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Aggregation of γ-globulin by *cis*-diamminedichloroplatinum(II): alteration of Fc region and restoration by diethyldithiocarbamate

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Abstract

Human γ-globulin containing IgGs generally shows a binding ability to protein A through its Fc region. When purified human γ-globulin was incubated with *cis*-diamminedichloroplatinum(II) (*cis*-DDP), reduced binding to protein A was observed. On the other hand, γ-globulin in human plasma showed only a slight decrease in protein A binding at similar doses, due probably to other internal substances in plasma by trapping *cis*-DDP. Reduction of γ-globulin pretreated with *cis*-DDP resulted in significantly decreased amounts of the H and HL components, whereas the L chain was normally detected. These results suggest that *cis*-DDP affects the disulfide(S-S) bond(s) in the inter H-H chains which locate in the Fc region. We have demonstrated that *cis*-DDP causes γ-globulin polymerization and its S-S bond cleavage (Chen et al., *Int. J. Pharm.*, 106 (1994) 249–253). Diethyldithiocarbamate (DDTC) partly restored these effects of *cis*-DDP on γ-globulin in terms of the decreased S-S bonds, polymerization, and the reduced binding ability of γ-globulin to protein A. Since DDTC is known to easily cleave the Pt-S bond, Pt-S bonds are likely to be responsible for the restoration of this γ-globulin-*cis*-DDP interaction.

Keywords: γ-Globulin; IgG; Disulfide bond; Drug binding; *cis*-Diamminedichloroplatinum(II); Cisplatin; Fc region; Diethyldithiocarbamate

1. Introduction

Cisplatin, *cis*-diamminedichloroplatinum(II) (*cis*-DDP), is one of the most widely used chemotherapeutic antitumor agents (Chu, 1994). Since the DNA adduct of *cis*-DDP is generally accepted to be responsible for its antitumor activity, much information is available for its binding to nitrogen atoms in DNA (Lempers and Reedijk, 1991). On the other hand, platinum has a high affinity for sulfur-containing molecules, including metallothionein or glutathione via the sulfhydryl group, α_2-antiplasmin via the methionine group, and the histidine group (Howe-Grant and Lippard, 1980; Geary and Gonias, 1989; Lempers and Reedijk, 1991; Pattanaik et al., 1992). Sulfur-containing biomolecules accelerate inactivation of *cis*-DDP and are involved in the resis-

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tance of normal or tumor cells to *cis*-DDP toxicity. Various compounds containing sulfur have been proposed as protecting agents to decrease the toxic side effects of *cis*-DDP without interfering with their antitumor activity. Such examples include WR 2721 (ethiofos, *S*-2-(3-aminopropylamino)ethylphosphorothioic acid), thiosulfate, and *N,N*-diethyldithiocarbamate (DDTC) which is the metabolic product of disulfiram used in the treatment of alcoholism (Agarwal et al., 1983; Schmalbach and Borch, 1990; Kobayashi et al., 1991; Treskes et al., 1991).

We have demonstrated that *cis*-DDP is able to bind proteins through the disulfide(S-S) bond and cause alterations in the secondary structures of human serum albumin, fibrinogen and γ-globulin (Ohta et al., 1992, 1993, 1995; Chen et al., 1994a,b). Interestingly, only γ-globulin was polymerized and changed to insoluble aggregates by *cis*-DDP. As compared to the extensively examined albumin, due to the lack of knowledge about competitive drug binding to γ-globulin and its polyclonal characteristics, little information is available about the interaction between γ-globulin and drugs from the pharmaceutical viewpoint. One of the functional sites of the IgG molecule other than the antigen-binding site is the Fc region consisting of C_H2 and C_H3 domains, which is known to bind specifically protein A from *Staphylococcus aureus* (Burton, 1985). Therefore, protein A is valuable for predicting the effect of a given drug to the Fc region.

In the present study, we investigated the effect of *cis*-DDP on this functional site of γ-globulin using affinity gel chromatography of protein A, and the restoration of the platinated γ-globulin by DDTC in terms of the S-S bond, polymerization and the protein A binding ability.

2. Materials and methods

2.1. Materials

cis-DDP was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Human γ-globulin (No. G4386) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Disodium 2-nitro-5-thiosulfobenzoate(NTSB) was synthesized according to the method of Thannhauser et al. (1984). Sodium *N,N*-diethyldithiocarbamate trihydrate (DDTC) was obtained from Wako Pure Chemical Ind. Ltd (Osaka). Affi-Gel protein A MAPS-II kit was purchased from Bio-Rad (Richmond, CA, USA). Whole blood was obtained from three healthy volunteers (Red Cross Blood Center, Nagoya).

2.2. Incubation of cis-DDP and γ-globulin

Incubation of γ-globulin with *cis*-DDP was carried out as previously described (Chen et al., 1994b). *cis*-DDP was dissolved in a phosphate buffer solution (50 mM, pH 7.4) with 0.1 M NaCl. Human γ-globulin was dissolved in the same medium. Each solution was filtered through a 0.2 μm sterilized filter (Toyo Roshi, Tokyo) and placed in sterilized test tubes with a screw cap after correcting the modified concentration of protein due to adsorption on the nitrocellulose membrane. The concentration of *cis*-DDP was varied from 0.1 to 0.6 mM, where the protein concentration was always kept at 3 mg/ml. The mixed solutions were incubated at 37°C, being protected from light.

2.3. Binding of IgG to protein A affinity gel

Binding of IgG in γ-globulin solution to protein A from *S. aureus* was conducted using the Affi-Gel protein A MAPS-II kit according to the method recommended by Bio-Rad. Since protein A specifically binds to the Fc region of immunoglobulins, mainly IgG, from different mammalian species (Langone, 1982), affinity chromatography, using protein A coupled to agarose gel, is widely used for the isolation of IgG. The isolation of IgGs was carried out by applying γ-globulin (2 ml) dissolved in pH 8.0 binding buffer to an Affi-Gel protein A column (bed volume 1 ml) and eluting the retained IgG1, IgG2, and IgG4 with pH 3.0–7.0 elution buffer (Burton, 1985). The IgG content in the eluted solution was measured at 280 nm.

2.4. Effect of cis-DDP on protein A binding ability to IgG in human plasma

Pooled human plasma was prepared by mixing an equal volume of plasma from three volunteers. The plasma was dialyzed against pH 7.4 buffer (50 mM phosphate) containing 0.1 M NaCl in order to compare with the protein A binding to purified γ-globulin in the same medium, since the reactivity of *cis*-DDP is sensitive to chloride concentration. Subsequently, the plasma was diluted to adjust the IgG concentration equal to 5 mg/ml. The concentration of IgG in human plasma was determined by the method of latex agglutination (Dezelic and Dezelic, 1970). Various concentrations of *cis*-DDP were added, and a 2 day incubation at 37°C was performed. Plasma (2 ml) diluted in pH 8.0 binding buffer was applied on Affi-Gel protein A as previously described.

2.5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of cis-DDP-treated and reduced γ-globulin

γ-Globulin was incubated with 0.1 or 0.6 mM *cis*-DDP at 37°C for 6 days. It was then solubilized in 25% urea and reduced using 4 mM dithiothreitol (DTT) according to the method of Davies et al. (1989). Alkylation of the reduced thiol groups was carried out using iodoacetamide. Carboxymethylated γ-globulin was obtained by separation through a column (1.0 cm i.d. × 18 cm) of Sephadex G-25 (Pharmacia, Uppsala, Sweden). Analysis by electrophoresis in the presence of SDS was performed on 7.5% polyacrylamide gel at pH 8.8.

2.6. Restoration of platinated γ-globulin by DDTC

Restoration experiments were performed on 2-day incubation mixtures of γ-globulin (3 mg/ml) and *cis*-DDP. Before adding DDTC, the reaction mixture of γ-globulin and *cis*-DDP was applied to a Sephadex G-25 column in order to remove excess *cis*-DDP, since DDTC has been shown to chelate with *cis*-DDP (Dedon and Borch, 1987). 3 mM DDTC was then added to the γ-globulin fraction and incubated at 37°C for 30 min. The number of S-S bonds, SDS-PAGE, and binding ability to protein A were then measured for γ-globulin.

2.7. Determination of S-S bond

The number of S-S bonds remaining in γ-globulin was determined using NTSB by a modified method of Kella and Kinsella (1985). γ-Globulin solution (0.5 ml) was mixed with NTSB assay solution (3 ml) containing 0.2 M Tris base, 0.1 M EDTA and 3 M guanidine thiocyanate with freshly prepared Na_2SO_3 solution (0.2 M). After 20 min the absorbance at 412 nm was monitored against an appropriate blank. The concentration of S-S was calculated from the extinction coefficient of 2-nitro-5-thiobenzoic acid of 13 600 M^{-1} cm^{-1} (Riddles et al., 1983).

Protein was assayed using the Coomassie Brilliant Blue G kit (Bio-Rad).

3. Results and discussion

The IgG molecule, a major component of γ-globulin, consists of two sets of light (L) and heavy (H) chains which are held together by S-S bonds as depicted in Fig. 1. An L chain is linked to an H chain by a single S-S bond. The molecular mass of the L and H chains is 25 and 50 kDa, respectively. Although inter H chain S-S bonds

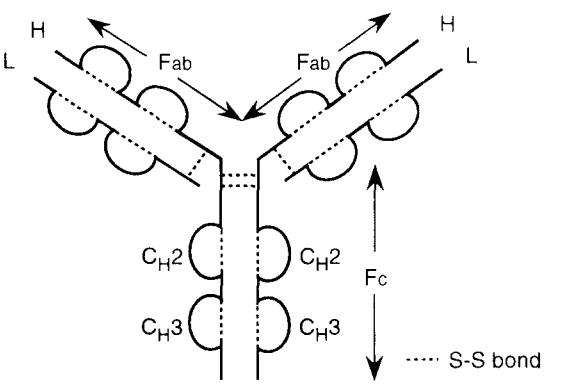

Fig. 1. Schematic diagram showing the immunoglobulin IgG1 molecule.

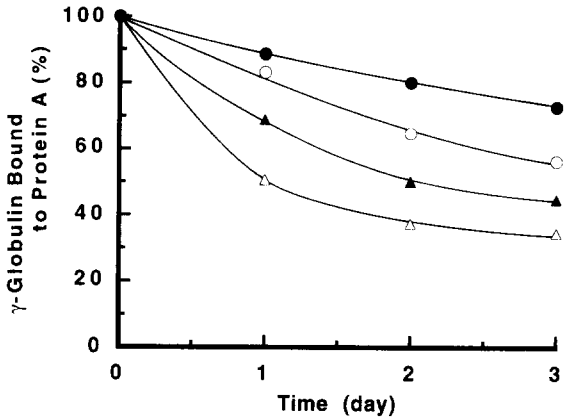

Fig. 2. Binding ability of *cis*-DDP-treated γ-globulin to protein A. γ-Globulin (3 mg/ml) was incubated with *cis*-DDP(mM): (●) 0.1, (○) 0.2, (▲) 0.4, (△) 0.6. The amount of native γ-globulin bound to protein A affinity gel (bed volume 1 ml) was regarded as 100%.

are different depending on the subclasses, the average number of S-S bonds is estimated to be 17.1 based on the assumption that the four subclasses were comparable to those in the blood, and contain no sulfhydryl groups (Nisonoff, 1984a; Chen et al., 1994b). The Fc region of IgG, which is composed of the C_H2 and C_H3 domain, plays an important role as functional sites, such as complement binding and binding to the Fc receptor on phagocytic cells (Burton, 1985). The interaction between staphylococcal protein A and human IgG1, IgG2, or IgG4 is mediated by this Fc region. The *cis*-DDP effect on the binding ability of the Fc region to protein A was evaluated.

3.1. Effect of cis-DDP on Fc region of γ-globulin

Fig. 2 shows the influence of incubation time on the extent of binding of protein A with γ-globulin, which was treated with various concentrations of *cis*-DDP at 37°C for 3 days. Bound γ-globulin to protein A without *cis*-DDP was regarded as 100%. Since the incubation solution gradually turned opaque after about 4 days incubation, a limited study was conducted in protein A binding within 3 days. The binding ability to protein A remaining in γ-globulin solution decreased with increasing *cis*-DDP and incubation time, as shown in Fig. 2. On incubation for 3 days with 0.6 mM *cis*-DDP, the γ-globulin fraction being able to bind with protein A was reduced to 35% of the untreated control. We reported that *cis*-DDP causes the formation of γ-globulin polymer, accompanying the cleavage of S-S bonds, in which 2.7 S-S bonds per mol of protein were cleaved and the polymerization proceeded to some extent by 3 days incubation with 0.6 mM *cis*-DDP (Chen et al., 1994b). Therefore, the changes in Fc induced by *cis*-DDP, rendering it unable to interact with protein A as shown in Fig. 2, might involve S-S cleavage and/or polymerization.

As shown by crystallographic studies, protein A binds between the C_H2 and C_H3 domains and His 435 in C_H3 plays an important role in protein A binding (Deisenhofer, 1981). The inability of human IgG3 to bind to protein A could be explained by the substitution of Arg for His 435. Therefore, the *cis*-DDP effect on protein A binding is assumed to be direct against His 435 or indirect, such as the cleavage of S-S bond which locates near His. The interaction between protein A and IgG1, however, is unaffected by inter H chain S-S reduction and alkylation (Klein et al., 1981). Thus, a greater perturbation of His 435 might have been induced by *cis*-DDP than interchain S-S reduction and alkylation.

3.2. Effect of cis-DDP on Fc region of γ-globulin in human plasma

In order to elucidate the effect of *cis*-DDP on the IgG Fc region in plasma, the binding ability of plasma IgG to protein A in plasma was compared with that in purified γ-globulin solution where the IgG concentrations were adjusted to be equivalent (5 mg/ml) in pH 7.4 buffer containing 0.1 M NaCl. Fig. 3 shows the effect of *cis*-DDP concentrations on the binding ability of IgG to protein A. Purified γ-globulin solution, upon incubation with 0.8 mM *cis*-DDP for 2 days, showed a decrease to about 39% in protein A binding, while plasma demonstrated a 60% decrease. This discrepancy may be attributed to internal substances contained in plasma that probably more preferentially interact with *cis*-DDP than IgG,

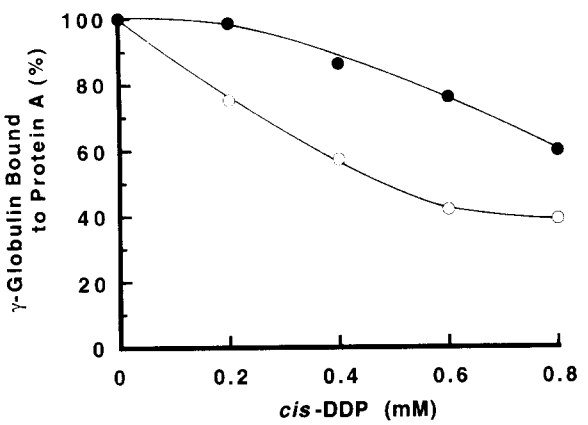

Fig. 3. Change of binding ability to protein A of *cis*-DDP-treated γ-globulin in human plasma. (○) Purified γ-globulin solution; (●) dialyzed human plasma. The concentrations of IgG in human plasma and IgG in purified γ-globulin solution were adjusted to be 5 mg/ml, and incubation with *cis*-DDP was performed at pH 7.4 and 37°C for 2 days.

such as albumin, and thus might trap *cis*-DDP before binding to IgG (Gonias and Pizzo, 1983; Ohta et al., 1992). Upon administration of *cis*-DDP, a similar degree of damage in the Fc region might not be feasible, since the *cis*-DDP concentration used in this experiment was greater than that found in plasma and the maximam plasma platinum was about 2×10^{-5} M even in high-dose therapy (Gandara et al., 1991).

3.3. SDS-PAGE of cis-DDP-treated γ-globulin prior to reduction

IgG has 12 intrachain S-S bonds and 4–13 interchain S-S bonds. Interchain S-S bonds are known to be more readily reduced by reducing agents than intrachain S-S bonds (Nisonoff, 1975). Partial reduction of an intact IgG would result in many fragments in order of molecular mass L, H, HL, or HHL as shown in Fig. 4. If any interchain S-S bonds were not altered by *cis*-DDP, fragments similar to those from intact IgG would be derived. However, SDS-PAGE of *cis*-DDP-treated γ-globulin prior to reduction with 1 or 4 mM DTT was significantly different from that of γ-globulin subjected to only reduction. In the case of *cis*-DDP-treated γ-globulin, the H chain (50 kDa), HL chain (75 kDa) which is a relatively large amount of intermediate breakdown product from intact IgG disappeared, and only the L chain (25 kDa) was clearly detected. The normal detection of L chain might indicate that the one S-S bond linked H and L chain is not involved in *cis*-DDP binding. Instead, the disappearance of H and HL chains demonstrates the possible involvement of the S-S interchain bond between H and H chains. This is consistent with the result showing *cis*-DDP induced damage in the Fc region, since the Fc region includes these inter H chain S-S bonds (Nisonoff, 1984b).

3.4. Restoration of platinated γ-globulin by DDTC

Several sulfur-based nucleophilic agents have been investigated as chemoprotectors against the side effects of *cis*-DDP, such as WR-2721 (Treskes et al., 1991), methiazole (Vail et al., 1993), thiourea, thiosulfate (Kobayashi et al., 1991) and DDTC (Dedon and Borch, 1987). The rescue mechanisms of these agents are likely to be different. DDTC can reduce the nephrotoxic-

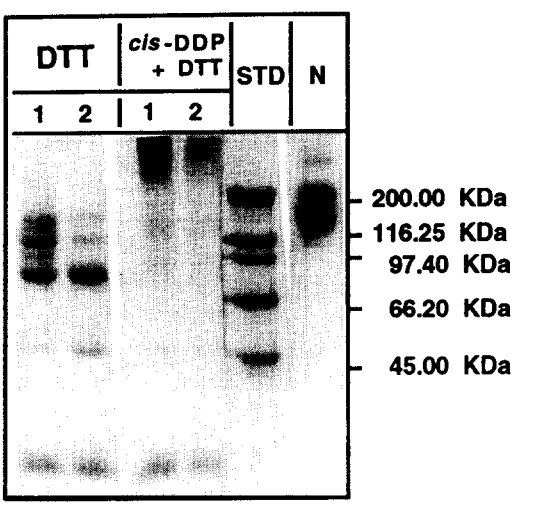

Fig. 4. SDS-PAGE of *cis*-DDP induced polymerized γ-globulin prior to reduction. (Left lanes) γ-Globulin was reduced by (1) 1 mM or (2) 4 mM DTT for 30 min, and alkylated. (Right lanes) γ-Globulin was incubated with *cis*-DDP (1) 0.1 mM or (2) 0.6 mM for 6 days, and then reduced by 4 mM DTT and alkylated. N, native γ-globulin; STD, standard SDS-PAGE molecular mass marker.

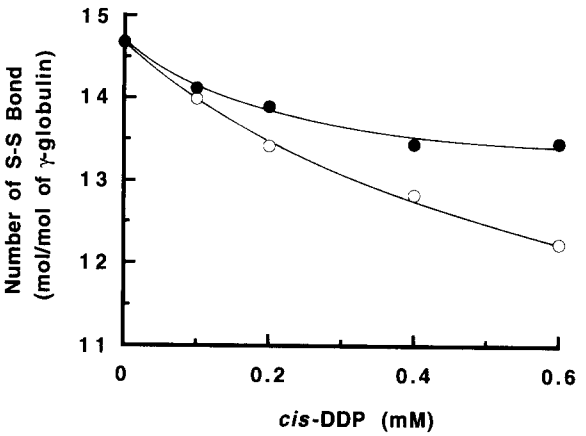

Fig. 5. Restoration of cleaved disulfide bond in *cis*-DDP-treated γ-globulin using 3 mM DDTC. (○) Before addition of DDTC; (●) after addition of DDTC. *cis*-DDP-treated γ-globulin (3 mg/ml) was prepared by 2 days incubation at 37°C with various concentrations of *cis*-DDP. The γ-globulin fraction in this incubation mixture was then incubated with 3 mM DDTC for 30 min at 37°C as described under Section 2.

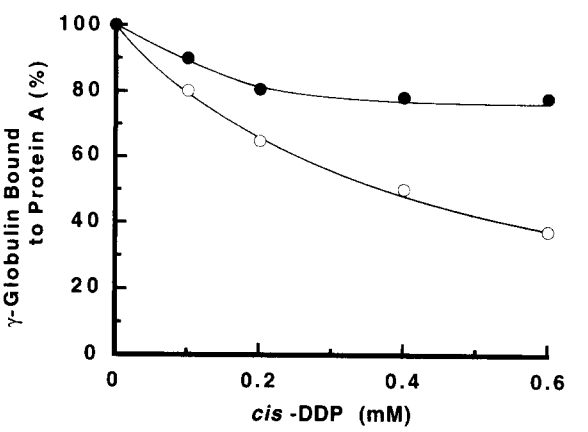

Fig. 7. Restoration of binding ability of *cis*-DDP-treated γ-globulin to protein A by 3 mM DDTC. (○) Before addition of DDTC; (●) after addition of DDTC. *cis*-DDP-treated γ-globulin was prepared by 2 days incubation at 37°C with various concentrations of *cis*-DDP. The γ-globulin fraction in this incubation mixture was then incubated with 3 mM DDTC for 30 min at 37°C.

ity by restoring platinated protein, while the problem of toxicity remains. Thus, DDTC restoring the alteration in γ-globulin caused by *cis*-DDP, i.e., S-S bond decrease, polymerization, and reduced binding to protein A, is possible.

Incubation of γ-globulin with *cis*-DDP resulted in a decrease of S-S bonds in γ-globulin. Fig. 5 demonstrates the number of S-S bonds cleaved in 3 mg/ml of γ-globulin during 2 days of incubation. When *cis*-DDP-treated γ-globulin was incubated with 3 mM DDTC for 30 min, DDTC was able to restore the decreased number to

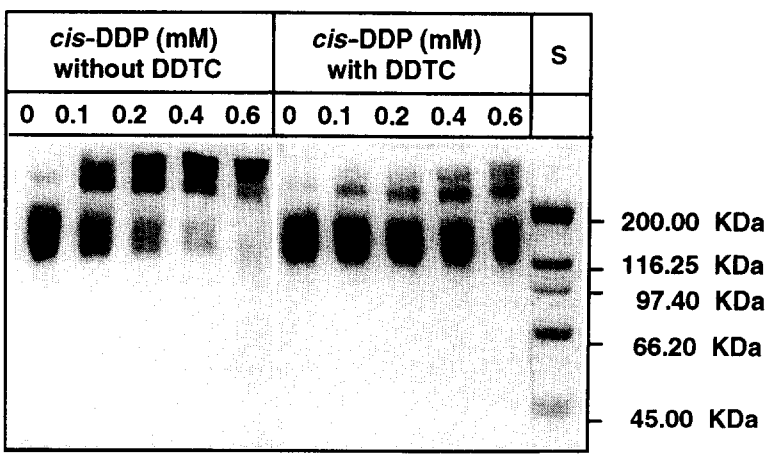

Fig. 6. SDS-PAGE of γ-globulin incubated with *cis*-DDP and restoration of monomer form using 3 mM DDTC. (Left lanes) *cis*-DDP-treated γ-globulin was prepared by 2 days incubation at 37°C with various concentrations of *cis*-DDP (0, 0.1, 0.2, 0.4, 0.6 mM). (Right lanes) The γ-globulin fraction in the incubation mixture was then incubated with 3 mM DDTC for 30 min at 37°C. S, standard SDS-PAGE molecular mass marker.

about 1 instead of the original value of 0. Prolonged incubation for 1 h did not appear to be effective for further restoration.

The effects of DDTC on polymerized γ-globulin induced by *cis*-DDP are shown in Fig. 6. The lanes of the left half show SDS-PAGE of γ-globulin with a higher molecular mass than the intact molecule caused by various amounts of *cis*-DDP. Subsequent treatments with 3 mM DDTC for 30 min restored the polymerized γ-globulin to the original molecular mass as shown in the right lanes. Upon incubation with *cis*-DDP at concentrations of 0.4 and 0.6 mM, intact γ-globulin is hardly observed before DDTC addition, whereas it is clearly evident after incubation with DDTC. Polymerized γ-globulin which is likely to be held together covalently via platinum might be restored to the monomeric form by dissociation of the bond(s) in the γ-globulin-*cis*-DDP adduct (Chen et al., 1994b).

Fig. 7 shows the restoration of binding ability of γ-globulin to protein A. After a 2 day incubation with *cis*-DDP, γ-globulin was significantly reduced in protein A binding. The addition of DDTC restored the binding ability to approx. 80% of its original state. No measurable effect of DDTC itself on the binding between γ-globulin and protein A was observed. As far as the restoration system by DDTC is concerned, complete restoration was not achieved in all three experiments after 30 min incubation with 3 mM DDTC.

The present study showed that incubation of γ-globulin with *cis*-DDP resulted in the reduced binding of protein A, in addition to S-S bond cleavage and polymerization (Chen et al., 1994ab). The partial restoration of these changes by DDTC might help to reveal the binding mechanism between *cis*-DDP and γ-globulin. As DDTC indeed induces dissociation of Pt-enzyme adducts by breaking the Pt-S bond, alterations of γ-globulin caused by *cis*-DDP might partly involve Pt-S bond formation (Lempers and Reedijk, 1990, 1991). The S generally represents the sulfur atom of cysteine, methionine or possibly cystine, although γ-globulin contains no cysteine as described above. The incomplete regeneration observed with DDTC suggests that the binding mechanism between *cis*-DDP and γ-globulin could not be explained by one type of bond. As demonstrated in the previous report, the binding of about 4.4 mol of *cis*-DDP at the ratio of one S-S cleavage in γ-globulin supports this explanation (Chen et al., 1994b). The incomplete regeneration might be also explained by some conformational perturbation, rendering γ-globulin inaccessible to DDTC, which was induced by *cis*-DDP.

Upon administration of *cis*-DDP, a relatively high level of platinum is seen in the γ-globulin fraction (Gullo et al., 1980). In the present paper we demonstrated that *cis*-DDP-treated γ-globulin is functionally deficient as well as the conformational change, and the Fc region contributes to these changes. The result is interesting, as little information is available about drug binding to the Fc region in IgG. Decreased biological activity of γ-globulin through the Fc region may be of medical relevance, for example, as regards the immune defense mechanism, although the functional deficiency in plasma was shown to occur to a smaller extent than in the buffer solution.

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References

Agarwal, P., McPherson, R.A. and Phillips, M., Rapid degradation of disulfiram by serum albumin. *Res. Commun. Chem. Pathol. Pharmacol.*, 42 (1983) 293–310.

Burton, D.R., Immunoglobulin G: Functional sites. *Mol. Immunol.*, 22 (1985) 161–206.

Chen, D., Ohta, N., Ukai, M., Masuda, M. and Yotsuyanagi, T., Binding and aggregation of human γ-globulin by *cis*-diamminedichloroplatinum(II) through S-S bond. *Biol. Pharm. Bull.*, 17 (1994b) 1561–1566.

Chen, D., Ohta, N., Yotsuyanagi, T. and Ikeda, K., Polymerized precipitation of human γ-globulin induced by *cis*-diamminedichloroplatinum(II). *Int. J. Pharm.*, 106 (1994a) 249–253.

Chu, G., Cellular responses to cisplatin. *J. Biol. Chem.*, 269 (1994) 787–790.

Davies, J.R., Gilbo, C.M., Marley, P.B. and Hearn, M.T.W.,

Gamma globulin-derived standards for the determination of molecular weights, transfer, and immunodetection efficiencies in protein blotting procedures. *Anal. Biochem.*, 176 (1989) 249–254.

Dedon, P.C. and Borch, R.F., Characterization of the reactions of platinum antitumor agents with biologic and nonbiologic sulfur-containing nucleophiles. *Biochem. Pharmacol.*, 36 (1987) 1955–1964.

Dezelic, N. and Dezelic, G., A photometric method for the determination of serum titers by latex particle agglutination. *Croatica Chem. Acta*, 42 (1970) 457–466.

Deisenhofer, J., Crystallographic refinement and atomic models of a human Fc fragment and its complex with fragment B of protein A from *Staphylococcus aureus* at 2.9- and 2.8-Å resolution. *Biochemistry*, 20 (1981) 2361–2370.

Gandara, D.R., Perez, E.A., Wiebe, W., DeGregorio, M.W. and Hoff, J., High dose cisplatin: Modulation of toxicity. In Howell, S.B. (Ed.), *Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy*, Plenum, New York, 1991, pp. 481–492.

Geary, W.A. and Gonias, S.L., Inactivation of α_2-antiplasmin by limited reaction with *cis*-dichlorodiammineplatinum(II). *Biochim. Biophys. Acta*, 994 (1989) 1–6.

Gonias, S.L. and Pizzo, S.V., Complexes of serum albumin and *cis*-dichlorodiammineplatinum(II). *J. Biol. Chem.*, 258 (1983) 5764–5869.

Gullo, J.J., Litterst, C.L., Maguire, P.J., Sikic, B.I., Hoth, D.F. and Woolley, P.V., Pharmaco-kinetics and protein binding of *cis*-dichlorodiammineplatinum(II) administered as one hour or as a twenty hour infusion. *Cancer Chemother. Pharmacol.*, 5 (1980) 21–26.

Howe-Grant, M.E. and Lippard, S.J., Aqueous platinum(II) chemistry; Binding to biological molecules. In Siegel, H. (Ed.), *Metal Ions in Biological Systems*, Dekker, New York, 1980, Vol. 11, pp. 63–125.

Kella, N.K.D. and Kinsella, J.E., A method for the controlled cleavage of disulfide bonds in proteins in the absence of denaturants. *J. Biochem. Biophys. Methods*, 11 (1985) 251–263.

Klein, M., Haeffner-Cavaillon, N., Isenman, D.E., Rivat, C., Navia, M.A., Davies, D.R. and Dorrington, K.J., Expression of biological effector functions by IgG molecules lacking the hinge region. *Proc. Natl. Acad. Sci. USA*, 78 (1981) 524–528.

Kobayashi, H., Taniguchi, S. and Baba, T., Improved efficacy of 'two-route chemotherapy' using cisplatin and its antidote, sodium thiosulfate, in combination with angiotensin II. In Howell, S.B. (Ed.), *Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy*, Plenum, New York, 1991, pp. 529–540.

Lempers, E.L.M. and Reedijk, J., Interactions of platinum amine compounds with sulfur-containing biomolecules and DNA fragments. *Adv. Inorg. Chem.*, 37 (1991) 175–217.

Lempers, E.L.M. and Reedijk, J., Reversibility of binding of cisplatin-methionine in proteins by diethyldithiocarbamate or thiourea: A study with model adducts. *Inorg. Chem.*, 29 (1990) 217–222.

Longone, J.J., Protein A of *Staphylococcus aureus* and related immunoglobulin receptors produced by *streptococci* and *pneumonococci*. *Adv. Immunol.*, 32 (1982) 157–252.

Nisonoff, A., Primary and three-dimensional structure of antibodies. *Introduction to Molecular Immunology*, 2nd Edn, Sinauer, Sunderland, MA, 1984b, pp. 67–89.

Nisonoff, A., Properties and evolution of classes of antibodies. *Introduction to Molecular Immunology*, 2nd Edn, Sinauer, Sunderland, MA, 1984a, pp. 45–65.

Nisonoff, A., Hopper, J.E. and Spring, S.B., Properties and interactions of the light and heavy chains of immunoglobulins. In Nisonoff, A. (Ed.), *The Antibody Molecule*, Academic Press, New York, 1975, pp. 238–263.

Ohta, N. and Yotsuyanagi, T., Alteration of fibrinogen secondary structure by *cis*-diamminedichloroplatinum(II) and calcium protection. *Biol. Pharm. Bull.*, 16 (1993) 631–634.

Ohta, N., Chen, D., Ito, S., Futo, T., Yotsuyanagi, T. and Ikeda, K., Effect of *trans*-diamminedichloroplatinum(II) on human serum albumin: conformational changes through partial disulfide bond cleavage. *Int. J. Pharm.*, (1995) in press.

Ohta, N., Yotsuyanagi, T., Chen, D., Ono, R., Ito, S. and Ikeda, K., Disulfide bond cleavage of human serum albumin and alterations of its secondary structure by *cis*-diamminedichloroplatinum(II). *Int. J. Pharm.* 85 (1992) 39–44.

Pattanaik, A., Bachowske, G., Laib, J., Lemkuil, D., Shaw III, C.F., Petering, D.H., Hitchcock, A. and Saryan, L., Properties of the reaction of *cis*-dichlorodiammineplatinum(II) with metallothionein. *J. Biol. Chem.*, 267 (1992) 16121–16128.

Riddles, P.W., Blakeley, R.L. and Zerner, B., Reassessment of Ellman's reagent. *Methods Enzymol.*, 91 (1983) 49–60.

Schmalbach, T.K. and Borch, R.F., Mechanism of diethyldithiocarbamate modulation of murine bone marrow toxicity. *Cancer Res.*, 50 (1990) 6218–6221.

Thannhauser, T.W., Konishi, Y. and Scheraga, H.A., Sensitive quantitative analysis of disulfide bonds in polypeptides and proteins. *Anal. Biochem.*, 138 (1984) 181–188.

Treskes, M., Holwerda, U., Klein, I., Pinedo, H.M. and Van der Vijgh, W.J.F., The chemical reactivity of the modulating agent WR2721 (ethiofos) and its main metabolites with the antitumor agents cisplatin and carboplatin. *Biochem. Pharmacol.*, 42 (1991) 2125–2130.

Vail, D.M., Elfarra, A.A., Cooley, A.J., Panciera, D.L., MacEwen, E.G. and Soergel, S.A., Methimazole as a protectant against cisplatin-induced nephrotoxicity using the dog as a model. *Cancer Chemother. Pharmacol.*, 33 (1993) 25–30.