

## Cisplatin에 의해 유도된 이독성에 대한 Melatonin의 효과

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### Effect of Melatonin on the Cisplatin Induced Ototoxicity in Rats

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#### ABSTRACT

**Background and Objectives** : Cisplatin (CP), an antitumor agent widely used in the treatment of head and neck cancers, has side effects such as ototoxicity and nephrotoxicity. These side effects are closely related to oxidative stress. In the present study, we attempted to suppress CP-induced ototoxicity in rats by administering melatonin, an antioxidant. **Materials and Method** : Male Sprague-Dawley rats were divided into different groups and were treated as follows : 1) saline control, 2) CP (16 mg/kg, i.p.), 3) CP plus melatonin (10 mg/kg, i.p.). The rats were sacrificed at the 6th day after CP treatment. **Results** : CP-treated rats showed increase in cochlear malondialdehyde, hydrogen peroxide, glutathione peroxidase and glutathione reductase levels, and the decrease in cochlear superoxide dismutase (SOD) and catalase levels. CP-treated rats showed markedly decreased in the number of stereocilia on the inner hair cells and mildly decreased in the number of outer hair cells in organ of Corti under the light and scanning electron microscopic examination. Light and electron microscopic findings, and cochlear hydrogen peroxide, malondialdehyde, SOD, catalase, glutathione peroxidase and glutathione reductase levels were restored in the rats injected with CP plus melatonin than those with CP alone. **Conclusion** : These results suggest that melatonin suppresses CP-induced ototoxicity via the suppression of the increased production of reactive oxygen species. (Korean J Otolaryngol 2003;46:808-14)

**KEY WORDS** : Cisplatin · Melatonin · Ototoxicity · Reactive oxygen species .

Cisplatin , , cisplatin , ,  
 , , , ,  
 가 , melatonin(N - acetyl -  
 5 - methoxytryptamine) peroxy radical  
 가 radical  
 , superoxide dismutase glutathione pe-  
 roxidase 가  
 superoxide dismutase, catalase glutathione system  
 가 .<sup>5)</sup> safrole DNA  
 .<sup>2)</sup> paraquat lipopo-  
 lysaccharide  
 : 2003 4 9 / : 2003 7 16  
 : , 700 - 712 194  
 : (053) 250 - 7711, 7714 · : (053) 250 - 7712  
 E - mail : mun@ dsmc.or.kr melatonin cisplatin

malondialdehyde

Cisplatin (300~370 g) 4 Sprague - Dawley 3

1) ( 1 ) 30 12  
5% ethanolic saline 4 mL 1 mL  
72 5% ethanolic saline  
4 mL . 2) Cisplatin ( 2 )  
55 Rybak 3) 12  
5% ethanolic saline 4 mL , kg  
16 mg cisplatin(Sigma, St. Louis, MO, )  
1 mL 72  
5% ethanolic saline 4 mL  
. 3) Cisplatin melatonin melatonin  
( 3 ) 50 12 Floreani  
7) 5% ethanolic saline 4 mL mg 10  
melatonin(Sigma, St. Louis, MO, ) kg  
mg kg  
16 mg cisplatin 1 mL  
72 5% ethanolic  
saline 4 mL melatonin kg 10 mg  
6 ,  
10 teflon  
glass homogenizer(chamber clearance 0.005~0.007  
inches, Wheaton Scientific, Milville, NJ, )  
10%(w/v)  
2,000 rpm 10

orange 560 nm

Malondialdehyde  
0.25 N 0.375% thiobarbituric acid  
15% trichloroacetic acid ,  
100 15 3,000 x  
g 5 535 nm  
malondialdehyde  
 $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$

Superoxide dismutase  
Sun 8) xanthine oxidase가 nitroblue  
tetrazolium , 1 unit  
nitroblue tetrazolium  
50%

Catalase  
Nelson Kiesow 9)  
25 30 240 nm  
가  
mg , 1 1

Glutathione peroxidase  
Paglia Valentine 10)  
glutathione, NADPH , glu-  
tathione reductase 25  
glutathione  
glutathione , glutathione  
reductase NADPH glutathione  
glutathione , NADPH가 NADP<sup>+</sup>  
340 nm time scan ,  
NADPH ( $E_{340\text{nm}} = 6.22 \text{ mM}^{-1}\text{cm}^{-1}$ )  
1 1 mg NADP<sup>+</sup>

Glutathione reductase  
Glutathione reductase Goldberg  
Spooner 11) glutathione NA-  
DPH 37 2

NADPH가 NADP<sup>+</sup> 340 nm  
 1 1 mg  
 NADPH  
 10%  
 5 μm  
 hematoxylin & eosin  
 0.5% paraformaldehyde 0.5% glutaraldehyde 가  
 , 1% OsO<sub>4</sub> 2  
 0.1 mol/L 25% dimethyl  
 sulfoxide (DMSO) 30 , 50% DMSO 30  
 (cracking) 50% DMSO 2% tannic acid  
 , 1% OsO<sub>4</sub>  
 , isoamyl acetate  
 Pt - Pd  
 Hitachi S - 4200  
 ±  
 Student 's t - test  
 paired t - test  
 0.05

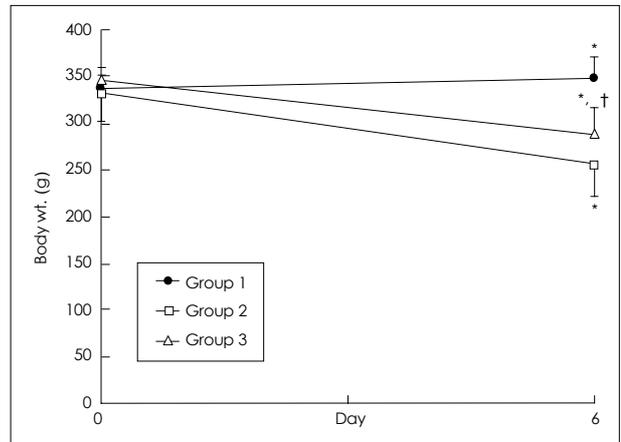


Fig. 1. Changes in mean body weight of animals during the experiment. Group 1 : control (n=6), group 2 : cisplatin treatment (16 mg/kg, i.p., n=11), group 3 : cisplatin+melatonin treatment (16 mg/kg, i.p., n=10). \*p<0.05 vs. day 0, † p<0.05 vs. group 2.

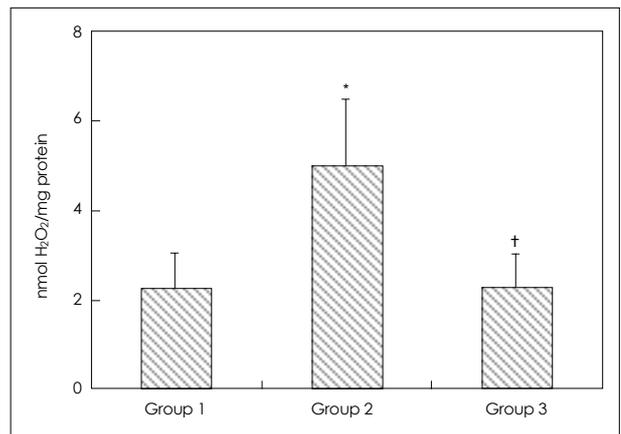
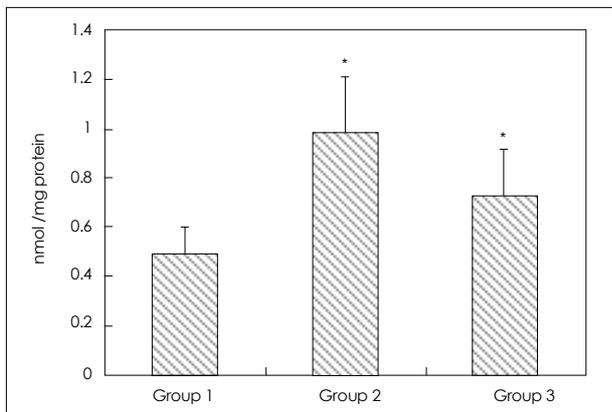


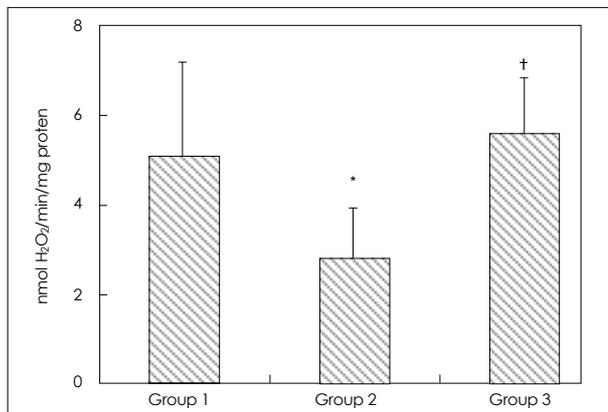
Fig. 2. Hydrogen peroxide levels in cochlear homogenate. Group 1 : normal control group, group 2 : cisplatin (16 mg/kg, i.p.) treated group, group 3 : cisplatin (16 mg/kg, i.p.) and melatonin (10 mg/kg, i.p.) treated group. \*p<0.05 vs. group 1, † p<0.05 vs. group 2.

100%  
 , cisplatin 37.5%, cisplatin mela-  
 tonin 48.2%  
 (Fig. 1) 337 ± 15 g 349 ±  
 22 g 가 , cisplatin 333  
 ± 30 g 256 ± 29 g . Mela-  
 tonin 346 ± 14 g 288 ± 35 g  
 (p<0.05), cisplatin (p<0.05).  
 2.24 ± 0.83 nmol H<sub>2</sub>O<sub>2</sub>/mg  
 protein , cisplatin 4.96 ± 1.54 nmol

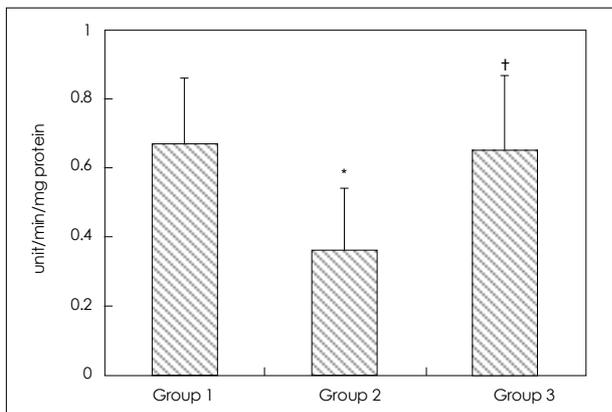
H<sub>2</sub>O<sub>2</sub>/mg protein 가  
 (p<0.05). Melatonin 2.26 ± 0.78 nmol  
 H<sub>2</sub>O<sub>2</sub>/mg protein 가  
 , cisplatin  
 (p<0.05)(Fig. 2).  
 Malondialdehyde  
 malondialdehyde 0.49 ± 0.11 nmol/mg  
 protein , cisplatin 0.98 ± 0.23 nmol/mg  
 protein 가  
 (p<0.05). Melatonin 0.73 ± 0.19 nmol/mg protein  
 가 (p<0.05),  
 cisplatin



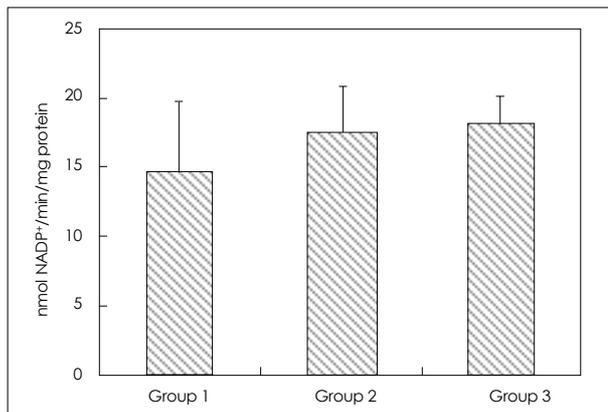
**Fig. 3.** Malondialdehyde levels in cochlear homogenate. Group 1 : normal control group, group 2 : cisplatin (16 mg/kg, i.p.) treated group, group 3 : cisplatin (16 mg/kg, i.p.) and melatonin (10 mg/kg, i.p.) treated group. \*p<0.05 vs. group 1.



**Fig. 5.** Catalase levels in cochlear homogenate. Group 1 : normal control group, group 2 : cisplatin (16 mg/kg, i.p.) treated group, group 3 : cisplatin (16 mg/kg, i.p.) and melatonin (10 mg/kg, i.p.) treated group. \*p<0.05 vs. group 1, †p<0.05 vs. group 2.



**Fig. 4.** Superoxide dismutase levels in cochlear homogenate. Group 1 : normal control group, group 2 : cisplatin (16 mg/kg, i.p.) treated group, group 3 : cisplatin (16 mg/kg, i.p.) and melatonin (10 mg/kg, i.p.) treated group. \*p<0.05 vs. group 1, †p<0.05 vs. group 2.



**Fig. 6.** Glutathione peroxidase levels in cochlear homogenate. Group 1 : normal control group, group 2 : cisplatin (16 mg/kg, i.p.) treated group, group 3 : cisplatin (16 mg/kg, i.p.) and melatonin (10 mg/kg, i.p.) treated group.

(Fig. 3).

Superoxide dismutase

superoxide dismutase 0.67 ± 0.19  
unit/min/mg protein, cisplatin 0.36 ±  
0.18 unit/min/mg protein  
(p<0.05). Melatonin 0.65 ± 0.22  
unit/min/mg protein 가  
, cisplatin 가  
(p<0.05)(Fig. 4).

Catalase

catalase 5.09 ± 2.11 nmol H<sub>2</sub>O<sub>2</sub>/  
min/mg protein, cisplatin 2.78 ± 1.16  
nmol H<sub>2</sub>O<sub>2</sub>/min/mg protein

. Melatonin 5.56 ± 1.27 nmol H<sub>2</sub>O<sub>2</sub>/  
min/mg protein 가  
, cisplatin 가  
(p<0.05)(Fig. 5).

Glutathione peroxidase

glutathione peroxidase 14.59  
± 5.11 nmol NADP<sup>+</sup>/min/mg protein, cisplatin  
17.43 ± 3.38 nmol NADP<sup>+</sup>/min/mg protein,  
melatonin 16.68 ± 3.43 nmol NADP<sup>+</sup>/min/mg  
protein (Fig. 6).

Glutathione reductase

glutathione reductase 22.04  
± 8.36 pmol NADPH/min/mg protein, cisplatin

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46.96 ± 12.90 pmol NADPH/min/mg protein  
 가 (p<0.05).  
 Melatonin 43.15 ± 11.29 pmol NADPH/min/  
 mg protein 가 (p<0.05), cisplatin  
 (Fig. 7).

stereocilia 가 cisplatin (Fig. 8C).

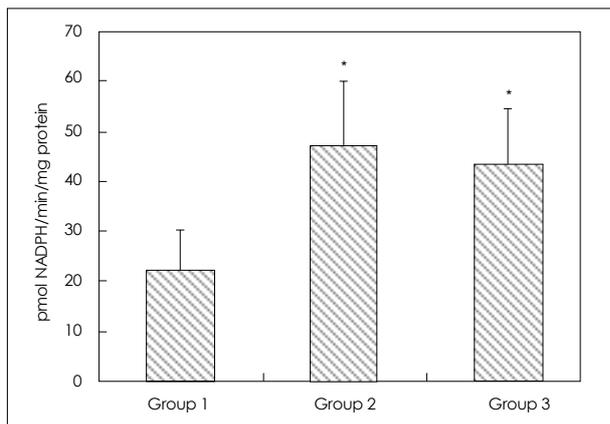
stereocilia 가 (Fig. 9A), cisplatin

(Fig. 9B). Melatonin (Fig. 9C).

stereocilia 가 (Fig. 8A). Cisplatin

stereocilia가 (outer hair cell)

(Fig. 8B). Melatonin



**Fig. 7.** Glutathion reductase levels in cochlear homogenate. Group 1 : normal control group, group 2 : cisplatin (16 mg/kg, i.p.) treated group, group 3 : cisplatin (16 mg/kg, i.p.) and melatonin (10 mg/kg, i.p.) treated group. \*p<0.05 vs. group 1.

superoxide radical

.<sup>1)</sup> , superoxide

superoxide dismutase

catalase glutathione peroxidase

가 , melatonin

가 .<sup>5)12)</sup> cisplatin

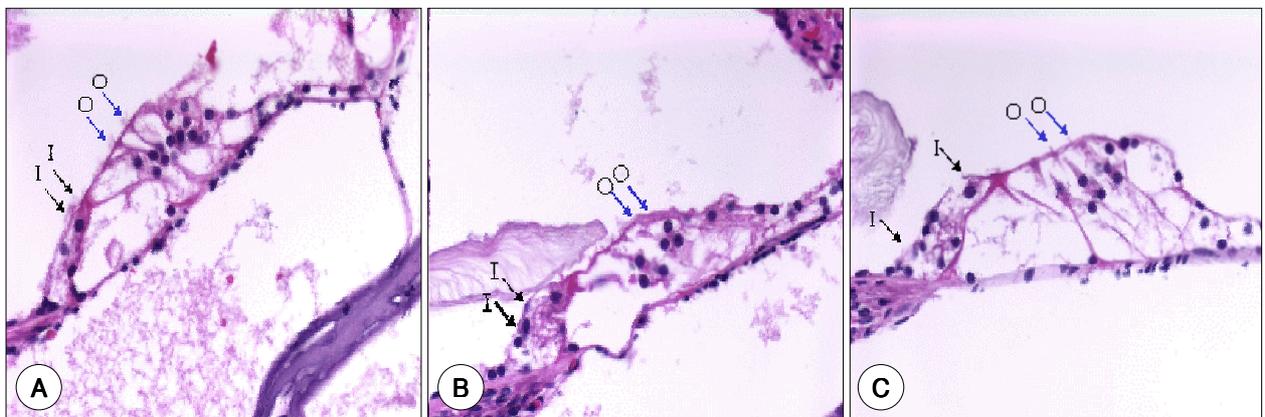
가 .<sup>3)13)14)</sup> Teranishi <sup>15)</sup> guinea pig - tocopherol

cisplatin Preyer

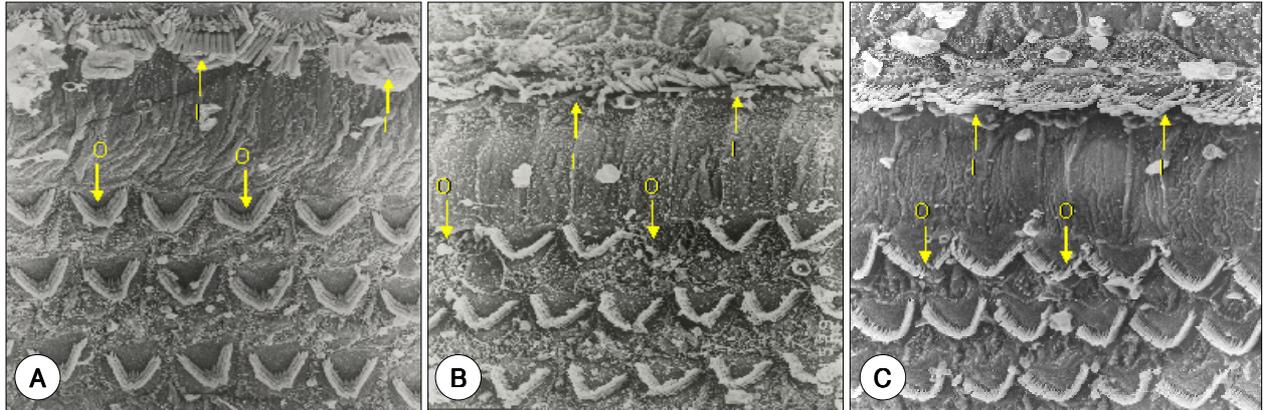
malondialdehyde

가 . Lopez - Gonzalez <sup>14)</sup>

cisplatin melatonin



**Fig. 8.** Light microscopic findings in organ of Corti (H & E, ×00). A : Control group (Group 1). Normal stereociliary arrangements on the inner (I) and outer (O) hair cells. B : Cisplatin (16 mg/kg, i.p.) treated group (Group 2). Markedly decreased in the number of stereocilia on the inner and mildly decreased in the number of outer hair cells comparing group 1. C : Cisplatin (16 mg/kg, i.p.) and melatonin (10 mg/kg, i.p.) treated group (Group C). A few stereocilia are present on the inner and outer hair cells when compared to group 2.



**Fig. 9.** Scanning electron microscopic findings in organ of Corti (H & E,  $\times 400$ ). A : Control group (Group 1). Inner (I) and outer (O) hair cells are well preserved. B : Cisplatin (16 mg/kg, i.p.) treated group (Group 2). Partial loss of inner hair cells and outer hair cells can be seen in the basal turn when compared to group 1. C : Cisplatin (16 mg/kg, i.p.) and melatonin (10 mg/kg, i.p.) treated group (Group C). Stereocilia are relatively well-preserved on the inner and outer hair cells when compared to group 2.

가 superoxide dismutase  
 cisplatin  
 superoxide dismutase catalase  
 superoxide 가  
 melatonin 가  
 19)  
 glutathione peroxidase  
 glutathione reductase  
 cisplatin melatonin  
 가 , cisplatin  
 melatonin  
 cisplatin  
 cisplatin  
 cisplatin  
 200% 가 , cisplatin  
 melatonin  
 electron donor  
 melatonin  
 17)  
 malondialdehyde  
 200% , Rybak  
 3)  
 Melatonin  
 malondialdehyde  
 3)  
 cisplatin melatonin  
 melatonin  
 stereocilia  
 melatonin cisplatin  
 reocilia 가 ste-  
 cisplatin  
 Superoxide dismutase catalase 가 가 , melatonin  
 cisplatin , melatonin  
 cisplatin 가  
 , cisplatin  
 melatonin superoxide dismutase ca-  
 talase cisplatin 가 supe-  
 roxide dismutase catalase , cochlea  
 . Ichikawa 18)  
 superoxide

cisplatin  
melatonin

melatonin cisplatin

, stereocilia

melatonin cisplatin

: Cisplatin · Melatonin ·

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