Current Topics in Biophysics 1997, 21(1), 46-49

THE PROTECTIVE EFFECTS OF SELENITE AGANIST CISPLATIN-INDUCED INHIBITION OF PLATELET ACTIVATION

BEATA OLAS, BARBARA WACHOWICZ, HALINA MALGORZATA ZBIKOWSKA

Department of General Biochemistry, Institute of Biochemistry, University of Łódz, Poland

(Received 10 February 1996, Accepted 3 December 1996)

The protective effects of sodium selenite aganist cisplatin-induced inhibition of pig blood platelets activation have been studied *in vitro*. Cisplatin (cis-diamminedichloroplatinum II, CDDP) inhibited ADP-induced platelet aggregation and decreased the G-actin content in pig platelets. The pretreatment of pig platelets with selenite (10 min) protected against inhibitory action of cisplatin on platelet aggregation and against GS-Pt complex formation in platelets. Treatment of platelets with selenite alone at a non-toxic dose (1 μM) had no effect on platelet aggregation.

INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum II, CDDP) is a widely used chemotheraupetic agent, highly effective in the treatment of various types of human malignancies. This agent causes also haematological toxicity and changes functions of blood cells (Ohno, Strebel, Stephens, Siddik, Baba, Makino, Khokhar & Bull, 1993; Pendyala & Creaven, 1993). In blood platelets cisplatin induces lipid peroxidation (Wachowicz, 1991; Wachowicz & Kustron, 1992), inhibits activities of antioxidative enzymes and reduces pathway of exogenous arachidonate (Wachowicz, 1993). Preliminary data from our laboratory shows that cisplatin also inhibits platelet aggregation and the platelet release reaction.

It has been reported that sodium selenite selectively reduced the side-effects of cisplatin without affecting its antitumor activity (Baldew, Cornelis, Van den Hamer, Vermeulen & de Goeij, 1989; Baldew, Mol, De Kanter, Van Baar, De Goeij & Vermeulen, 1991; Vermeulen, Baldew, Los, Mevie & De Goeij, 1992). The mechanism by which sodium selenite protects against the toxicity of cisplatin is not yet understood. It seems that in this process -SH groups play an important role. It has been proposed that the interactions of cisplatin with intracellular sulfur-containing species such as glutathione and other sulfhydryl-containing compounds and/or proteins (including cytoskeletal protein-actin) are the points of attack of cisplatin that are responsible for at least some of side effects associated with the use of this anticancer drug (Beaty, Jones & Ma, 1992; Ishikawa & Ali-Osman, 1993). Cisplatin may form a complex with platelet glutathione (Wachowicz, Krajewski, Olas & Zbikowska, 1995). The present study was undertaken to determine the effects of cisplatin, and sodium selenite on ADP-induced platelet aggregation, and G-actin level in blood platelets. Actin is the major protein in blood platelet cytoskeleton (20-35% of the total protein content). Activation of platelets (aggregation) is associated with polimerization of G-actin (Lefebvre, White, Krumwiede & Cohen, 1993).

MATERIAL AND METHODS

Materials

Cisplatin, sodium selenite, benzamidine, phenylmethylsulfonyl fluoride (PMSF), DN-ase I, DNA type I, guanidine HCL and adenosine triphosphate were obtained from Sigma Chemical Co.

Isolation of blood platelets

Human blood was collected into one-tenth volume of sodium citrate from a forearm vein through an 18 gauge needle. Platelets were isolated by differential centrifugation (20 min, at 200×g). Then, the platelet-rich plasma was collected and centrifuged for 20 min at 1000×g to sediment platelets. The resulting pellet was resuspended in the modified Tyrode's buffer (140 mM NaCl,

10 mM glucose and 15 mM Tris/HCl, pH 7.4), and the platelets were subsequently washed three times with the same buffer. The entire washing procedure was performed in plastic tubes and carried out at room temperature. Platelet protein concentrations was estimated by a modified Lowry method (Vatassary & Smith, 1987).

Platelet aggregation

The aggregation of platelets in response to ADP was recorded at 37°C at a stirring rate of 1000 rpm using a Labor APPACT duel-channel aggregometer. For this purpose, 275 µl samples of platelet-rich plasma (2.7×10^8 platelets/ml) were preincubated for 30 min at 37°C with cisplatin (0.1 µM), selenite (1 µM, 10 min) or without these compounds (control). 10 µl ADP was then added to the final concentration of 10 µM and the extent of platelet aggregation was measured.

Analysis of G-actin content

Control platelets and platelets after 30 min preexposure to cisplatin (20 µM, 30 min) or selenite (1 µM, 10 min) at 37°C were activated by thrombin (0.1 µ/ml, 2 min) , then lysed with an equal volume of pH 7.4 buffer containing 2% Triton X-100, 10 mM EDTA, 100 mM Tris-HCl, 2 mg/ml leupeptin, 100 mM benzamidine and 2 mM PMSF. For unactivated samples, 100 µl of platelet suspension in a small microcentrifuge tube was lysed as above. Samples were assayed in triplicate immediately after lysis by mixing a small aliquot with a DNase buffer, pH 7.4, at a ratio of 1:1, containing 10 µg/ml deoxyribonuclease I, 0.1 mM $CaCl_2$, 10 µM PMSF and 50 mM Tris-HCl. Twenty µl was immediately mixed with 3 ml of a deoxyribonucleic acid buffer, pH 7.4, containing 40 µg/ml DNA type I, 1.8 mM $CaCl_2$, 4 mM $MgSO_4$ and 100 mM Tris-HCl. The reaction rate was approximately 10^{-3} absorbance units/sec at 260 nm, and the change in absorbance was recorded in a Model UV (Beckman DU62 Spectrofotometr). The total actin content of the sample was determined by incubating a lysed sample with an equal volume of denaturing buffer, pH 8.4, containing 1.5 mM guanidine HCl, 1 M sodium adenosine triphosphate, 1 M sodium acetate, 6 mM $CaCl_2$ and 20 mM Tris-HCl for 5 to 15 min on ice, and assaying as above. The denaturing buffer causes disassembly of F-actin into G-actin. Percent G-actin was calculated by dividing the percent inhibition of DNase activity of the lysed samples by the percent inhibition of the denatured sample (Fox , Docker & Philips, 1981).

Isolation of GS-platinum complex by anion-exchange chromatography

After incubation of platelets with CDDP (20 µM, 1 h, 37°C) alone or after preincubation with selenite (20 µM, 10 min, 37°C) the GS-platinum complex from the acid-soluble fraction was isolated by anion-exchange chromatography (Ishikawa & Ali-Osman, 1993). Briefly, to the acid-soluble fraction obtained as described above 7.5 % perchloric acid was added (v/v) and after centrifugation (16,000×g, 10 min) 1 ml of the supernatant was applied to a QAE-Sephadex equlibrated with 10 mM Tris-HCl (pH 7.5). The

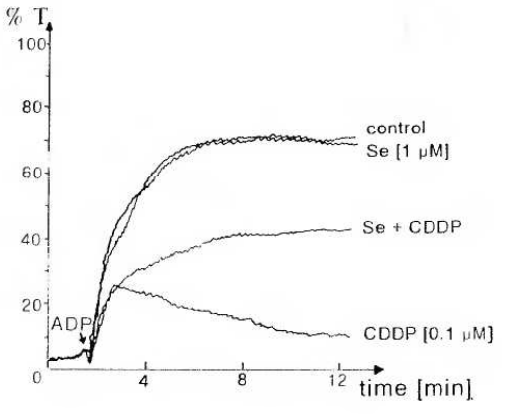

Fig. 1 Platelet aggregation patterns in response to ADP (10 µM): aggregation of control platelets, platelets incubated with selenite (1 µM), with cisplatin (0.1 µM, 30 min, 37°C), and with cisplatin (0.1 µM, 30 min, 37°C) after preincubation with selenite (1 µM)

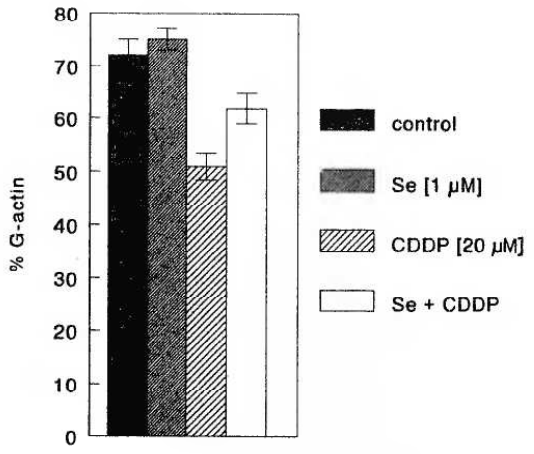

Fig. 2 The effects of preincubation of platelets with selenite (1 µM), cisplatin (20 µM, 30 min, 37°C) and cisplatin (20 µM, 30 min, 37°C) after preexposure to selenite (1 µM, 10 min, 37°C) on the platelet G-actin percent, measured by DN-ase I inhibition assay

column was washed with 5-7 ml deionized water. The GS-platinum bound to the QAE-Sephadex was eluted with 0.2 M HCl. In the eluent the GS-Pt complex was estimated spectrophotometrically (at 280 nm). The molar extinction coefficient at 280 nm was 8.05 mM^{-1} cm^{-1} (Ishikawa & Ali-Osman, 1993).

The data are presented as the means of the averaged replicates ± SD. Statistical analysis was performed using the Student's t-test.

RESULTS AND DISCUSSION

Blood platelets are anucleated blood elements without DNA. The mechanism by which cisplatin affects tumor cells is believed to be the results of an interaction with DNA (Keppler, 1993). The exact molecular mechanism of cisplatin action on blood platelets remains unclear. The present study demonstrated that selenite had no effect on the ADP-induced platelet aggregation (73% aggregation), whereas cisplatin caused a significant inhibition of this process. Fig. 1 represents platelet aggregation patterns in response to ADP. The treatment of platelets with cisplatin (0.1 μM) decreased the extend of ADP-induced aggregation to 15%. After preexposure of platelets to selenite (1 μM, 10 min) the inhibitory effect of cisplatin on platelet aggregation was reduced (42% aggregation) (Fig. 1). We observed, that after incubation of platelets with cisplatin (20 μM, 30 min, 37°C) the amount of G-actin in platelets was also reduced. Pretreatment of platelets with selenite had a slight protective effect on this process (Fig. 2). Cisplatin may inhibit the platelet response to agonists probably by interfering with cytoskeletal proteins *via* -SH groups and in this way may affect the polymerization of platelet actin. The studies showed that in pig blood platelets after incubation with cisplatin (20 μM, 1 h, 37°C; molar ratio of GSH to cisplatin was 2:1) the GS-platinum complex was formed (Fig. 3). The treatment of platelets with cisplatin after preexposure (10 min) to selenite (20 μM) caused a decrease of GS-Pt complex level in platelets as compared to cisplatin alone (Fig. 3).

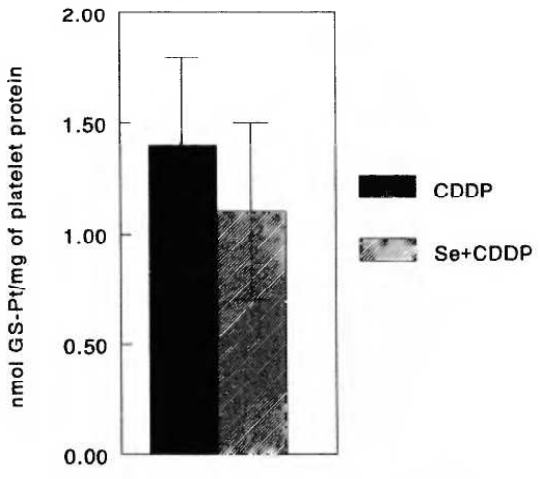

Fig. 3 The levels of GS-cisplatin complex in blood platelets incubated with cisplatin (20 μM, 1 h, 37°C) alone and with cisplatin and selenite (20 μM, 10 min, 37°C) ($p < 0.05$)

The glutathione conjugate of cisplatin, excreted by the GS-X pump plays an important role in cytotoxicity of this agent (Ishikawa and Ali-Osman, 1993). Selenite, like cisplatin reacts with free sulfhydryl groups, mainly with GSH present in blood platelets (Zbikowska, Wachowicz, Krajewski & Olas, 1994, Wachowicz *et al* 1995). From our previous studies appeared that the concentration of free thiols (GSH and -SH groups of proteins) in pig blood platelets treated with cisplatin or selenite distinctly decreased. The treatment of platelets with CDDP (20 μM, 30 min) caused reduction of GSH about 20% (reduction of -SH groups in proteins about 25 %). After incubation of cells (30 min) with Se (0.1 μM) the amount of platelet thiols was also reduced (Wachowicz *et al* 1995). The present study demonstrated that in the presence of selenite the level of formed GS-PT complex in platelets was decreased (Fig. 3). A possible explanation for the protective effect of selenite might, therefore, be the competition between selenite and cisplatin for glutathione present in these cells. Complex GSH with cisplatin (GS-Pt) is much more toxic than cisplatin alone and it inhibits significantly aggregation of platelets (Olas and Wachowicz, 1996, in press). Pretreatment of platelets with Se caused the decrease of both GSH and GS-Pt complex. The cell response to selenite depends on the dose of selenite and this compound can function as a cytostatic or as a cytotoxic agent. Selenite reacts rapidly with GSH and forms selenodiglutathione, which is then further transformed to hydrogen selenite in the presence of additional GSH. Selenite at the concentration used in our experiments (1 μM) *in vitro* is not toxic. It had no inhibitory effect on ADP-induced aggregation of pig platelets. Our data presented in this paper confirm that GSH, a peptide essential in detoxication in blood platelets may react as well with selenite as cisplatin and therefore platelet glutathione seems to be the primary target for action of the tested compounds.

Acknowledgements

We are grateful to Prof. C. Cierniewski and the staff of Dept. Biophysics, Medical University of Łódź for their help in the aggregation studies.

REFERENCES

Baldew G. S., Cornelis J. A., Van den Hamer, Los G., Vermeulen N. P. E., de Gocij J. J. M. & Mc Vie J. G. (1989). Selenium-induced protection against cis-diamminedichloroplatinum (II) nephrotoxicity in mice and rats. *Cancer Res.*, **49**, 3020-3023.

Baldew G. S., Mol J. G. J., De Kanter F. J. J., Van Baar B., De Goeij J. J. M. & Vermeulen N. P. E. (1991). The mechanism of interaction between cisplatin and selenite. *Biochem. Pharmacol.*, **41**, 1429-1437.

Beaty J. A., Jones M. M. & Ma l. (1992). The reactions of cis-$(Pt(NH_3)_2(H_2O)_2)^{2+}$ with L-(+)-cystathionine and seleno-L-methionine: potential relevance to the molecular basis of cisplatin toxicity. *J. Am. Chem. Soc.*, **5**, 647-653.

Fox, J. E. B., Dockter M. E. & Philips D. R. (1981). An improved method for determining the actin filament content of nonmuscle cells by the DNase I inhibition assay. *Anal. Biochem.*, **117**, 170-171.

Ishikawa T. & Ali-Osman F. (1993). Glutathione associated cis-diamminedichloroplatinum (II) metabolism and ATP-dependent efflux from leukemia cells. *J. Biol. Chem.*, **27**, 20116-20125.

Keppler K. (1993). *Metal complexes in cancer chemotherapy.* VCH.

Lefebvre P., White J. G., Krumwiede M. D. & Cohen I. (1993) Role of actin in platelet function. *Eur. J. Cell Biol.*, **62**, 194-204

Ohno S., Strebel F. R., Stephens L. C., Siddik Z. H., Baba H., Makino M., Khokhar A. R. & Bull J. M. C. (1993). Haematological toxicity of carboplatin and cisplatin combined with whole body hyperthermia in rats. *Br. J. Cancer*, **68**, 469-474.

Olas B. & Wachowicz B. (1996). Cisplatin-induced changes of biological activity of blood platelets; thiol-related mechanisms. *Anti-Cancer Drug* (in press)

Pendyala L. & Creaven P. J. (1993). In vitro cytotoxicity, protein binding, red blood cell partitioning, and biotransformation of oxaliplatin. *Cancer Research*, **53**, 5970-5976.

Vastassary G. T. & Smith W. E. (1987). Determination of α-tocopherolquinone (vitamin E quinone) in human serum, platelets, and cell membrane samples. *Analyt. Biochem.*, **167**, 411-417.

Vermeulen N. P. E., Baldew G. S., Los G., Mcvie & De Goeij J. J. M. (1992). Reduction of cisplatin nephrotoxicity by sodium selenite. *Drug Metab.*, **21**, 30-36.

Wachowicz B. (1991). Effect of cisplatin on lipid peroxidation in blood platelets. *Acta Biochim. Pol.*, **38**, 87-90.

Wachowicz B. (1993). Inhibitory effect of cisplatin on the enzymatic peroxidation of platelet endogenous arachidonate. *Cytobios*, **75**, 157-162.

Wachowicz B. & Kustron J. (1992). Effect of cisplatin on lipid peroxidation in pig blood platelets. *Cytobios*, **70**, 41-47.

Wachowicz B., Krajewski T., Olas B. & Zbikowska H. M. (1995). Effects of cisplatin and selenite on the level of thiols in pig blood platelets. *J. Physiol. Pharmacol.*, **46**, 97-103

Zbikowska H. M., Wachowicz B., Krajewski T. & Olas B. (1994). Effect of sodium selenite on the sulphydryl groups of pig blood platelets. *Biomed. Let.*, **50**, 203-210.