The preventive effect of bismuth and vitamin E combination on cisplatin hepatotoxicity in cancer patients

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Abstract

Background and objectives: The objective of this study was to explore the optimal combination of agents with their doses used along with cisplatin for the protection of hepatotoxicity.

Methods: This experiment was carried out on a patients suffering from different solid types of tumor divided into two groups: cisplatin group receiving cisplatin in a dose of 90 mg/ m² body surface area(BSA) and the therapy group receiving cisplatin in a dose of 90 mg/m² BSA and bismuth subcitrate (200mg/day) with vitamin E (400mg/day).

Ten healthy subjects were taken as a control group.Total serum bilirubin (TSB), serum glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT) and alkaline phosphatase (ALP) were tested for the assessments of liver function.

Results: The level of TSB, GPT, GOT and ALP in healthy subjects did not changed significantly with respect to baseline value along the entire period of the study ; while cisplatin group showed a sustain and significant elevation in TSB, GPT, GOT and ALP level in comparison with baseline along the entire period of the study, $P<0.05$. Meanwhile, the hepatotoxic effect of cisplatin was shown to be slightly but however significantly decreased in patients received bismuth-vitamin E therapy ($P<0.05$) along the entire period of the study.

Conclusion: Bismuth and vitamin E combination play a beneficial role for prevention of cisplatin hepatotoxicity. The potentiated actions for prevention of cisplatin hepatotoxicity could be achieved via combined use of these agents .

Key words: Cisplatin; Side effects; Protective agents; Oxidative stress; Hepatotoxicity

Introduction

Cisplatin (*cis*-diamine-dichloroplatinum) is a prominent member of the effectivebroad-spectrum antitumor drugs. However, its clinical usage is restricted due to some adverse side effects, such as ototoxicity and nephrotoxicity. [1–3]The cisplatin-induced ototoxicity and nephrotoxicity have been very well studied inboth clinical and animal researches; however hepatotoxicity has been rarely paid attention to. Recent studies around the world suggested that hepatotoxicity is also amajor dose -limiting side effect in cisplatin-based chemotherapy. [4–7] Continued aggressive high-dose cisplatin chemotherapy necessitates the investigation of ways for prevention of the dose-limiting side effects that inhibit the cisplatin administration at tumoricidal doses. Until now a large number of studies have been focused on the ways for prevention of cisplatin side effects via supplementation of preventive agents simultaneously [8-10] although the mechanism underlying the side effects induced by cisplatin are not understood clearly, it was considered to be attributed to the combination of multi- ways [5,11,12], such as the generation of reactive oxygen species (ROS), which could interfere with the antioxidant defense system and result in oxidative damage in different tissues [6,13,14], and reaction with thiols in protein and glutathione, which could cause

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cell dysfunction. On the other hand, it has been proposed that the antitumor activity of cisplatin is due to its ability to form adducts with DNA, which could cause cross-linking of DNA strands. Bismuth is known to induce the synthesis of Metallothionein [15], and it has been shown that pretreatment with bismuth complexes can prevent the toxic side effects of the anti-cancer drug cisplatin without compromising its anti-tumor activity. [16,17] Recent studies have on the role of anti oxidants in CP toxicity. Administration of antioxidants such as vitamin C [18], vitamin E and selenium [19-21] before treatment with CP has been used to protect against toxicity inhuman and experimental animals. As the antitumor activity and side effects in cisplatin- based chemotherapy are mediated in part by different mechanisms, the actions on selective inhibition of certain side effects could be achieved while the antitumor activity is not altered. [22] This study attempted to explore the preventive effect of the combination of vitamin E and bismuth for the prevention of cisplatin hepatotoxicity.

Methods

All healthy subjects and patients were informed and signed consent before the study. Ten (10) healthy subjects aged between 25-65 years are taken as a control group, sixty (60) patients suffering from different types of solid tumors aged between 25-65 years attending Rizgari Teaching Hospital were included in this study, these patients were divided randomly into two groups, the first group represents cisplatin group , 30 patients (12) male, (18) female treated with cisplatin in a dose of 90 mg/ m² at 21days interval and the second group represents therapy group treated with cisplatin in a dose of 90 mg/ m² at 21days interval and bismuth subcitrate (200mg/day) with vitamin E (400mg/day).Blood samples were taken at baseline before the initiation of therapy and 21 day intervals after that for three cycles. Each sample was transferred with plastic centrifuge tube, sera were separated by centrifugation at 1000 rpm for 10 minutes and analyzed for TSB, GPT, GOT and ALP. Total serum bilirubin (TSB) was measured with Reflotron using its special strips, REF 1 0905321.Serum glutamic pyruvic transaminase (GPT) was measured with Reflotron using its special strips, REF 1 0745138. Serum glutamic oxaloacetic transaminase (GOT) was measured with Reflotron using its special strips, REF 1 0745120.Serum alkaline phosphatase (ALP) was measured with Reflotron using its special strips, REF 1 1622773.Statistical evaluation: All data are expressed as mean ± SD. Differences between mean levels with baseline along the entire period of the study were evaluated statistically using students t-test. A value of $P< 0.05$ was considered statistically significant.

Results

1. TSB :

The level of TSB in healthy subjects did not changed significantly with respect to baseline value along the entire period of the study; while patients received cisplatin showed a gradual and significant elevation ($P<0.05$) in comparison with baseline values, reaching maximum after 63 days (36.36%); meanwhile, patients received bismuth-vitamin E combination therapy show a slight but however significant elevation in TSB levels in comparison with baseline value ($P<0.05$), reaching maximum after 63 days (6.52%), as shown in (Table (1) and (Figure (1).

2. GPT :

The level of GPT in healthy subjects did not changed significantly with respect to baseline value along the entire period of the study; while patients received cisplatin showed a gradual and significant elevation ($P<0.05$) in comparison with baseline values, reaching maximum after 63 days (30.63%);meanwhile, patients received bismuth-vitamin E combination therapy show a slight but however significant elevation in GPT levels in comparison with baseline value ($P<0.05$), reaching maximum

after 63 days (3.02%), as shown in (Table 2) and (Figure 2).

3. GOT :

The level of GOT in healthy subjects did not changed significantly with respect to baseline value along the entire period of the study; while patients received cisplatin showed a gradual and significant elevation (P<0.05) in comparison with baseline values, reaching maximum after 63 days (30.66%); meanwhile, patients received bismuth-vitamin E combination therapy show a slight but however significant elevation in GOT levels in comparison with baseline value (P<0.05), reaching maximum after 63 days (3.02%), as shown in (Table 3) and (Figure 3). The level of ALP in healthy subjects did not changed significantly with respect to baseline value along the entire period of the study; while patients received cisplatin showed a gradual and significant elevation (P<0.05) in comparison with baseline values, reaching maximum after 63 days (27.99%); meanwhile, patients received bismuth-vitamin E combination therapy show a slight but however significant elevation in ALP levels in comparison with baseline value (P<0.05), reaching maximum after 63 days (4.54%), as shown in (Table 4) and (Figure 4).

4. ALP :

The level of ALP in healthy subjects did not changed significantly with respect to baseline value along the entire period of the study; while patients received cisplatin showed a gradual and significant elevation (P<0.05) in comparison with baseline values, reaching maximum after 63 days (27.99%); meanwhile, patients received bismuth-vitamin E combination therapy show a slight but however significant elevation in ALP levels in comparison with baseline value (P<0.05), reaching maximum after 63 days (4.54%), as shown in (Table 4) and (Figure 4).

Table (1): Total serum bilirubin level in control and patients received bismuth-vitamin E combination therapy.

	Total serum bilirubin (< 1.0 mg/dl)			
	Baseline	After 21 days	After 42 days	After 63 days
Healthy group (n=10)	0.429 ± 0.04	0.436 ± 0.04	0.435 ± 0.03	0.435 ± 0.03
Cisplatin group (n30)	0.440 ± 0.07	0.530 ± 0.08 *	0.570 ± 0.09	0.600 ± 0.10*
Therapy group (n=30)	0.460 ± 0.08	0.470 ± 0.08 *	0.480 ± 0.08 *	0.490 ± 0.08 *
P-value	0.24 [NS]	0.034 [S]	0.005 [S]	0.002 [S]

Data are expressed as mean ± SD.
n= number of patients.
*P<0.05 with respect to baseline value.
S = significant difference.
NS = no significant difference.

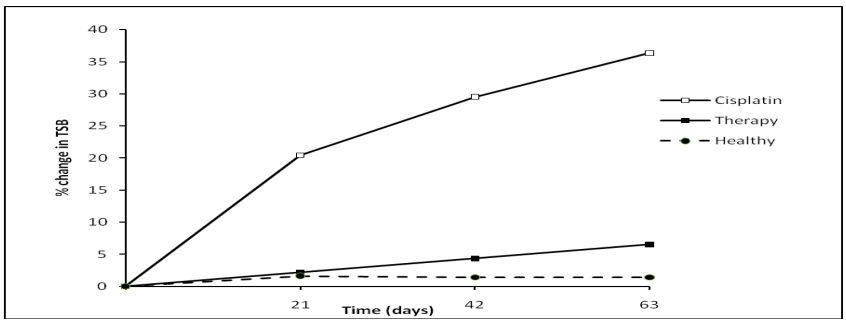

Figure 1: Percent change in TSB in control and patients received bismuth-vitamin E combination therapy along the study period

Table 2: Serum GPT level in control and patients received bismuth-vitamin E combination therapy.

	Serum GPT (< 41 IU/L)			
	Baseline	**After 21 days**	**After 42 days**	**After 63 days**
Healthy group (n=10)	20.00 ± 0.04	20.10 ± 0.39	20.14 ± 0.48	20.28 ± 0.51
Cisplatin group (n=30)	20.81 ± 3.05	22.48 ± 3.30*	24.95 ± 3.66*	26.45 ± 3.88*
Therapy group (n=30)	18.56 ± 3.37	18.75 ± 3.40 *	18.79 ± 3.41 *	19.12 ± 3.47 *
P-value	0.37 [NS]	0.15 [NS]	0.04 [S]	0.03 [S]

Data are expressed as mean ± SD.
n= number of patients.
*P<0.05 with respect to baseline value.
S = significant difference.
NS = no significant difference.

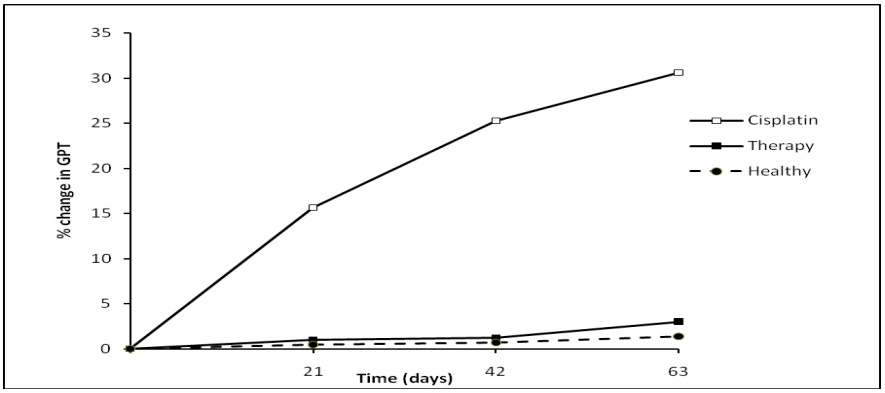

Figure 2: Percent change in GPT level in control and patients received bismuth-vitamin E combination therapy along the study period.

Table 3: Serum GOT level in control and patients received bismuth-vitamin E combination therapy.

	Serum GOT (< 45 IU/L)			
	Baseline	**After 21 days**	**After 42 days**	**After 63 days**
Healthy group (n=10)	20.00 ± 0.04	20.19 ± 0.48	20.06 ± 0.77	20.19 ± 0.65
Cisplatin group (n=30)	20.81 ± 3.05	24.08 ± 3.53*	26.08 ± 3.83*	27.19 ± 3.99*
Therapy group (n=30)	20.22 ± 8.29	20.42 ± 8.38 *	20.47 ± 8.40 *	20.83 ± 8.54 *
P-value	0.37 [NS]	0.032 [NS]	0.003 [S]	0.001 [S]

Data are expressed as mean ± SD. n= number of patients.
*P<0.05 with respect to baseline value. S = significant difference.
NS = no significant difference.

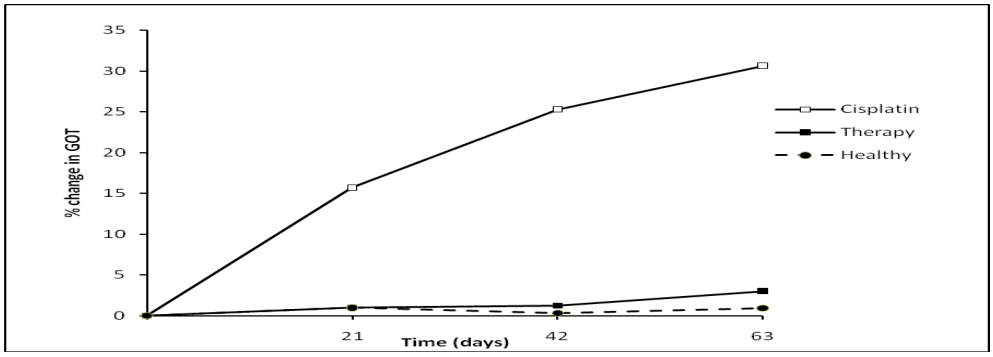

Figure 3: Percent change in GOT in control and patients received bismuth-vitamin E combination therapy along the study period.

Table 4: Serum ALP level in control and patients received bismuth-vitamin E combination therapy.

	Serum ALP (30-85 IU/L)			
	Baseline	**After 21 days**	**After 42 days**	**After 63 days**
Healthy group (n=10)	55.34 ± 11.20	55.78 ± 11.10	56.20 ± 12.83	56.11 ± 10.84
Cisplatin group (n=30)	83.99 ± 10.64	96.17 ± 12.19 *	104.14 ± 13.2 *	107.5 ± 13.62 *
Therapy group (n=30)	86.42 ± 14.40	88.41 ± 14.73 *	89.29 ± 14.88 *	90.34 ± 15.06 *
P-value	0.28 [NS]	0.058 [NS]	0.004 [S]	0.001 [S]

Data are expressed as mean ± SD. n= number of patients.
*P<0.05 with respect to baseline value. S = significant difference.
NS = no significant difference.

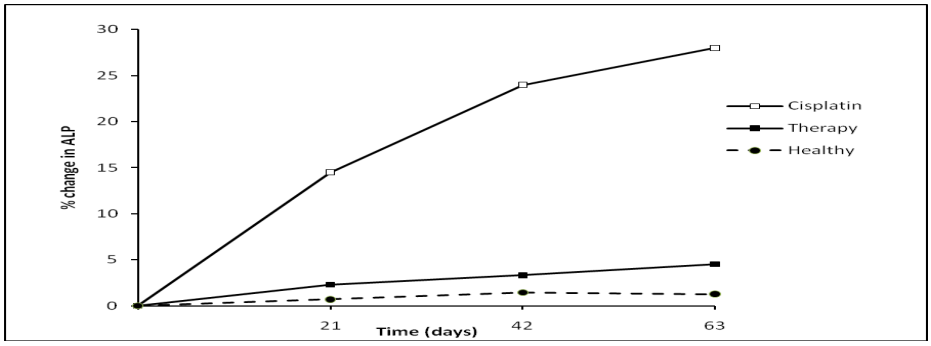

Figure 4: Percent change in ALP in control and patients received bismuth-vitamin E combination therapy along the study period

Discussion

Hepatotoxicity in this study was gauged by the measurements of total serum bilirubin (TSB), the levels of GPT, GOT and ALP in serum. Recent studies have been focused on the ways for protection of cisplatin hepatotoxicity. [2, 7, 13, 14] However, little is reported regarding the combined actions of agents against cisplatin hepatotoxicity.The findings disclosed that each agent used in this study could play a beneficial role for prevention of cisplatin hepatotoxicity; however none could play the crucial role. Metallothionein (MT) is a protein with low molecular weight, and one-third of its amino acids are cysteine residues therefore, can easily trap and sequester metal ions with its thiols. MT was reported to be the main mechanism underlying the action of bismuth against cisplatin toxicity. [23, 24] Several in vitro studies have shown that elevation of MT levels in certain cultured cells can result in resistance to cisplatin. [25, 26] It has been reported that the active metabolites of cisplatin can react quickly with the thiols in glutathione (GSH) and small proteins such as MT, then in high molecular weight proteins such as albumin through covalent link. Thereby, the levels of GSH and MT can play an important role in switching the mode of cell death induced by cisplatin. [27] On the other hand, it has been reported that intracellular levels of GSH and induction of MT were directly involved in the resistance to cisplatin in tumor cells. [28-30] It has recently been shown that pretreatment with bismuth compounds reduced markedly the toxic side effects of various anticancer drugs such as cisplatin [31-33], Adriamycin [33,34] and bleomycin [35], tumor necrosis factor [36], and -y-irradiation [37] without compromising their antitumor activities. Bismuth compounds induce specifically MT synthesis in normal tissues such as kidney, liver, heart, and bone marrow but not in tumors [31-38]. Several studies suggest that supplemental antioxidants can reduce cisplatin-induced hepatotoxicity. [39-42]

There are many studies which have demonstrated the involvement of oxidative stress, lipid peroxidation and mitochondria dysfunction in CP-induced liver toxicity. [41-45] A mechanism by which CP exerts its cytotoxicity is through the generation of reactive oxygen species (ROS). [45, 46] The administration of CP causes an increase in lipid peroxide levels and a decrease in the activity of antioxidant defense enzymes, as well as in the concentrations of non-enzymatic components of Anti oxidative stress that prevent, or protect against, lipid peroxidation in the tissues [47].It is accepted that both correlate to oxidative stress and cause an imbalance between the generation of oxygen derived radicals and the organism's antioxidant potential. [48] Supplementation of the antioxidant vitamin E has been reported to inhibit lipid peroxide in various conditions such as CP-induced nephrotoxicity and hepatotoxicity. [47]

Recent studies have focused on the role of antioxidants in CP toxicity. Administration of antioxidants such as vitamin E and vitamin C before and during treatment with CP has been used to protect against toxicity in humans and experimental animals [43-47, 49, 50].In conclusion, the findings of this study would provide a more promising strategy for prevention of hepatotoxicity in cisplatin-based chemotherapy. However, this study is a pilot study and the results are preliminary, starting with bismuth and vitamin E therapy before the initiation of cisplatin therapy may be of additional benefits and additional studies using other combinations of agents with animals and patients are needed.

References

1. Ekborn A, LindbergA, Laurell G,Wallin I, Eksborg S, Ehrsson H Ototoxicity,nephrotoxicity and pharmacokinetics of cisplatin and its Monohydrated complex in the 1guinea pig. Cancer Chemother Pharmacol 2003;51:36–42
2. Iraz M, KalciogluMT, Kizilay A, Karatas E. Amino guanidine prevents ototoxicity induced by ciplatin in rats. Ann Clin Lab Sci 2005;35(3):329–35.
3. Yao X, Panichpisal K, Kurtzman N, Nugent K. Cisplatin nephrotoxicity: a review. Am J Med Sci 2007;334(2):115–24.
4. Liao YJ, Tang H, Jin YP. Study of toxic effects on hearing, kidney and liver of mice induced by anticancer agent of cisplatin and their mechanisms.Chin Pharmacol Bull 2004;20(1):82–5.
5. Hong KO, Hwang JK, Park KK, Kim SH. Phosphorylation of c-Jun N-terminal Kinases (JNKs) is involved in the preventive effect of Xanthorrhizol on cisplatin-induced hepatotoxicity. ArchToxicol 2005;79(4):231–6.
6. Pratibha R, Sameer R, Rataboli PV, Bhiwgade DA, Dhume CY. Enzymatic studies of cisplatin induced oxidative stress in hepatic tissue of rats. Eur J Pharmacol 2006;532(3):290–3.
7. Iseri S, Ercan F, Gedik N, Yuksel M, Alican I. Simvastatin attenuates cisplatin-induced kidney and liver damage in rats. Toxicology 2007;230(2-3):256–64.
8. Ali BH, Al Moundhri MS. Agents ameliorating or augmenting the Nephrotoxicity of cisplatin and other platinum compounds: a reviewof some recent research. Food Chem Toxicol 2006;44 (8):1173–83.
9. 9.Blakley BW, Cohen JI, Doolittle ND, Muldoon LL, Campbell KC, Dickey DT, et al. Strategies for prevention of toxicity caused by platinum-based chemotherapy. Laryngoscope 2002;112(11):1997–2001.
10. Kim SH, Hong KO, Hwang JK, Park KK. Xanthor rhizol has a Potential to attenuate the high dose cisplatin-induced nephrotoxicity in mice. Food Chem Toxicol 2005;43(1):117–22.
11. Ramesh G, Reeves WB.TNF-alpha mediates chemokine and Cytokine expression and renal injury in cisplatin nephrotoxicity. J Clin Inves 2002;110(6):835–42.
12. Xiao T, Choudhary S, Zhang W, Ansari NH, Salahudeen A. Possible involvement of oxidative stress in cisplatin-induced apoptosis in LLC-PK1 cells. J Toxicol Environ Health A 2003;66(5):469–79.
13. Koc A, Duru M, Ciralik H, Akcan R, Sogut S. Protective agent, erdosteine, against cisplatin-induced hepatic oxidant injury in rats. Mol Cell Biochem 2005;278(1–2):79–84.
14. Iraz M, Ozerol E, Gulec M, Tasdemir S, Idiz N, Fadillioglu E, et al. Protective effect of caffeic acid phenethyl ester (CAPE) administration on cisplatin-induced oxidative damage to liver in rat. Cell Biochem Funct 2006;24(4):357– 61.
15. Disilvestro, R. A., Liu, J., and Klaassen, C. D. *Res. Commun. Mol. Pathol. Pharmacol. 1996;* 93, 163–170
16. Naganuma, A., Satoh, M., and Imura, N. *Cancer Res. 1987;* 47, 983–987
17. Satoh, M., Aoki, Y., and Tohyama, C. *Cancer Chemother. Pharmacol.1977;* 40, 358–362.
18. Antunes, L. M. G., Darin, J. D. C., and M. L. P. Bianchi. Protective effects of vitamin C against cisplatin-induced nephrotoxicity and lipid peroxidation in adult rats: a dose-dependant study. Pharmacol. Res. 2000; 41, 405-411. 20.Antunes, L. M. G., Darin, J. D. C., and M. L. P. Bianchi. Effects of the antioxidants curcumin or selenium on cisplatin-induced nephrotoxicity and lipid peroxidation in rats. Pharmacol. Res. 2001; 43, 145-150.
19. Antunes, L. M. G., Darin, J. D. C., and M. L. P. Bianchi. Effects of the antioxidants curcumin or selenium on cisplatin-induced nephrotoxicity and lipid peroxidation in rats. Pharmacol. Res. 2001; 43, 145-150.
20. Caffrey, P. B., and G. D. Frenkel. Selenium compounds prevent the induction of drug resistance by cisplatin in human ovarian tumor xenografts in vivo. Cancer Chemother. Pharmacol. 2000; 46, 74-78
21. Naziroğlu, M., Karaoglu, A., and A. O. Askoy. Selenium and high dose vitamin E administration protects cisplatin-induced oxidative damage to renal, liver and lens tissues in rats. Toxicology 2004; 195, 221-230.
22. Leonetti C, Biroccio A, Gabellini C, Scarsella M, Maresca V, Flori E, et al. Alpha-tocopherol protects against cisplatin-induced toxicity Without interfering with antitumor efficacy. Int J Cancer 2003;104:243–50.
23. Satoh M, Shimada A, Zhang B, Tohyama C. Renal toxicity caused By cisplatinum in glutathione -depleted metallothionein-null mice.

Biochem Pharmacol 2000;60(11):1729–34.
24. Hosokawa O, Okabe M, Saito S, Saito T, Kurasaki M. Protective role of metallothionein on DNA damage in rat kidney caused by cisdiammine-dichloroplatinum. Pharmacol Toxicol 2000;86 (6):276–82.
25 .Bakka. A.. Endresen, L.. Johnsen. A. B. S.. Edminson. P. D.. and Rugstad. H. E. Resistance against ns-diamminedichloroplatinum in cultured cells with a high content of metallothionein. Toxicol. Appi. Pharmacol.. 61: 215-226. 1981.
26.Kasahara, K.. Fujiwara. Y, Nishino, K.. Ohmori. T.. Sugimoto. Y.Komiya. K. Matuda, T., and Saijor, N. Metallothionein content correlates with the sensitivity of human small cell lung cancer cell lines to cisplatin. Cancer Res.1991; 5: 323- 3242.
27.Zhang K, Chew M, Yang EB, Wong KP, Mack P. Modulation of Cisplatin cytotoxicity and cisplatin-induced DNA cross-links in HepG2 cells by regulation of glutathione-related mechanisms. Mol Pharmacol 2001;59(4):837 –43.
28.Ikeda K, Miura K, Himeno S, Imura N, Naganuma A. Glutathione Content is correlated with the sensitivity of lines of PC12 cells to cisplatin without a corresponding change in the accumulation of platinum. Mol Cell Biochem 2001;219(1–2):51–6.
29. Cherian MG, Howell SB, Imura N, Klaassen CD, Koropatnick J,Lazo JS,et al. Role of metallothionein in carcinogenesis. Toxicol Appl Pharmacol 1994;126(1):1–5.
30.Satoh M, Cherian MG, Imura N, Shimizu H. Modulation of resistance to anticancer drugs by inhibition of metallothionein synthesis. CancerRes1994;54(20):5255–7.
31.Naganuma, A.. Satoh. M.. and Imura. N. Prevention of lethal and renal toxicity of cis-diamminedichloroplatinum(II) by induction of metallothionein synthesis without compromising its antitumor activity in mice. Cancer Res.1987; 47: 983-987.
32.Kondo. Y. Satoh, M.. Imura. N.. and Akimoto. M. Effect of bismuth nitrate given in combination with cis-diamminedichloroplatinum(ll) on the antitumor activity and renal toxicity of the latter in nude mice inoculated with human bladder tumor. Cancer Chemother Pharmacol.1991; 29: 19-23.
33.Satoh. M.. Naganuma. A., and Imura. N. Metallothionein induction prevents toxic side effects of cisplatin and Adriamycin used in combination. Cancer Chem. Pharmacol.1988; 21: 176-178.
34.Satoh, M., Naganuma. A., and Imura. N. Involvement of cardiac metallothionein in prevention of Adriamycin induced lipid peroxidation in the heart. Toxicology, 53:231-237.
35. Imura. N.. Satoh. M.. and Naganuma. A. Possible application of metallothionein in cancer therapy. In: C. D. Klaassen and K. T. Suzuki (eds.). Metallothionein in Biology and Medicine, Boca Raton. FL: CRC Press, 1992: 375-382.
36.Satomi. N.. Sakurai. A.. Haranaka. R., and Haranaka. K. Preventive effects ofseveral chemicals against lethality of recombinant human tumor necrosis factor. J. Biol. Response Modif.1988, 7: 54-64
37.Satoh. M.. Miura. N., Naganuma. A.. Matsuzaki. N.. Kawamura. E.,and Imura. N. Prevention of adverse effects of "y-ray irradiation by metallothionein induction by bismuth subnitrate in mice. Eur. J. Cancer Clin. Oncol.1989 25: 1727-1731.
38.Naganuma. A., Satoh. M.. and Imura. N. Specific reduction of toxic side effects of Adriamycin by induction of metallothionein in mice. Jpn. J. Cancer Res.1988, 79:406-411
39.Zicca, A., Cafaggi, S., Mariggio, M. A., Vannozzi, M. O., Ottone, M., Bocchini, V., Caviglioli, G., and M. Viale. Reduction of cisplatin hepatotoxicity by procainamide hydrochloride in rats. Eur. J. Pharmacol. 2004; **442,** 265-272.
40.Koc, A., Duru, M., Ciralik, H., Akcan, R., and S. Sogut. Protective agent, erdosteine, against cisplatin-induced hepatic oxidant injury in rats. Mol. Cell Biochem. 2005; **278,** 79-84.
41.Mansour, H. H., Hafez, F. H., and N. M. Fahmy. Silymarin modulates cisplatin-induced oxidative stress and hepatotoxicity in rats. J. Biochem. Mol. Biol. 2006; **39,**656-661.
42.Pratibha, R., Sameer, R., Rataboli, P. V., Bhiwgade, D. A., and C. Y. Dhume. Enzymatic studies of cisplatin-induced oxidative stress in hepatic tissueof rats. Eur. J. Pharmacol. 2006; **532,** 290-293.
43.Antunes, L. M. G., Darin, J. D. C., and M. L. P. Bianchi. Protective effects of vitamin C against cisplatin-induced nephrotoxicity and lipid peroxidation in adult rats: a dose-dependant study. Pharmacol. Res. 2000; **41,** 405-411.
44.Jordan, P., and M. Carmo-Fonseca. Molecular mechanisms involved in cisplatin cytotoxicity. Cell Mol. Life Sci. 2000; **57,** 1229–1235.
45.Atasayar, S., Gurer-Orhan, H., Orhan, H., Gurel, B., Girgin, G., and H. Ozguneş. Preventive effect of aminoguanidine compared to vitamin E and C on cisplatin-induced nephrotoxicity in rats. Exp. Toxicol. Pathol.2009; 61, 23-32.
46.Antunes, L. M. G., Darin, J. D. C., and M. L. P. Bianchi. Effects of the antioxidants curcumin or selenium on cisplatin-induced nephrotoxicity and lipid peroxidation in rats. Pharmacol. Res. 2001; **43,** 145-150.
47.Naziroğlu, M., Karaoglu, A., and A. O. Askoy (2004).Selenium and high dose vitamin E administration protects cisplatin-induced oxidative damage to renal, liver and lens tissues in rats. Toxicology 2004; 195, 221-230.
48.Halliwell, B., and J. M. C. Gutteridge. Free Radicals in Biology and Medicine, 4rd Ed. Oxford University Press, New York. 2007.
49.Spallhoz, J. E. On the nature of selenium toxicity and carcinostatic activity. Free Radic. Biol. Med. 1994; **17,**45-64.

50. Rybak, L. P., Whitworth, C., and S. Somani. Application of antioxidants and other agents to prevent cisplatin ototoxicity. Laryngoscope **109,** 1740–1744 [50] Caffrey, P. B., and G. D. Frenkel (2000). Selenium compounds prevent the induction of drug resistance by cisplatin in human ovarian tumor xenografts in vivo. Cancer Chemother. Pharmacol 1999; 46, 74-78.