Physiol. Res. 41: 129-134, 1992

Effect of Cisplatin, Carboplatin and Stobadine on Lipid Peroxidation of Kidney Homogenate and Phosphatidylcholine Liposomes

D. GERGEĽ, V. MIŠÍK, K. ONDRIAŠ

Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava

Received July 22, 1991
Accepted November 6, 1991

Summary

The effects of the nephrotoxic, anticancer agents cisplatin (CDDP) and carboplatin (CBDCA), and the free radical scavenger, stobadine, were investigated on lipid peroxidation (LPO) of rat kidney homogenates and phosphatidylcholine (PC) liposomes. Kidney homogenates were incubated in air at 37 °C for 6-48 h and lipid peroxidation was detected spectroscopically as absorbance (533 nm) of the thiobarbituric acid-malondialdehyde (TBA-MDA) complex. CDDP (0.3-10 mmol.l^{-1}) increased LPO of the homogenate. CBDCA decreased the TBA-MDA absorbance, yet was found to interfere with MDA, TBA and/or with the TBA-MDA complex. Thus when CBDCA is involved, the TBA-MDA method for detection of LPO is not suitable. Stobadine (0.1 mmol.l^{-1} and 1 mmol.l^{-1}) inhibited LPO either in the control homogenate and in the homogenate where peroxidation was increased by CDDP. The effect of CDDP and CBDCA on peroxidation of PC liposomes was monitored as oxygen consumption using a Clark-type oxygen electrode. CDDP increased but CBDCA decreaed the rate of oxygen consumption during the peroxidation of liposomes induced by $FeSO_4$. The results suggest that the effects of CDDP and CBDCA on LPO may be linked with their nephrotoxicity.

Key words

Cisplatin – Carboplatin – Stobadine – Kidney Homogenate – Liposomes – Lipid Peroxidation

Introduction

CDDP is an effective anticancer chemotherapeutic agent used mostly for the treatment of testicular, urinary bladder, and ovarian tumours, as well as cancers of the head and neck (Rozencweig *et al.* 1977). The most important adverse effect of CDDP is its nephrotoxicity (Goldstein and Mayor 1983, Daugaard and Abildgaard 1989). The exact mechanism of the nephrotoxicity caused by CDDP is not yet known. CBDCA is a less nephrotoxic analog of CDDP (Daugaard and Abildgaard 1989) with antitumour activity, especially in lung and ovarian cancer (Smith *et al.* 1985, Wiltshaw *et al.* 1983).

It was found in previous studies that CDDP stimulates phagocytes to produce the superoxide radical (Oyanagui 1977). Superoxide dismutase and antioxidants decrease the nephrotoxicity caused by CDDP (Sugihara and Gemba 1986, McGinness *et al.* 1978). The increase of LPO in renal tissue was prevented by antioxidants (Sugihara *et al.* 1987, Hannemann and Baumann 1988). These results suggested that free radicals or LPO generated by CDDP might contribute to the nephrotoxic effect. Several authors showed that CDDP inhibited the activity of many enzymes (Daley-Yates and McBrien 1982, Kohl *et al.* 1979), especially sulfhydryl-group containing enzymes. Depletion of glutathion (GSH), which is a potent factor in the control of LPO (Younes and Siegers 1980) was found to potentiate LPO induced by CDDP in rat kidney slices (Nakano and Gemba 1989).

The aim of this study was to compare the effect of CDDP and CBDCA on lipid peroxidation in kidney homogenates. Since a new drug, stobadine, was found to be very effective in inhibiting lipid peroxidation (Staško *et al.* 1990), we studied the influence of this drug in modulating the effect of CDDP on lipid peroxidation of the homogenate and liposomes.

Abbreviations

CDDP, cisplatin, cis-diamminedichloroplatinum(II); CBDCA, carboplatin, cis-diammine-cyclobutane-1,1-dicarboxylato platinum(II); stobadine, (-)-cis-2,8-dimethyl-2,3,4,4a,5,9b-hexahydro-1-H-yrido(4,3b)indole; BHT, butylated hydroxytoluene; PC, egg yolk phosphatidylcholine; TBA, 2-thiobarbituric acid; MDA, malondyaldehyde; LPO, lipid peroxidation;

Materials and Methods

Wistar rats of either sex, weighing about 200 g, were decapitated, and their kidneys were rapidly removed, washed and homogenized (1 g wet tissue/20 ml) in a buffer solution (values in mmol.l^{-1}): KCl 100, Tris 5, pH 7.4. The samples contained 30 mg wet tissue, 600 μl buffer with or without CDDP, CBDCA or stobadine. Peroxidation of the samples was performed at 37 °C by incubation under air during 6 – 48 h. The relative LPO in the samples was monitored by measuring the formation of TBA reactive products, mainly of the TBA-malondialdehyde (TBA-MDA) complex, according to a modified method (Ondriaš *et al.* 1989) of Haeman and Bast (1983). Briefly: Butylated hydroxytoluene (BHT) (0.55 mg BHT in 360 μl ethanol) was added to 300 μl of homogenates to prevent further peroxidation. Then 3.6 ml of the TBA solution (2.1 g TBA, 84 g trichloroacetic acid, 3.57 ml of 37 % HCl diluted with H_2O to 500 ml) was added and the samples were incubated at 80 °C for 15 min. The samples were cooled at 10 °C in a water bath and centrifuged at 1600 x g for 5 min. The supernatant was analyzed spectroscopically, where the absorption at 533 nm was taken as the relative value of LPO.

To study the effect of the drugs on LPO, egg phosphatidylcholine (PC) multilamellar liposomes were used. PC (25 mg) dissolved in chloroform/methanol (2:1) was mixed with the drugs tested. The solvent was evaporated in a stream of nitrogen followed by evacuation. The dry samples were hydrated with 4.5 ml of the buffer (in mmol.l^{-1}): KCl 100, Tris 5, pH 7.4. Multilamellar liposomes were prepared by vortexing the suspension for 3 min and sonicating it in a bath for 30 s. Stobadine in the given buffer was added to the prepared liposomes. The peroxidation of the liposomes was induced by the Fenton reaction by addition of 250 μl $FeSO_4$ (0.1 mmol.l^{-1} final concentration) with or without 250 μl H_2O_2 (0.05 mmol.l^{-1}). The lipid/buffer ratio in the samples was 25 mg/5 ml. LPO was measured by monitoring the oxygen consumption using a Clark-type oxygen electrode (Green and Hill 1984, Hitchman 1978). The liposomes were stirred at 22 °C during the measurement.

Cisplatin (CDDP) {Cis-diammine-dichloropla-tinum(II)} and carboplatin (CBDCA) {cis-diamminecy-clobutane-1,1-dicarboxylatoplatinum(II)} were obtained from Lachema (CSFR), stobadine {(-)-cis-2,8-dimethyl-2,3,4,4a,5,9b- hexahydro-1H-pyrido(4,3b)indole} was provided by courtesy of Dr. Beneš (Institute of Experimental Pharmacology, Bratislava). Butylated hydroxytoluene (BHT) was from Sigma (U.S.A.). 2-thiobarbituric acid (TBA) was from Fluka AG (Switzerland), egg yolk phosphatidylcholine, isolated according to the method of Singleton *et al.* (1965) was provided by courtesy of Dr. Švajdlenka (Faculty of Pharmacy, Comenius University, Bratislava). All other chemicals were of analytical grade from commercial sources.

Results

Time-dependent LPO of homogenates evaluated as the absorbance change at 533 nm caused by the formation of TBA-reactive products is shown in Fig. 1.

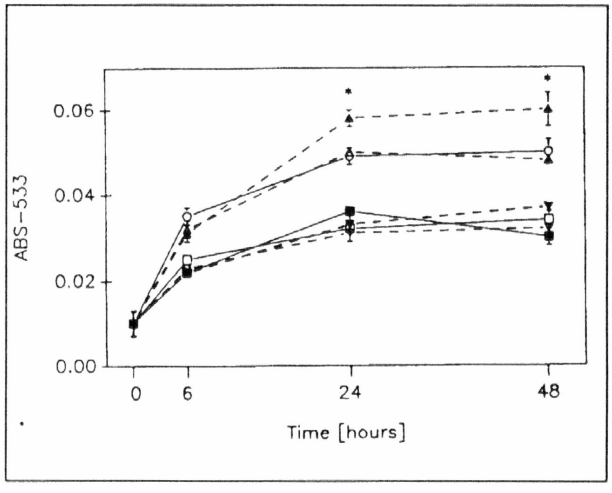

Fig. 1

Dependence of absorbance of TBA-reactive products (ABS-533) on the incubation time of kidney homogenates at 37 °C. Broken lines are the samples containing CDDP. Control (circles); 0.1 mmol.l^{-1} (open triangles) and 1 mmol.l^{-1} (filled triangels) CDDP; 0.1 mmol.l^{-1} (open squares) and 1 mmol.l^{-1} (filled squares) stobadine; 1 mmol.l^{-1} CDDP with 0.1 mmol.l^{-1} stobadine (inversed filled triangels) and 1 mmol.l^{-1} CDDP with 1 mmol.l^{-1} stobadine (inversed open triangels). The data represent means ± S.D. of at least four experiments. * $P<0.01$ vs. the control.

In the control samples, in the absence of drugs, a continuous progress of LPO was found. CDDP increased LPO in the concentration of 1 mmol.l^{-1} yet failed to do so in the concentration of 0.1 mmol.l^{-1}. Stobadine (0.1 and 1 mmol.l^{-1}) inhibited LPO in the samples with or without CDDP, i.e. prevented CDDP from increasing LPO. Even at 0.1 mmol.l^{-1} concentration, stobadine was effective in depressing LPO induced by 1 mmol.l^{-1} of CDDP. Incubation of the homogenates for 24 hours with CDDP increased the production of TBA-reactive products in a concentration-dependent manner (Fig 2).

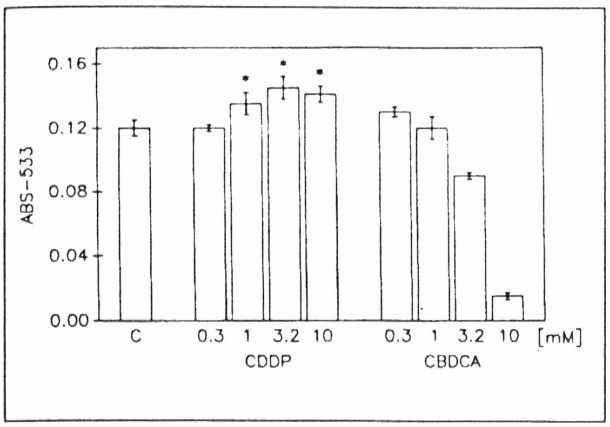

Fig. 2
Relative effect of CDDP and CBDCA on TBA-reactive product formation (ABS-533) in kidney homogenates. The homogenates were incubated at 37 °C for 24 hours. The data represent means ± S.D. of at least seven experiments. *P < 0.02 vs. the control.

On the other hand, CBDCA decreased the absorbance of the TBA-reactive products. In order to establish whether CDDP or CBDCA did or did not interfere with MDA, the main product of LPO, or with the TBA-MDA complex, we used an authentic MDA to measure absorption of the TBA-MDA complex. The results are shown in Fig. 3. CDDP did not change the absorption of the complex, whereas CBDCA decreased it.

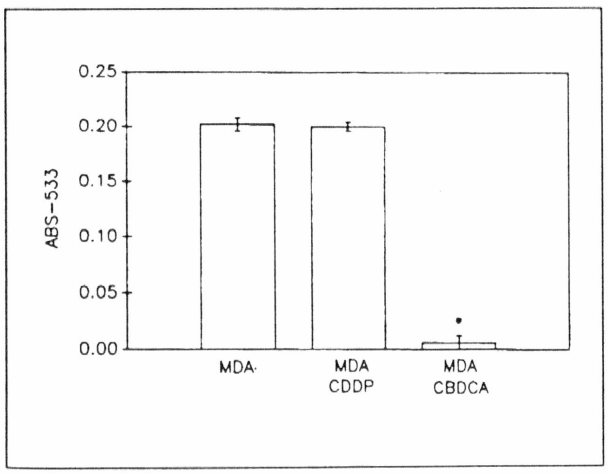

Fig. 3
Effect of CDDP and CBDCA on absorbance (ABS-533) of TBA-MDA complex. The samples were incubated at 37 °C for 24 hours in a medium containing authentic MDA 0.5 mmol.l^{-1} with CDDP 10 mmol.l^{-1}, or with CBDCA 10 mmol.l^{-1}. The data represent means ± S.D. of at least four experiments. *P < 0.001 vs. the control.

Fig. 4a shows an example of oxygen consumption in PC liposomes during peroxidation. No oxygen uptake was observed in the control liposomes incubated for up to 3 hours. CDDP, CBDCA or $FeCl_3$ did not induce oxygen uptake in the liposomes. When $FeSO_4$ (0.1 mmol.l^{-1} final concentration) with or without H_2O_2 (0.05 mmol.l^{-1}) was added, oxygen consumption occurred. An example of the effect of CDDP, CBDCA and of the antioxidant BHT on oxygen uptake during peroxidation of PC liposomes induced by $FeSO_4$ (0.1 mmol.l^{-1}) is shown in Fig. 4b.

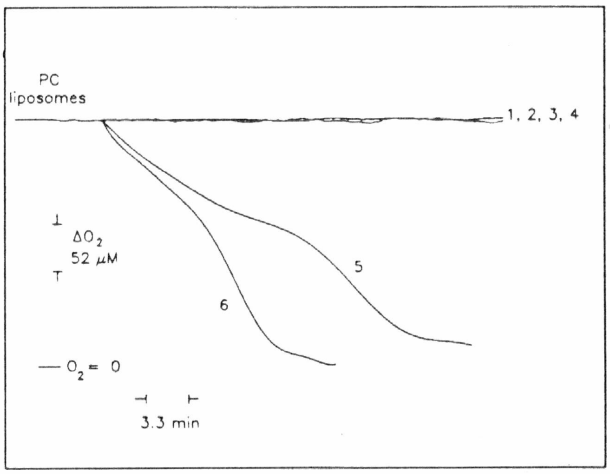

Fig. 4a
Example of the effect of drugs on oxygen consumption in egg yolk PC liposomes. The samples of 6 mmol.l^{-1} PC liposomes were incubated at 22 °C without (1) or with 10 mmol.l^{-1} CDDP (2), 10 mmol.l^{-1}CBDCA (3), 0.2 mmol.l^{-1} $FeCl_3$ (4), 0.1 mmol.l^{-1} $FeSO_4$ (5), 0.1 mmol.l^{-1} $FeSO_4$ + 0.05 mmol.l^{-1} H_2O_2 (6)

Oxygen uptake was accelerated in the samples with CDDP (10 mmol.l^{-1}) in comparison to control samples. However, when the samples contained CBDCA (10 mmol.l^{-1}) the rate of oxygen consumption was decreased. Stobadine (0.2 mmol.l^{-1}) and BHT (0.2 mmol.l^{-1}) prevented oxygen uptake by liposomes. The time dependence of oxygen uptake in the liposomes induced by FeSO4 (0.1 mmol.l^{-1}) and modulated by CDDP (10 mmol.l^{-1}) and CBDCA (10 mmol.l^{-1}) is shown in Fig. 5a. CDDP increased the rate of oxygen uptake, while CBDCA decreased it.

When LPO was induced by the Fenton reaction (0.1 mmol.l^{-1} of $FeSO_4$ with 0.05 mmol.l^{-1} of H_2O_2) CDDP increased the rate of oxygen uptake about two fold values as compared to the system where only $FeSO_4$ was used (Fig. 5b).

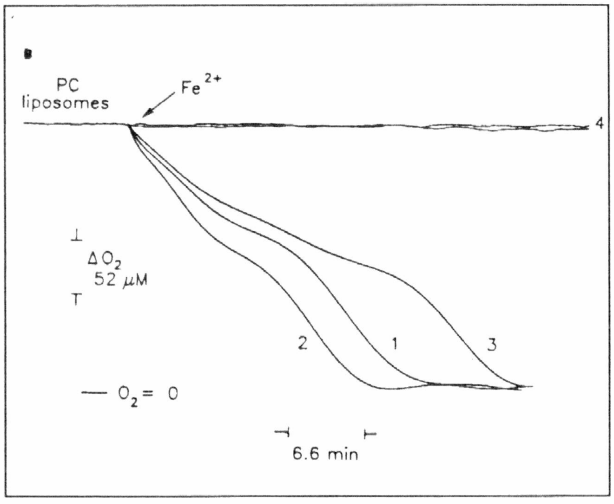

Fig. 4b
Example of oxygen consumption of 6 mmol.l^{-1} PC liposomes following addition of: $FeSO_4$ (0.1 mmol.l^{-1}) alone (1), or with 10 mmol.l^{-1} CDDP (2), or with 10 mmol.l^{-1} CBDCA (3), or with 0.2 mmol.l^{-1} BHT (4). Temperature 22 °C.

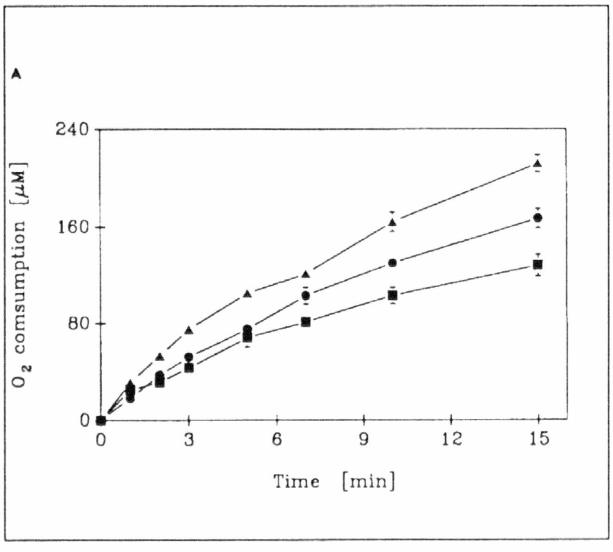

Fig. 5a
Comparison of the effect of CDDP and CBDCA on the oxygen consumption of 6 mmol.l^{-1} PC liposomes induced by $FeSO_4$ (0.1 mmol.l^{-1}) without (circles) and with 10 mmol.l^{-1} DDP (triangles), or 10 mmol.l^{-1} CBDCA (squares). Temperature 22 °C. The data represent means ± S.E.M. (n = 5).

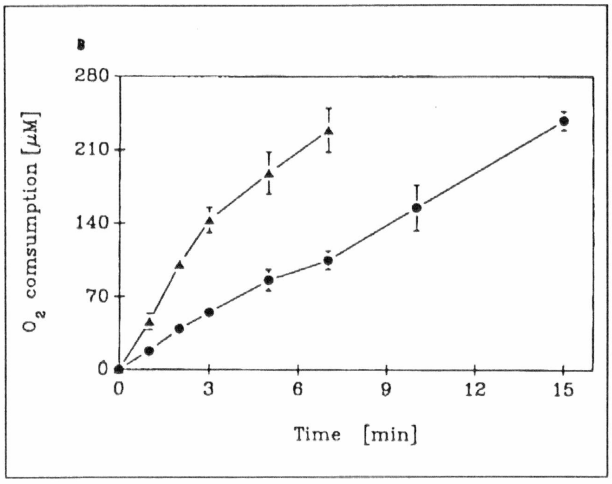

Fig. 5b
Effect of CDDP on oxygen consumption during PC liposome peroxidation induced by $FeSO_4$ (0.1 mmol.l^{-1}) with H_2O_2 (0.05 mmol.l^{-1}). Circles - 6 mmol.l^{-1} PC liposomes. Triangles - 6 mmol.l^{-1} PC liposomes + 10 mmol.l^{-1} CDDP. The data represent means ± S.E.M. (n = 5).

Discussion

Previous *in vivo* studies demonstrated that SOD and antioxidants reduced the nephrotoxic effect of CDDP (Sugihara and Gemba 1986, McGinness *et al.* 1978). Pretreatment of rats with the antioxidants α-tocopherol or N,N'-diphenyl-p-phenyl-enediamine prevented the rise in MDA level in the renal tissue after CDDP administration (Sugihara *et al.* 1987). In the present study, CDDP increased the LPO in kidney homogenates. These results are in agreement with previous *in vitro* studies on rat renal cortical slices (Sugihara *et al.* 1987), where authors investigated the stimulatory effect of CDDP on LPO and its inhibition by antioxidants. Stobadine, a potent scavenger of OH radicals (Staško *et al.* 1990), which was found to be very effective in depressing LPO in PC liposomes (Ondriaš *et al.* 1989), decreased CDDP induced LPO. The effect of stobadine in preventing kidney homogenate peroxidation may presumably be accounted for by its free radical scavenging activity.

CBDCA inhibited the absorption of the TBA-MDA complex at 533 nm in kidney homogenates (Fig. 2). However, since CBDCA was found to interfere with TBA-MDA complex absorption when authentic MDA was used (Fig. 3), the lowered absorbance at 533 nm induced by CBDCA might not be associated with the inhibition of LPO. Therefore, when CBDCA is involved, the TBA-MDA method for detection of LPO did not prove to be suitable. However, since we found that CBDCA decreased the rate of oxygen consumption in PC liposomes, an inhibitory effect of CBDCA on LPO in the homogenate seems to be plausible.

In experiments on animal tissues, CDDP may interact with lipids and/or affect enzymes responsible for the redox state of the body, which may lead to an increase of LPO. We therefore chose PC liposomes as a nonenzymatic system to study the effect of CDDP or CBDCA on LPO.

It is known that oxygen consumption is involved in LPO (Subczynski and Kusumi 1985, Yamamoto and Niki 1988, Yamamoto *et al.* 1984). The Clark-type oxygen electrode was conveniently used in monitoring this process (Ross and Barclay 1988). In our study, we used the Fenton-type reaction, which produces OH radicals, and which has also been supposed to be a source of OH radicals *in vivo* (Floyd and Levis 1983) where Fe^{2+} and generation of H_2O_2 are involved in many physiological events. Yamamoto and Niki (1988) reported that the oxygen consumption in lipid liposomes caused by Fe^{2+} where the iron catalyzed the decomposition of lipid hydroperoxides can be the primary driving force of LPO. In our study, CDDP or CBDCA alone failed to induce oxygen uptake in PC liposomes but they affected the rate of peroxidation induced by Fe^{2+}. It may be supposed that CDDP and CBDCA alone did not induce LPO in this system, yet they affected the process of peroxidation induced by Fe^{2+}.

Nakano and Gemba (1989) found that CDDP decreased GSH levels in rat renal slices, simultaneously increasing LPO. They observed that diethylmaleate, a GSH depletor, although inducing depletion of GSH, it did not increase LPO, and that antioxidants inhibited CDDP-induced LPO when diethylmaleate was present in the samples. They suggested that CDDP in addition to depletion of GSH induced LPO also by another mechanism. In supporting this hypothesis, our results indicate that the increase of LPO in the homogenates induced by CDDP may be partly due to a direct effect of the drug on membrane lipids.

In conclusion, CDDP increased lipid peroxidation in kidney homogenates and increased the rate of liposome peroxidation, whereas CBDCA decreased the rate of the peroxidation and affected TBA, MDA and/or the TBA-MDA complex. These results correlate with the nephrotoxicity of the drugs tested, with CBDCA being the less nephrotoxic analog of CDDP (Daughaard and Abildgaard 1989). The increased peroxidation of PC liposomes caused by CDDP suggests that, in addition to its effect on enzymatic systems, this drug also acts by another mechanism inducing lipid peroxidation.

References

DALEY-YATES, P. T., McBRIEN, D. C. H.: The inhibition of renal ATPase by cisplatin and some biotransformation products. *Chem.- Biol. Interactions* **40:** 325–334, 1982.

DAUGAARD, G., ABILDGAARD, U.: Cisplatin nephrotoxicity: experimental and clinical studies. *Chemother. Pharmacol.* **25:** 1–9, 1989.

FLOYD, R. A., LEVIS, C. A.: Hydroxyl free radical formation from hydrogen peroxide by ferrous iron-nucleotide complexes. *Biochemistry* **22:** 2645–2649, 1983.

GOLDSTEIN, R. S., MAYOR, G.H.: The nephrotoxicity of cisplatin. *Life Sci.* **32:** 685–690, 1983.

GREEN, M. J., HILL, H. A. O.: Chemistry of dioxygen. In *Methods Enzymol.* **105:** 3–21, 1984.

HAEMAN, G. R. M. M., BAST, A.: Protection against lipid peroxidation by a microsomal glutathione-dependent labile factor. *FEBS-lett.* **159:** 24–28, 1983.

HANNEMANN, J., BAUMANN, K.: Cisplatin-induced lipid peroxidation and decrease of gluconeogenesis in rat kidney cortex: Different effects of antioxidants and radical scavengers. *Toxicology* **51:** 119–132, 1988.

HITCHMAN, M. L.: Measurement of disolved oxygen. John Wiley and Sons, Inc., New York, 1978

KOHL, H. H., FRIEDMAN, M. E., MELIUS, P., MORA, E. C. McAULIFFE, C. A. : Enhanced inhibition of both cellular protein synthesis and malate dehydrogenase by aged aquaoplatinum (II) complexes. *Chem.-Biol. Interactions* **24:** 209–215, 1979.

McGINNESS, J. E., PROCTOR, P. H., DEMOPOULOS, H. D., HOKANSON, J.A., KIRKPATRICK, D. S.: Amelioration of cis-platinum nephrotoxicity by orgotein (superoxide dismutase). *Physiol. Chem. Physics* **10:** 267–277, 1978.

NAKANO, S., GEMBA, M.: Potentiation of cisplatin-induced lipid peroxidation in kidney cortical slices by glutathione depletion. *Japan. J. Pharmacol.* **50:** 87–92, 1989.

ONDRIAŠ, K., MIŠÍK, V., GERGEĽ, D., STAŠKO, A.: Lipid peroxidation of phosphatidylcholine liposomes depressed by the calcium channel blockers nifedipine and verapamil and by the antiarrhythmic-antihypoxic drug stobadine. *Biochim. Biophys. Acta* **1003:** 238–245, 1989.

OYANAGUI, Y.: Stimulatory effect of platinum (IV) ion on the production of superoxide radical from xantin oxidase and macrofages. *Biochem. Pharmacol.* **26:** 473, 1977.

ROSS, L., BARCLAY, C.: The cooperative antioxidant role of glutathione with a lipid-soluble and a water-soluble antioxidant during peroxidation of liposomes initiated in the aqueous phase and in the lipid phase. *J. Biol. Chem.* **263:** 16138–16142, 1988.

ROZENCWEIG, M., VON HOFF, D. D., SLAVIK, M., MUGGIA, F.M.: Cisdiamine-dichloroplatinum(II). A new anticancer drug. *Ann. Intern. Med.* **86:** 803–812, 1977.

SINGLETON, W. S., GRAY, M. S., BROWN, M. L., WHITE, J. L.: Chromatographically homogenous lecithin from egg phospholipids. *J. Am. Oil. Chem. Soc.* **42:** 53–56, 1965.

SMITH, I. E., HARLAND, S. J., ROBINSON, B. A., EVANS, B. D., GOODHARD, L. C., CALVERT, A. H., YARNOLD, J., GLEES, J.P., BAKER, J., FORD, H. T.: Carboplatin: a very active new cisplatin analog in the treatment of small cell lung cancer. *Cancer. Treat. Rep.* **69:** 43, 1985.

STAŠKO, A., ONDRIAŠ, K., MIŠÍK, V., SZOCSOVÁ, H., GERGEĽ, D.: Stobadine – a novel scavenger of free radicals. *Chem. Papers* **44:** 493–500, 1990.

SUBCZYNSKI, W. K., KUSUMI, A.: Detection of oxygen consumption during very early stages of lipid peroxidation by ESR nitroxide spin probe method. *Biochim. Biophys. Acta* **821:** 259–263, 1985.

SUGIHARA, K., GEMBA, M.: Modification of cisplatin toxicity by antioxidant. *Japan. J. Pharmacol.* **40:** 353–355, 1986

SUGIHARA, K., NAKANO, S., GEMBA, M.: Effect of cisplatin on in vitro production of lipid peroxides in rat kidney cortex. *Japan. J. Pharmacol.* **44:** 71–76, 1987.

WILTSHAW, E., EVANS, B.D., JONES, A. C., BAKER, J. W., CALVERT, A. H.: JM8, successor to cisplatin in advanced ovarian carcinoma? *Lancet* **I** : 587 1983.

YAMAMOTO, K., NIKI, E.: Interaction of α-tocopherol with iron: antioxidant and prooxidant effects of α-tocopherol in the oxidation of lipids in aqueous dispersions in the presence of iron. *Biochim. Biophys. Acta* **958:** 19–23, 1988.

YAMAMOTO, Y., NIKI, E., KAMIYA, Y., SHIMASHAKI, H.:Oxidation of lipids. 7. Oxidation of phosphatidylcholines in homogenous solution and in water dispersion. *Biochim. Biophys. Acta* **795:** 332–340, 1984.

YOUNES, M., SIEGERS, C. P.:Lipid peroxidation as a consequence of glutathione depletion in rat and mouse liver. *Res. Commun. Chem. Pathol. Pharmacol.* **27:** 119–128, 1980.

Reprint requests

Dr. D. Gergeĺ, Institute of Experimental Pharmacology, Slovak Academy of Sciences, CS-842 16 Bratislava, Dúbravská cesta 9.