused by the hydrocephalus related stretching of cerebral vessels. Mori et al. (1993) indicated that the production of superoxide dismutase, one of the scavengers of active oxygen species, was congenitally reduced in the congenitally hydrocephalic rat brain. They proposed that this reduction might promote the impairment of the function of choroid plexus and cilia due to increased active oxygen species. In their studies on pathophysiology of periventricular tissue in hydrocephalus, various authors indicated that cerebral blood flow was significantly diminished in the periventricular white matter (Rosenberg et al. 1983; Higashi et al. 1986; Del Bigio and Bruni 1988). Periventricular white matter seems to be the primary site where, oxidative biochemical reactions are triggered by the ischemic changes.

Unlike cerebral trauma and ischemic injury, in which damage occurs over a period of seconds to minutes, hydrocephalus might be particularly amenable to supplemental pharmacological therapy, because the hydrocephalus

is gradual and progressive over a period of days to months. It is therefore of interest to study the effects of antioxidants, free radical scavengers or trapping agents for use as potential cerebroprotective agents of periventricular white matter injury in hydrocephalus.

Various experimental studies have shown that green tea polyphenols reduced free radical-induced lipid peroxidation in the central nervous system (Matsuoka et al. 1995; Inanami et al. 1998; Lin et al. 1998; Hong et al. 2000; Skrzydlewska et al. 2002). In their study, Hong et al. (2000) found that the pretreatment of 0.5% green tea extract for 3 weeks resulted in inhibitory effects on the ischemia/reperfusion-induced increase of eicosanoid concentration, hydrogen peroxide, lipid peroxidation and oxidative DNA damage levels in the rat. Inanami et al. (1998) reported that oral administration of catechin prevented the delayed hippocampal neuronal death after ischemia-reperfusion induction. Lin et al. (1998) investigated the antioxidative property of green tea against iron-induced oxidative stress in the rat brain and they reported that the potent antioxidative action of green tea extract protected the nigrostriatal dopaminergic system from the iron-induced oxidative injury. In another study, Matsuoka et al. (1995) showed that tea catechins protected the cultured newborn-mouse cerebral nerve cells from death induced by glucose oxidase and they proposed that tea catechins might become useful for protecting human from senile disorders such as dementia.

These data suggest that brain penetrating property of polyphenols, as well as their antioxidant and iron-chelating properties may make such compounds an important class of drugs to be developed for treatment of neurodegenerative diseases where oxidative stress has been implicated. The fact that catechins are rapidly and extensively metabolized emphasizes the importance of demonstrating their antioxidant activity in vivo. The effects of green tea catechins on biomarkers of oxidative stress, especially oxidative DNA damage, appear very promising in animal models, but data on biomarkers of in vivo oxidative stress in humans are limited. In humans, modest transient increases in plasma antioxidant capacity have been demonstrated following the consumption of green tea catechins (Higdon and Frei 2003).

Green tea contains many polyphenolic antioxidants. (−)-Epigallocatechin gallate (EGCG) was indicated to be the key polyphenolic antioxidant responsible for cancer chemoprevention, anti-inflammation, and neuroprotection (Valcic et al. 1999). Being the main constituent of green tea polyphenols, EGCG was found to be the most effective scavenger among tea catechins for the superoxide anion, hydroxyl radical, and 1, 1-diphenyl-3-picrylhydrazyl radical (Nanjo et al. 1999). Recently, the antioxidant effects of EGCG in various models of central nervous system injury were extensively studied. Lee et al. (2000) demonstrated that EGCG significantly reduced neuronal cell damage in the hippocampal CA1 region after transient global ischemia in the gerbils. Levites et al. (2001) demonstrated neuroprotective property of EGCG in mice model of Parkinson's disease. EGCG was also shown to increase the activities of superoxide dismutase and catalase (endogenous antioxidant enzymes that can effectively scavenge superoxide radicals) in the brain. In a recent study, Chan et al. (2002) indicated that catechin was an effective antioxidant to increase the activity of superoxide dismutase in rat brain astrocytes, which would be beneficial to neurons subjected to oxygen free radical damage.

These data have shown EGCG to be of some protective value in various models of neurological injury as a free oxygen radical scavenger and it was proposed that EGCG might be useful in the prevention and treatment of diseases due to increased free radical production (Alvarez et al. 2002). However, to our knowledge, there is no previous study on the

neuroprotective effect of EGCG against periventricular oxidative damage in hydrocephalus. In the present study, we examined the effect of EGCG on lipid peroxidation levels of periventricular white matter in hydrocephalus induced infantile rats. It has been reported that tea brews generally consumed contain 708 μg/ml EGCG (Wang et al. 1992). In this study, EGCG was administered at the same dose (50 mg/kg) that Lee et al. (2000) have indicated in their study.

Periventricular white matter MDA values of the EGCG treated hydrocephalic rat pups were found to be significantly reduced ($p<0.05$) when compared to non-treated hydrocephalic animals. These data indicate that EGCG may have a protective effect against periventricular white matter oxidative damage in hydrocephalus induced infantile rats. This study is the first to report the neuroprotective effects of EGCG in the kaolin induced hydrocephalus model. These results need to be confirmed by further studies.

Up to date, numerous laboratory experiments have suggested protective effects of several antioxidants against cancer and cardiovascular disease. However, large scale human intervention trials have already refuted the roles of β-carotene and α-tocopherol in the prevention of these diseases. Considering these experiences, one must be cautious in inferring the potential protective effects of another antioxidant.

Although the mechanisms underlying the neuroprotective effect of EGCG are not fully understood, EGCG has several possible pharmacological evidences for neuroprotection as an antioxidant. Valcic et al. (2000) suggested that EGCG reacted with peroxyl radicals generated by thermolysis of the azo initiator 2, 2′-azobis (2, 4-dimethylvaleronitrile) to produce several oxidation products. An other study has shown that in the cultured rat brain astrocytes, the activity of superoxide dismutase was markedly increased by incubation with catechin (Chan et al. 2002). Lee et al. (2000) suggested the action mechanisms which, might contribute to the potent antioxidant and putative neuroprotective actions of EGCG were as follows: (a) free radical scavenging activity or attenuation of lipid peroxidation due to various forms of radicals, (b) inhibition of xanthine oxidase activity, and (c) blockade of inducible nitric oxide synthase and neuronal nitric oxide synthase induction.

In this study we emphasize the importance of investigating the free oxygen radical mediated neuronal damage of periventricular tissue in the early stage of hydrocephalic process and the role of free oxygen radical scavengers as an adjunct medical treatment possibility. We suggest that EGCG may have a neuroprotective effect through its antioxidative property on hydrocephalus-induced periventricular damage.

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