THE EFFECT OF ANTIOXIDANT CAFFEIC ACID PHENETHYL ESTER (CAPE) ON SOME ENZYME ACTIVITIES IN CISPLATIN-INDUCED NEUROTOXICITY IN RATS

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Aim: Neurotoxicity is the next common side effect of cisplatin (CDDP)-based chemotherapeutics following nephrotoxicity. We investigated the effect of CDDP on some brain metabolic enzyme activities such as hexokinase (HK), glucose-6-phosphate dehydrogenase (G6PD), lactate dehydrogenase (LDH), and malate dehydrogenase (MDH) in an experimental model of CDDP toxicity, and examined the protective role of Caffeic acid phenethyl ester (CAPE), a phenolic antioxidant derived from the honeybee propolis, on the enzyme activities mentioned above.

Methods: Female Wistar albino rats were divided into three groups: sham operation group (n:6), CDDP group (n:9), and CAPE + CDDP group (n:8). All the chemicals used were applied intraperitoneally. HK, G6PD, LDH, and MDH activities were determined spectrophotometrically in the brain supernatant at the end of the surgical procedures.

Results: There were decreased G6PD activities and increased MDH activities in CDDP group compared to control group ($p<0.05$, $p<0.05$). LDH activities were increased in CAPE+CDDP group compared to CDDP group ($p<0.001$).

Conclusion: These results provide a new point of view on the glucose metabolizing enzymes of brain tissue affected from CDDP and the protective effects of CAPE on these enzymes.

Key words: Caffeic acid phenethyl ester, cisplatin, neurotoxicity, metabolic enzymes.

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INTRODUCTION

Caffeic acid phenethyl ester (CAPE), an active component of propolis extract, (1) has been synthesized by esterification of caffeic acid (2). At a concentration of 10 μM, it completely blocks production of reactive oxygen species (ROS) in human neutrophils and the xantine/xantine oxidase system (3). Besides its well-known antioxidant activity (2,4). CAPE inhibits certain enzyme activities such as lipoxygenases, cyclooxygenase and glutathione S-transferase (GST) (5-7). CAPE has been reported to have anti-inflammatory (8), cytostatic (1), antiviral (9), antibacterial (10) and antifungal (11) properties.

The antitumor drug cisplatin [Cis-dichlorodiammineplatinum (II), CDDP] is widely used in the treatment of solid tumors. The major side effects renal toxicity, neurotoxicity, and hepatotoxicity are dose limiting and occur acutely or after repeated doses (2,13). Beside its nephrotoxicity CDDP causes senrory neuropathy about %20 of patients taking full course of CDDP therapy (14). Since hyperhydration significantly reduces the incidence of renal toxicity, neurotoxicity remains as the only major dose-limiting toxicity. The underlying mechanism of neurotoxicity is in argument. There is a report that CDDP causes apoptosis of dorsal root ganglion neurons in vitro and in vivo (15,16) that are not protected by the blood-brain barrier. Earlier studies have shown that cisplatin generates oxygen radicals, which are known as one of the pathogenic intermediates following chemothrapy (17) and that antioxidants effectively protect neurons from CDDP-induced neurotoxicity (18,19).

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Mitochondrial dysfunction and oxidative damage seem to play important role in cell death induced by CDDP (20).

Glucose-6-phosphate dehydrogenase (G6PD) is the key regulatory enzyme in the pentose phosphate pathway (PPP), whose major physiological role is to supply NADPH by the conversion of glucose 6-phosphate to 6-phosphogluconate. NADPH is also generated by the next step in the PPP, the conversion of 6-phosphogluconate to ribulose 5-phosphate by 6-phosphogluconate dehydrogenase (21). NADPH plays an important role in antioxidant reactions together with glutathion (GSH), glutathion-peroxidase (GS-Px) and catalase (CAT). G6PD, normally thought of as a "housekeeping" enzyme whose expression is constitutive, is, in fact, induced by agents which cause oxidative stress and is essential for resistance to oxidative stress. Hexokinase (HK) is important in the metabolism of glucose (the only form of sugar accepted in the brain). This enzyme catalyzes oxidative phosphorylation of glucose to glucose-6-phospate using the gamma phosphate of ATP. Malate dehydrogenase (MDH), an important intermediate in several biosynthatic processes, oxidizes L-malate to oxaloacetate in the last step of citric acide cycle. The reaction yields a NADH molecule, equivalant to 3 ATP. Lactate dehydrogenase (LDH), catalyzes lactate to piruvate and NAD to NADH (the reaction is reversible). LDH is found in many body tissues, especially in heart, liver, kidney, skeletal muscle, brain, blood cells, and lungs. These enzymes together with G6PD are important enzymes in production of ATP, NADPH and GSH.

The effects of CAPE on CDDP induced brain toxicity have not been examined to date. The objective of our study was to investigate the effects of CAPE on the activities of HK, G6PD, MDH, and LDH which are important enzymes mainly in glycolysis, citric acid cycle and PPP in damaged brain tissue by CDDP in rats and possible role of these enzymes in brain.

MATERIAL AND METHODS

Animals

Female Wistar albino rats weighing 200-250 g were purchased from experimental research center, University of Erciyes (Kayseri, TURKEY) and housed in animal laboratory. The animals were fed with a standard diet and kept on a physiological day-night rhythm. The rats were divided into three groups. Group I (control): Isotonic saline solution (an equal volume of CDDP) was injected intraperitoneally (n:6). $NaHCO_3$ served as control, dissolved with distilled water was given intraperitoneally (as equal volume of CAPE) 24 hours prior to the saline injection, followed by once a day at the same dose until sacrifice. Group II (CDDP): The animals (n: 9) received intraperitoneal injection of CDDP (cisplatinum Ebewe, 0.5 mg/ml) at a dose of 7 mg/kg body wt and were sacrificed 5 days after CDDP injection (22). Brains were obtained for the various measurements. Group III (CAPE+CDDP): CAPE was administered at a dose of 10 µmol/kg body wt (n:8). First dose of CAPE was given 24 hours prior to CDDP injection and continued until sacrifice.

Tissue preperation for biochemical analysis

After brain tissues were obtained for biochemical analysis, the specimens were washed out from contaminating blood with ice-cold buffered saline. They were weighed and cut very thinly with a clean scalpel blade and then homogenized in 0.15 M ice-cold KCl for 3 min at 16,000 rpm with a homogenizer (Ultra Turrax Type T-25-B; Labortechnic, Staufen, Germany). The homogenates were then centrifuged for 1 h at 4° C at 5000 x g. All measurements were done at the supernatant.

Enzyme activity determinations

The activities of HK (EC 2.7.1.1), G6PD (EC 1.1.1.49), MDH (EC 1.1.1.37), and LDH (EC 1.1.1.27) enzymes were determined spectrophotometrically (23), from the oxidation of NADH (for MDH and LDH) or the reduction of $NADP^+$ (for HK and G6PD) by taking the decrease or increase of the absorbance (A_{340}), in reaction mixtures at 25°C for 1-min period. Tissue protein levels were determined by the Lowry procedure, with bovine serum albumin as a standard (24). Activities were given in miliunits (mU) and units (U) per miligram protein (mg $protein^{-1}$). All samples were assayed in duplicate.

Statistical Analysis

Data were expressed as means ± standard error. The one-way ANOVA analysis of variance and post-hoc multiple comparison tests (LSD) were performed on the data of biochemical variables to examine differences among groups. A p-value <0.05 was considered as statistically significant.

Table1. Metabolic enzyme activities in the brain tissues of study groups.

	HK mU/mg protein	G6PD mU/mg protein	LDH U/mg protein	MDH U/mg protein
I- Control (n=6)	39.2±4	14.07 ± 1	4.91 ± 0.5	11.97 ± 0.5
II- CDDP (n=9)	44.6±2	10.17 ± 1	5.12 ± 0.4	13.04 ± 1.1
III- CDDP+CAPE (n=8)	33.4 ± 4	7.77 ± 1	7.91 ± 0.8	17.34 ± 1.7
P values				
I-II	ns	0.019	ns	ns
I-III	ns	0.001	0.004	0.039
II-III	ns	ns	0.003	ns

Results were given in mean ± standard error. ns: not significant.

RESULTS

Results were summarized in Table 1. G6PD activity was found to be decreased in CDDP group compared to control group ($p<0.019$). CAPE application decreased the G6PD activity compared to control group significantly ($p<0.001$). LDH and MDH activity in CDDP group was not changed compared to control group but CAPE increased the LDH activity compared to control and CDDP groups. On the other hand, MDH activity in CDDP+CAPE group increased compared to control group ($p=0.039$). The difference in MDH activity between CDDP and CDDP plus CAPE groups was not statistically significant.

DISCUSSION

Neurotoxicity in cancer patients treated with CDDP has limited the use of CDDP at effective doses as nephrotoxicity. Reducing CDDP-induced neurotoxicity has clinical importance in cancer chemotherapy. The underlying mechanism of the toxicity remains unknown. CDDP-induced neurotoxicity includes peripheral sensory polineuropathy, ototoxicity, and rarely, focal encepahalopathy (25). Some evidence indicates that CDDP-induced side effects are, at least in part, the result of the formation of oxygen free-radicals and studies in human focused that CDDP treatment induces a fall in plasma antioxidant levels because of oxidative stress (26). Brain tissue is highly sensitive to damage by free radicals because of its high use of oxygen, its high concentration of polyunsaturated fatty acids and its low concentration of cytosolic antioxidants.

So far various drugs have been used in an attempt to prevent or reduce CDDP-induced neurotoxicity (27-29). Many clinical studies suggested that GSH offers neuroprotection from the toxic effects of CDDP (19,30,31). Oxidative stress caused by various stimuli lead to oxidation of GSH, the major redox power of the cell. A previous study showed that CAPE, a potent free radical scavenger, caused a neuroprotective effect on biochemical status in pentylenetetrazol-induced oxidative damage in mice (32). Similar results are suggested in various studies used CAPE as a nueroprotective agent (33-35).

Cellular events involving oxidative damage mediated by reactive oxygen species (ROS) may evoke neurodegeneration. It has been known that CDDP is a potent redox cycler that generates toxic ROS and, hence, causes oxidative injury of various cells and tissues (36). As mitochondria is the major site of ROS, oxidative stress in and around these organelles would be increased (37,38). CDDP binds to mitochondrial DNA, interacts with phospholipids and phosphatidylserine in membranes, disrupts the cytoskleton, and affects the polimerization of actin. Considerable evidence indicates that CDDP can kill cells through the induction of apoptosis characterized by unique morphological and biochemical features depends on the energy supplied by ATP as well as on active protein syntesis (39). A mitochondrial step involving outermembrane permeabilization is controlled by the pro- and antiapoptotic members of the bcl-2 family and leads to the cytosolic release of mitochondrial intermembrane space proteins that can trigger either caspase activation or caspase-independent pathways (40). Kruidering et al. showed that CDDP-induced mitochondrial dysfunction was the result of inhibition of the enzymatic complexes of the respiratory chain by CDDP, with decreased ATP levels as a consequence. CDDP caused inhibition of glutathione-reductase leading decreased GSH levels. They also found that, LDH was only slightly inhibited by CDDP, glucose-6-phosphatase, located in the endoplasmatic reticulum, CAT (peroxisomes), and acid phosphatase (lysosomes) were not altered significantly (38). In our study LDH was significantly increased in CAPE+CDDP group compared to control and CDDP group. Increases of LDH activities may refer to increased antioxidant defence system of the brain tissue.

The essentiality of G6PD in mammalian

cells has been well recognized. It has been shown to be necessary for cell growth (41). The role of G6PD in the cell response to oxidative stress is well established in human erythrocytes (42). Within these cells, which have a very limited biochemical apparatus and where gene expression cannot be regulated because a nucleus is lacking, G6PD is the only source of NADPH (in other cells there are different pathways that produce NADPH); furthermore, an impaired production of this reductive agent directly affects the production of both GSH and catalase, thus inhibiting both pathways that dispose of hydrogen peroxide. Ursini et al. found that a pulse of oxidative stress, obtained by the direct addition of H_2O_2 to the cells, stimulates the expression of G6PD in a rapid and transient manner in the various human cell lines of different tissue specificity that have been tested. When they inhibited the activity of a potent H_2O_2 cellular detoxification agent, CAT, they did not observe any detectable increase in G6PD expression, which suggests that the effect is not due to general degenerative conditions (43). In this study, CDDP administration at a dose of 7 mg/kg body wt resulted in a significant decrease in G6PD activity in the rat brain, maybe due to depletion of GSH. But CAPE did also decrease (nonsignificantly) G6PD activity compared to CDDP and control group. This effect of CAPE seems to be an unexpected result and may be a moot point. On the other hand there is a report that CAPE inhibits the activity of GST (7). GST is required in detoxifying and metabolizing of agents such as chemotherapeutics. Reduced GST can prevent the konjugation of CDDP with glutathion and thus increased CDDP can cause more and more oxidative stress by inhibiting the activity of G6PD.

It was shown that in CDDP-treated rats, although statistically insignificant, MDH activity was found to be decreased in the kidney tissues (44).We found here increased MDH activity both in CDDP (insignificant) and CDDP+CAPE (significant) group. MDH plays an important role in producing ATP in the last step of citric acid cycle. ATP is essential in many metabolizing processes. So increased MDH may refer to increased antioxidant defence system in the brain tissues.

The resultant oxidative stress by CDDP, activates the transcription factor NF-κB, which in turn stimulates the production of tumor necrosis factor alpha (TNF-α) Anda et al. evaluated the effect of TNF-α on the transport of CDDP or high molecular weight dextran labeled with fluorescein isothiocyanate (FITC-dextran) across bovine brain microvessel endothelial cell (BMEC) monolayers that was conducted on side-by-side diffusion chambers in vitro. TNF-α selectively promoted the in vitro permeability of the blood-brain barrier to CDDP without disrupting tight junctions (45). So, CDDP can enter and cause neurotoxicity in the brain tissue in this way. The agents that can downregulate the activation of NF-κB have potential for therapeutic interventions. CAPE has been shown to block specifically and completely the activation of NF-κB induced by a wide variety of inflamatory agents including tumor necrosis factor and H_2O_2 (46).

In this study CAPE caused significant increase in the activity of LDH compared to CDDP. Decreases in the activities of HK, G6PD, and increase in the activity of MDH were not significant. CAPE seem to be ligthly effective on the activities of these enzymes in CDDP-induced effect to rat brain tissue. Different experimental studies are needed to understand the real effect of CAPE on these metabolizing enzymes. Nonetheless this study was a new approach to the neuroprotection against CDDP with CAPE via affecting some metabolizing enzymes, which are important in glycolysis, citric acid cycle and PPP consequently important in antioxidant defence system.

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