Original Article

Antioxidant and Apoptotic Effect of Edaravone on Cisplatin-Induced Brain Injury in Rats

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Abstract

Purpose: This study aims to investigate the effect of edaravone in preventing cisplatin-induced brain damage. **Methods:** Forty female Wistar albino rats were included in the study. 4 groups were created. In group 1 (control group) (n=10), neither any drugs were given nor anything was performed. Group 2 (cisplatin group) (n=10), single dose 7.5 mg/kg cisplatin was given. In group 3 (edaravone group) (n=10), single dose 1 mg/kg edaravone was administered. Group 4 (cisplatin+ edaravone group) (n=10), single dose 7.5 mg/kg cisplatin and 1 mg/kg edaravone were given. Brain tissue was removed in all rats after 3 days. Blood samples taken from heart tissue were examined for malondialdehyde (MDA) and nitric oxide (NO) levels. Brain tissue was evaluated for damage with p53, GFAP and Ki 67. **Results:** Edaravone reduced cisplatin-induced brain damage. MDA and NO levels in the cisplatin group were significantly higher than the other groups ($p< 0.05$). Likewise, tissue damage in the cisplatin group was significantly higher than in the other groups ($p< 0.05$). The immunohistochemical staining which was done by using p53, GFAP and Ki 67 was shown that tissue damage was higher in cisplatin group than cisplatin+ edaravone group and this difference was found to be statistically significant (p< 0.05). **Conclusion:** The findings of our study suggest that edaravone therapy may be effective in the prevention and treatment of cisplatin-induced brain injury.

Keywords: Cisplatin, edaravone, apoptosis, brain, rat

Introduction

Cisplatin has been widely used for years, especially in the treatment of solid organ tumors. However, it has been shown to have a toxic effect on many organs and systems, especially the kidney, ear, liver and nervous system.[1] Cisplatin forms a bond with DNA inside the cell and a cytotoxic effect occurs.[2] The toxic effect of cisplatin is dose dependent, more side effects are observed at higher doses.

The synthesis of free radicals and reactive oxygen species increase and normal cells other than cancer cells are also affected by this situation.[3] As a result, the harmful environment called oxidative stress occurs. Neurotoxic conditions caused by cisplatin include peripheric neuropathy, ototoxicity, vestibulopathy and encephalopathy.[4,5]

A number of protective mechanisms have been developed to protect against the toxic effect of oxidative stress. These include enzymatic and non-enzymatic defense systems. Edaravone is a pyrazoline derivative and has been used as a free radical scavenger for cerebral ischemia.[6] Edaravone neutralizes the hydroxyl radicals, diminishes the lipid peroxidation and the final effect is neuroprotection.[7,8] Because of these described beneficial properties and its successful use in the treatment of cerebral ischemia since 2001, Edaravone could be useful in the prevention of cisplatin-induced brain injury. To the best of our knowledge, there is no other study showing the effect of edaravone in preventing and treating cisplatin-induced brain injury.

Material and Methods

This study was carried out in Erciyes University Faculty of Medicine, Department of Histology- Embryology. The study

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protocol was reviewed and approved by Erciyes University Animal Experiments Local Ethics Committee (ERU-HADYEK). The date and number of the ethical report was 07.07.2021 and 21/159, respectively. 8-10 weeks old female Wistar albino rats weighing 150-220 g were used in relation with Helsinki declaration of animal rights. All the rats were kept in cages at a room temperature of 22-24 °C, and they were treated to 12 h of daylight. Ad libitum feeding method with free access to water and food was applied.

The animals included in the study were randomly numbered. The experiment was conducted according to the guidelines stated on the website www.randomization. com. Random distribution was made to 4 groups on the day of the experiment.

Experimental study

Animals were allocated to create four groups. Each group consisted of 10 animals. In control group, no procedure was performed. In cisplatin group, single dose 7.5 mg/kg cisplatin was administered. In edaravone group, single dose 1 mg/kg edaravone was given and no procedure was performed. Cisplatin+ edaravone group, single dose 7.5 mg/kg cisplatin and 1 mg/kg edaravone were administered. Cisplatin and edaravone were given via intraperitoneal route. Animals were anesthetized by using ketamine hydrochloride (45 mg/kg, Ketalar, Eczacibasi, Istanbul, Turkey) and xylazin hydrochloride (5 mg/kg, Rompun, Bayer, Leverkusen, Germany). Blood samples were taken by entering the heart with a needle. Then, sacrification was performed by applying cervical dislocation procedure. Brain tissues of rats were removed.

Tissue samples were fixed in 10% formaldehyde solution. Then dehydration and parafin blocking processes were performed, respectively. The tissues were cut with a thickness of 5 micrometers and stained with hematoxylin and eosin (H&E) after deparaffinization and rehydration. The specimens were then examined and photographed by a single clinician by using light microscopy (Olympus® Inc. Tokyo, Japan). A modified semi-quantitative scoring was performed for the microscopic evaluation of the brain damage and four categories, Grade 0: None (0%) 1: Minimal (0-5%) 2: Mild (5-25%) 3: Moderate (25-50%) 4: Severe (more than 50%) were defined. To grade the damage to the brain, vascular changes, necrosis, edema, inflammation, neuronal degeneration, neuronal loss in the cortex were included as the parameters of the scoring system.

p53, GFAP, and Ki67 determination

Expressions of p53, GFAP, Ki67 in brain tissue were investigated immunohistochemically. Expression levels were graded using the 0-3+ range. (0: no staining, 1: less than 10% nuclear staining, 2: 10-30% nuclear staining, 3: more than 30% nuclear staining).

Biochemical measurement

The MDA kit (Cat. No: E0156Ra, Bioassay Technology Laboratory) was studied by using ELİSA method and their amounts were determined as ng/ml at 450 nm in the ELISA reader. The NO levels were detected by using the NO kit (Cat. No: E0703Ra, Bioassay Technology Laboratory) and the measurement was explained as µmol/ l at 450 nm in the ELISA reader.

Statistical analysis

Statistical Package for the Social Sciences (18.00 SPSS Inc., Chicago, IL) was used for statistical analyses. One-way ANOVA test and Post hoc Tukey HSD multiple comparison test were used for levels of MDA and NO. Tissue damage scores were compared by Kruskal Wallis test. Evaluation of caspases was determined by Fisher's Exact Test as p value. p value < 0.05 was accepted as statistically significant.

Results

Blood NO and MDA levels are shown in Table 1. The MDA levels were significantly higher in the cisplatin group than the cisplatin+ edaravone group (p=0.037). The NO levels were found to be lower in the cisplatin group than the cisplatin+ edaravone group, and the difference was statistically significant (p=0.026).

Brain tissue damage was significantly higher in the cisplatin group than the cisplatin+ edaravone group (p< 0.05) (Table 2). Tissue damage was also assessed by measuring the expression of p53, GFAP, and Ki67. GFAP expression was significantly higher in the cisplatin group than in the cisplatin+resveratrol group (p< 0.05). There was no significant difference between the groups in terms of other immunostains (Table 3). Macroscopically, there was no significant difference between the groups. In the control group, the cerebral tissue had normal morphologic structure with its' connective tissue components. (Figure 1A). In the cisplatin group, vascular changes, edema, and inflammation were more prominent than other groups (Figure 1B). However, other parameters such as necrosis, neuronal degeneration were similar as other groups. The parameters demonstrating

Table 1. Nitric Oxide (NO), Malondialdehyde (MDA) measurements in serum samples of the groups.

	NO (nmol/lt)	MDA (nmol/mg)
Group 1 (n=10)	17.48±2.43[a]	0.48±0.04[a]
Group 2 (n=10)	9.75±0.95	0.85±0.09[b]
Group 3 (n=10)	19.28±2.76[b]	0.51±0.05[a]
Group 4 (n=10)	13.56±2.08[a]	0.49±0.04[a]
p value*	0.026	0.037

Table 2. Distribution of histological damage according to the groups.

	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=10)	Group 4 (n=10)
Vascular changes	0.00	2.00*	0.00	1.10±0,3*
Necrosis	0.00	0.00	0.00	0.00
Edema	0.00	3.00*	0.00	1.60±0,5*
Inflammation	0.00	2.00*	0.00	1.00±0,2*
Neuronal degeneration	0.00	0.00	0.00	0.00
Neuronal loss in the cortex	0.00	0.00	0.00	0.00

*Kruskal Wallis test, the difference between group 2 and group 4 was statistically significant (p<0.05).

histopathologic damage were not observed in the edaravone group and the connective tissue was containing normal histological features (Figure 1C). In the cisplatin+edaravone group, addition of edaravone reversed the injury in relation with vascular changes, edema, and inflammation (Figure 1D).

There was no difference between the groups in terms of p53 and Ki67 (Figure 2 and Figure 3). However, the cisplatin group had more intense GFAP immunoreactivity than cisplatin+edaravone group (Figure 4).

Discussion

In this experimental study, the effect of edaravone on brain injury due to cisplatin was investigated. MDA levels appear to be increased and NO levels appear to be decreased according to our study. The tissue damage scores such as vascular changes, edema, and inflammation were more intense in the cisplatin group than cisplatin+edaravone group. Immunostaining with GFAP gave the same histological findings as light microscopy. Addition of edaravone reversed cisplatin-related adverse effects.

The platinum inside cisplatin combines with DNA of the cell. The amount of this platinum-DNA complex increases with the dose and duration of administration of cisplatin. As a result, intracellular toxicity occurs. In addition, oxidative stress caused by cisplatin leads to an increase in free radical

Table 3. Distribution of immunhistochemical damage according to the groups.

	Group 1 (*n*=10)	Group 2 (*n*=10)	Group 3 (*n*=10)	Group 4 (*n*=10)
p53	0.00	0.00	0.00	0.00
Ki67	0.00	0.00	0.00	0.00
GFAP	0.00	2.00*	0.00	1.05±0,2*

*Kruskal Wallis test, the difference between group 2 and group 4 was statistically significant ($p<0.005$).

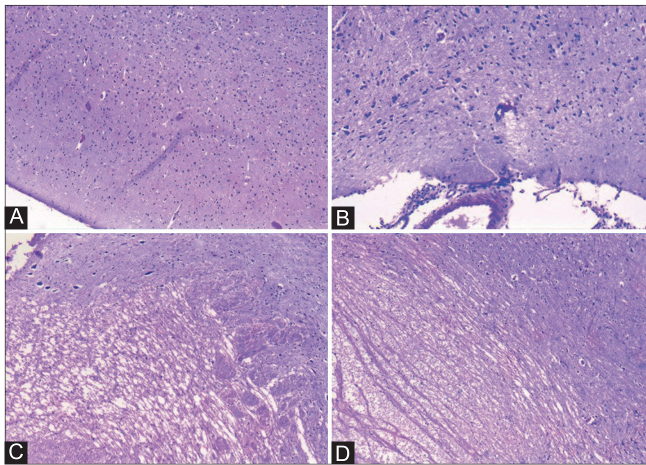

Figure 1: Light microscopic appearance of cerebral tissue by using Hematoxylin & Eosin staining. A. Control group, B. Cisplatin group, C. Edaravone group, D. Cisplatin + edaravone group.

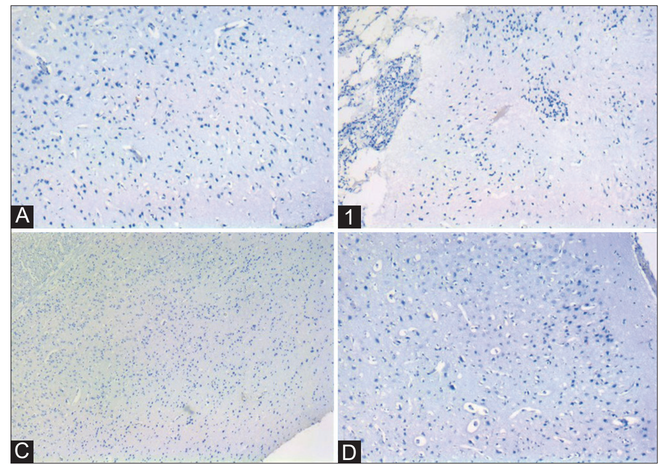

Figure 2: Immunohistochemical staining with p53 dye. A. Control group, B. Cisplatin group, C. Edaravone group, D. Cisplatin + edaravone group.

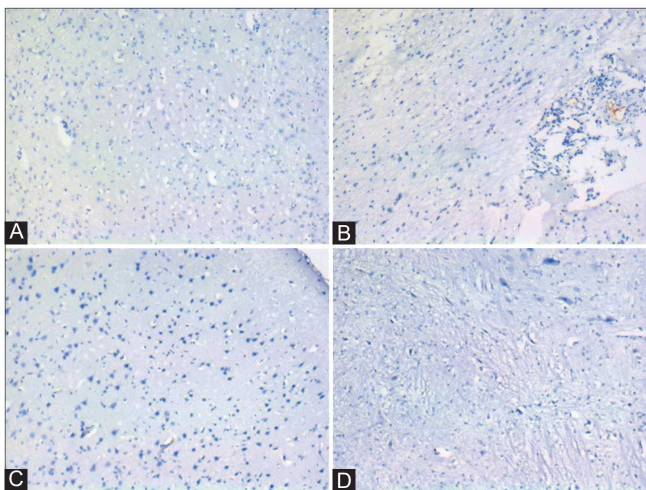

Figure 3: Immunohistochemical staining with Ki67 dye. A. Control group, B. Cisplatin group, C. Edaravone group, D. Cisplatin + edaravone group.

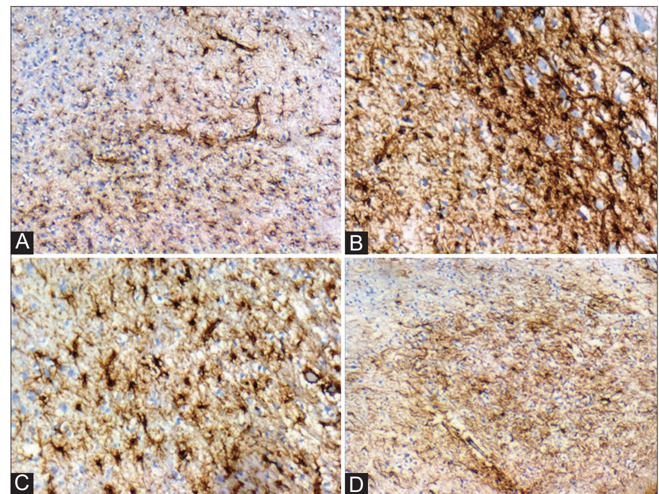

Figure 4: Immunohistochemical staining with GFAP dye. A. Control group, B. Cisplatin group, C. Edaravone group, D. Cisplatin + edaravone group.

and ROS levels in the cell.[9] p53 gene expression is increased as a result of DNA damage. All these protective mechanisms are geared towards repairing DNA damage.[10]

Resveratrol is an antioxidant that is found in many plants and has an antioxidant effect due to the phenol extract of it. Due to its antioxidant effect, resveratrol reduces free radicals formed as a result of oxidative stres.[11] Subsequently, effects of vitamin C, vitamin E have been demonstrated against the testicular injury in the rats administered valproic acid.[12] Abe *et al* reported that edaravone reversed the harmful effects due to liver ischemia.[13] Later, Kara *et al* reported that edaravone could be an effective agent in the short-term treatment and prevention of ovarian ischemia and reperfusion damage.[14] Kawasaki *et al* reported that edaravone might have a neuroprotective effect by reducing levels of OH- metabolites, increasing NO production and decreasing nNOS expression in brain cells.[15] Theories about the mechanism of action of edaravone are relation with neutralizing free radicals, scavenging ROS, inhibiting lipid peroxidation, and detoxifying hydroxyl radicals.[16] For the reasons listed, it was thought that edaravone may be useful in the prevention of cisplatin-induced brain damage.

In this study, serum MDA levels were found to be significantly higher in the cisplatin group than the cisplatin+edaravone group ($p<0.05$). On the contrary, NO levels were significantly lower in the cisplatin group than the cisplatin+edaravone group ($p<0.05$). It was also observed that edaravone diminished the histopsthologic damage. Cerebral injury criteria such as vascular changes, edema, and inflammation were evaluated. Edaravone improved the morphology and microscopic appearance of the cerebral tissue. The improvement in the morphology and structural characteristics was prominent in the cisplatin+edaravone group. Limitations of our study are the difficulty in adapting the findings in rats to humans and the relatively small sample size.

In conclusion, edaravone, a novel free-radical scavenger, was evaluated as a protective chemical on cisplatin induced cerebral injury. According to our short- term findings, edaravone seems to reverse cerebral injury due to cisplatin. However, large prospective, randomized trials are required.

Declarations

Ethical approval

The study protocol was reviewed and approved by Erciyes University Animal Experiments Local Ethics Committee (ERU-HADYEK). The date and number of the ethical report was 07.07.2021 and 21/159, respectively.

Competing interests

None declared.

Authors' contributions

Ozlem Kara: Project development, Data Collection, Manuscript writing Asuman Kilitci: Project development, Data management, Manuscript writing.

Data availability statement

The data used to support the findings are included within the article.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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