RESEARCH ARTICLE

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The Role of Edaravone on Cisplatin-Induced Lung Tissue Damage

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Objective: We aimed to assess the role of edaravone on cisplatin-induced lung tissue damage.

Materials and Methods: 4 groups were formed using 40 Wistar albino rats. In group 1 (control group) (n=10), neither any drugs were given nor anything was performed. In group 2 (cisplatin group) (n=10), only 7.5 mg/kg cisplatin was given. In group 3 (edaravone group) (n=10), only 1 mg/kg edaravone was given. In group 4 (cisplatin+edaravone group) (n=10), 7.5 mg/kg cisplatin and 1 mg/kg edaravone were administered. Blood malondialdehyde (MDA) and nitric oxide (NO) levels were studied. Lungs were removed in all groups. Tissue damage was evaluated by using a light microscop. In addition, immunohistochemistry was used for evaluation.

Results: In the cisplatin group, MDA and NO levels were found to be higher than the other groups ($p<0.05$). Histopathologic damage was more prominent in the cisplatin group than other groups and the difference was statistically significant ($p<0.05$). Immunstaining with CD68 and bcl 2 were indicated that immunoexpression was significantly higher in the cisplatin group than cisplatin+ edaravone group.

Conclusion: According to our short-term findings, edaravone is effective in the prevention of cisplatin-induced lung injury.

Key Words: *Rat, lung, cisplatin, edaravone*

Cisplatin'in Yol Açtığı Akciğer Hasarında Edaravon'un Rolü

Amaç: Cisplatin'in neden olduğu akciğer dokusu hasarında edaravonun rolünü değerlendirmeyi amaçladık.

Gereç ve Yöntemler: 40 adet Wistar albino rat kullanılarak 4 grup oluşturuldu. Grup 1'de (kontrol grubu) (n=10) herhangi bir ilaç verilmedi ve herhangi bir işlem yapılmadı. Grup 2'de (cisplatin grubu) (n=10) sadece 7,5 mg/kg cisplatin verildi. Grup 3'te (edaravon grubu) (n=10) sadece 1 mg/kg edaravon verildi. Grup 4'e (cisplatin+edaravon grubu) (n=10) 7,5 mg/kg cisplatin ve 1 mg/kg edaravon verildi. Kan malondialdehid (MDA) ve nitrik oksit (NO) düzeyleri çalışıldı. Tüm gruplarda akciğerler çıkarıldı. Doku hasarı ışık mikroskobu kullanılarak değerlendirildi. Ayrıca değerlendirme için immünohistokimya kullanıldı.

Bulgular: Cisplatin grubunda MDA ve NO düzeyleri diğer gruplara göre daha yüksek bulundu ($p<0.05$). Histopatolojik hasar cisplatin grubunda diğer gruplara göre daha belirgindi ve aradaki fark istatistiksel olarak anlamlıydı ($p<0.05$). CD68 ve bcl 2 ile immün boyama, sisplatin grubunda immün ekspresyonun cisplatin+edaravon grubuna göre anlamlı derecede yüksek olduğunu gösterdi.

Sonuç: Kısa dönem bulgularımıza göre edaravon, cisplatin kaynaklı akciğer hasarının önlenmesinde etkilidir.

Anahtar Kelimeler: *Sıçan, akciğer, cisplatin edaravon*

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Introduction

Congenital small bowel atresia (duodenal, jejunal and ileal atresia) is a well-recognized, frequent cause of Cisplatin, a potent antineoplastic, has negative effects on many organs and systems, especially kidney, liver and nervous tissue (1). Platinum compounds in cisplatin form complexes with DNA. Besides, cisplatin leads to an increase in the levels of reactive oxygen species and free radicals due to elevated lipid peroxidation. The direct cell-damaging effect is also responsible for the undesirable effect. As a result of all these interactions, the net effect is cell damage and oxidative stress (2). When cisplatin is given in high doses, normal cells are negatively affected as well as cancer cells. These side effects can also be seen in the lungs, pancreas, ovarium and intestines (3-5).

Numerous drugs and molecules such as simvastatin, quercetin, and silymarin have been introduced to reverse the cisplatin induced cell toxicity (6-8). These chemical agents protect the cell by using enzymatic and non-enzymatic systems. Edaravone is a free radical scavenger and has been utilized for years for treatment of ischemic events such as cerebral infarct and stroke (9). Edaravone, mainly is an antioxidant, decreases lipid peroxidation by inhibiting lipooxyenase. In addition, edaravone contributes to the neutralization of free radicals by increasing electron transport (10). For all these

reasons, we thought that edaravone may be effective in preventing cisplatin-induced lung injury. To the best of our knowledge, there is currently no other study investigating the protective effect of edaravone on cisplatin-induced lung injury.

Materials and Methods

Research and Publication Ethics: The ethical consent and protocol was approved by Erciyes University Animal Experiments Local Ethics Committee with the date of 07.07.2021 and number of 21/160.

Forty adult Wistar albino rats were used for the study. The animals were taken from the Department of Erciyes University Animal Experiments. The study was carried out in Erciyes University Faculty of Medicine, Department of Histology-Embryology. The rats were fed with free access to the food and water and treated to a heat between 20 and 22°C and to a 12 hour sunlight during the day. All the animals were randomly allocated into the 4 groups. The instructions at the website of www.randomization.com were followed.

4 groups were created. In group 1 (n=10, control group), nothing was given. In group 2 (n=10, cisplatin group), single dose 7,5 mg/kg cisplatin was administered. In group 3 (n=10, edaravone group) single dose 1 mg/kg edaravone was given. In group 4 (n=10, cisplatin+ edaravone group), single dose 7.5 mg/kg cisplatin and 1 mg/kg edaravone were administered. Ketamine hydrochloride (50 mg/kg, Ketalar, Eczacibasi, Istanbul, Turkey) and xylazin hydrochloride (5 mg/kg, Rompun, Bayer, Leverkusen, Germany) were given via intraperitoneal route for anesthesia. Blood samples were yielded by puncturing the cardiac tissue. Lung tissue was extirpated and then, sacrification procedure was executed by applying cervical dislocation.

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 lung 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 (11). To grade the damage to the lung; neutrophyle infiltration, congestion, narrowing of alveolar spaces and inflammation were utilized as the parameters of the scoring system.

CD 68 and Bcl 2 Measurement: Expressions of macrophage CD 68 and Bcl 2 were fulfilled by 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) (11).

Biochemistry: The MDA kit (Cat. No: E0156Ra, Bioassay Technology Laboratory, Türkiye) 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, Türkiye 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. Kruskal Wallis test and Mann-Whitney U test were used for analyses. p-value <0.05 was considered as significant.

Results

There was no death in the groups. In total 40 rats were included into the study and serum NO and MDA levels were demonstrated in Table 1. The MDA levels significantly increased in the cisplatin group than control group ($p<0.05$). A significant decrease was observed in the cisplatin+ edaravone group compared to the cisplatin group ($p<0.05$). The NO levels were significantly higher in the cisplatin group than the cisplatin+ edaravone group ($p<0.05$).

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	0.48±0.04
Group 2 (n=10)	9.75±0.95*	0.85±0.09*
Group 3 (n=10)	19.28±2.76	0.51±0.05
Group 4 (n=10)	13.56±2.08*	0.49±0.04*
p value	0.026	0.037

* The difference between group 2 and group 4 was statistically significant ($p<0.05$).

There was no difference among the groups in relation with the macroscopic features. Total tissue damage scores were demonstrated in Table 2. The histopathological tissue injury was found to be significantly higher in the cisplatin group than the cisplatin+ edaravone group ($p<0.05$). Also, macrophage CD 68 and bcl 2 expressions were significantly higher in the cisplatin group than in the cisplatin+resveratrol group ($p<0.05$) (Table 3).

The morphology of the lung tissue was detected as normal in the control group (Figure 1A). Exposure to the cisplatin led to an increase in the neutrophyle infiltration, congestion, narrowing of alveolar spaces. Moreover, inflammation was prominently seen in the cisplatin group (Figure 1B). In the edaravone group, the appearance and microscopy of the lung tissue was similar to the control group (Figure 1C). In the cisplatin+edaravone group, addition of edaravone improved the congestion, and inflammation (Figure 1D).

In the cisplatin group, the expression of the CD 68 and bcl 2 were more common in the cisplatin group than in the cisplatin+ edaravone group (Figure 2 and 3).

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)
Neutrophyle infiltration	0.80	1.60*	1.00	1.20*
Congestion	1.40	2.80*	1.50	1.60*
Narrowing of alveolar spaces	0.00	1.00*	0.00	0.00*
Inflammation	0.00	2.00*	0.00	1.00*

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

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)
Macrophage CD 68	0.00	2.00*	0.00	0.00*
Bcl 2	0.00	1.00*	0.00	0.00*

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

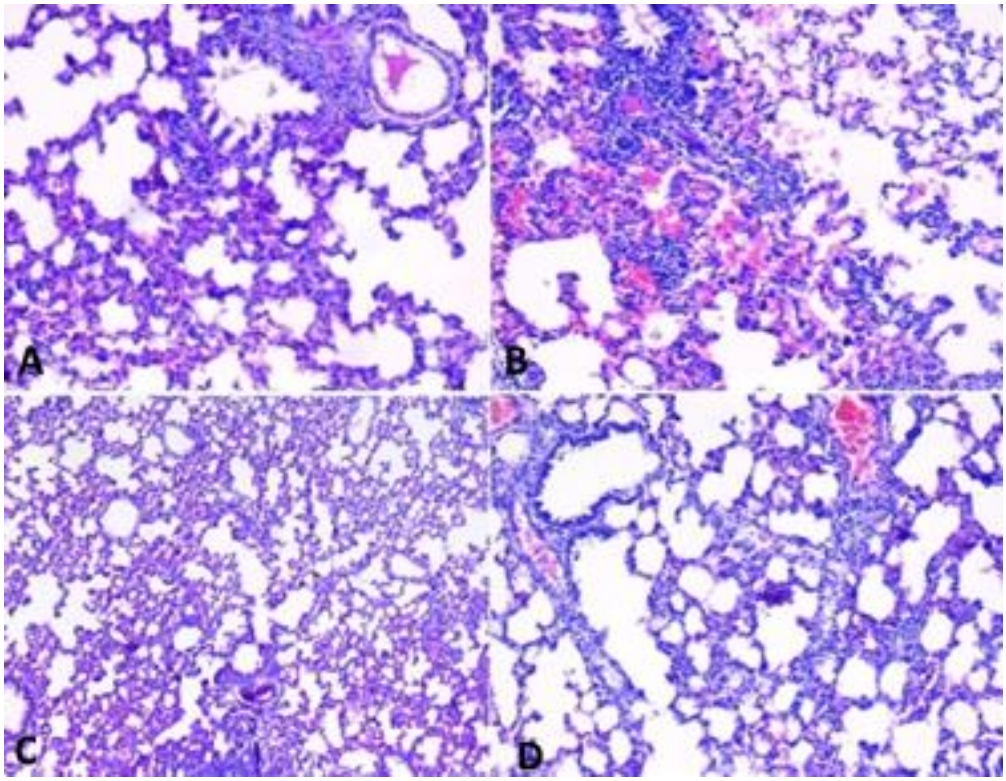

Figure 1. Light microscopic appearance of lung tissue by using Hematoxylin & Eosin staining. **A.** Control group, **B.** Cisplatin group, **C.** Edaravone group, **D.** Cisplatin + edaravone group.

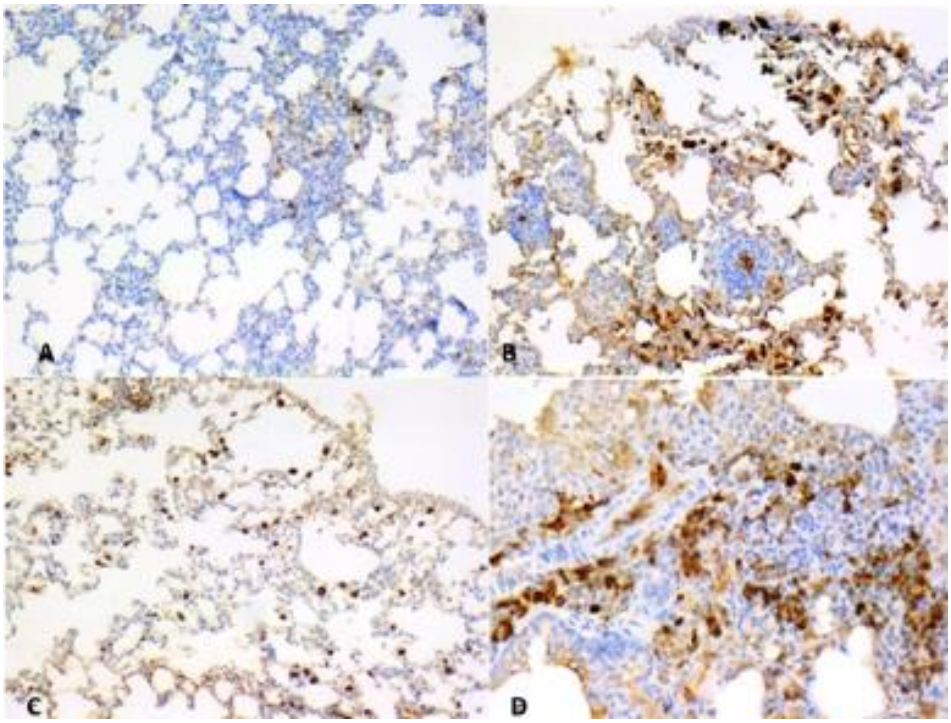

Figure 2. Immunohistochemical staining with CD 68 dye. **A.** Control group, **B.** Cisplatin group, **C.** Edaravone group, **D**. Cisplatin + edaravone group

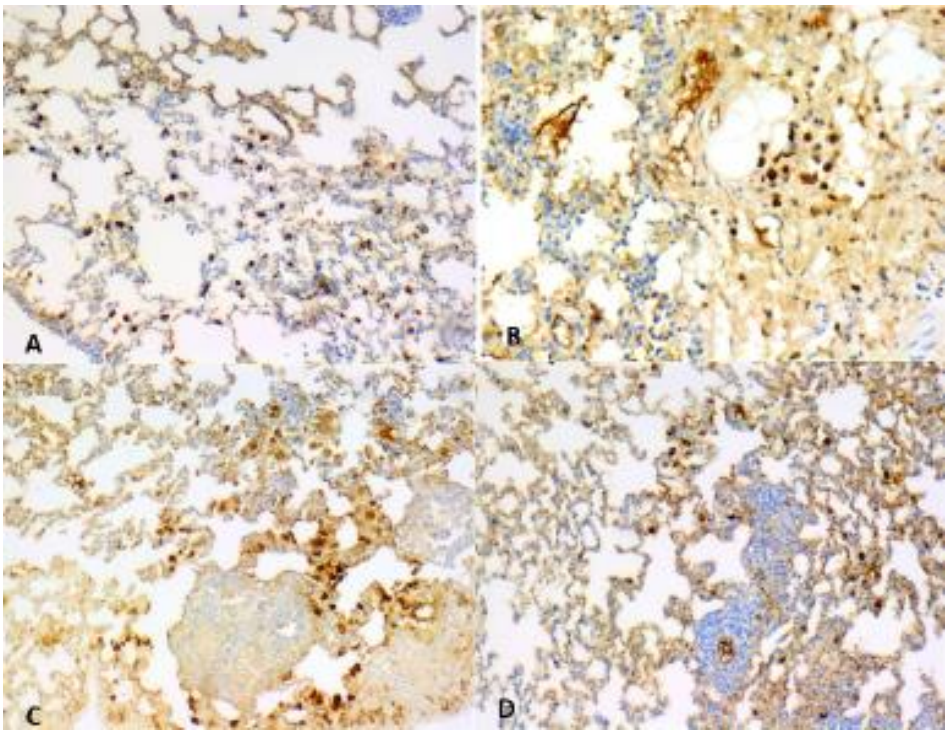

Figure 3. Immunohistochemical staining with bcl 2 dye. **A.** Control group, B. Cisplatin group, **C.** Edaravone group, **D.** Cisplatin + edaravone group

Discussion

In this randomized controlled study, the effect of edaravone on ciplatin induced lung toxicity was studied. Our study has revealed that, serum MDA and NO levels tend to increase due to cisplatin and edaravone seems to reduce these MDA and NO levels. Furthermore, tissue damage was more prominent in cisplatin group than

cisplatin+edaravone group, and the difference was found to be statistically significant ($p<0.05$).

Antineoplastic drugs such as cisplatin have been used in the treatment of cancers of ovarium, lung and solid organs (12). The improvement and combination of cancer therapy led to an increase in survival rates. This has made it important to prevent cisplatin-related toxicity. The toxicity of the cisplatin is dose dependent. Borovskaya et al reported that the dose and duration of cisplatin were the major contributed factors in terms of the toxicity of cisplatin (13). Cisplatin behaves as a direct cellular toxin, increases the activity of lipooxygenase and finally causes an increase in the levels of free radicals and reactive oxygen species (14).

Since oxidative stres has been introduced an important cause of cisplatin toxicity many antioxidants were used to reverse the adverse effects due to cisplatin (15). Kara et al. (16) reported that an antioxidant chemical, resveratrol, reduces free radicals and decreases the harmful effect of oxidative stres. Abe et al. (17) reported that edaravone reversed the harmful effects due to liver ischemia. Later, Kara et al. (18) reported that edaravone could be an effective agent in the short-term treatment and prevention of ovarian ischemia and reperfusion damage. Theories about the mechanism of action of edaravone are relation with neutralizing free radicals, scavenging ROS, inhibiting lipid peroxidation, and detoxifying hydroxyl radicals (19). In the present study, intraperitoneal administration of edaravone decreased the MDA and NO levels and enhanced the morphology and microscopic appearance of the lung tissue. The improvement in the morphology and structural characteristics was prominent in the cisplatin+edaravone group.

In this study, a promising free-radical scavenger, edaravone, was assessed on cisplatin induced lung injury. Edaravone led to an improvement both histogically and biochemically. The levels of MDA and NO significantly decreased. Also, histopathological damage due to cisplatin was reduced with edaravone treatment.

In conclusion, according to our short-term findings, edaravone seems to reverse lung injury due to cisplatin. However, large prospective, randomized trials are required.

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