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EFFECTS OF THE MODULATING AGENT WR2721 AND ITS MAIN METABOLITES ON THE FORMATION AND STABILITY OF CISPLATIN–DNA ADDUCTS *IN VITRO* IN COMPARISON TO THE EFFECTS OF THIOSULPHATE AND DIETHYLDITHIOCARBAMATE

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Abstract—The influence of the modulating agent WR2721, its active thiol-metabolite WR1065 and the symmetrical disulphide WR33278 on the *in vitro* formation and stability of *cis*-diamminedichloroplatinum(II) (cisplatin, CDDP)–DNA adducts was investigated and compared with the effects of the highly nucleophilic modulating agents diethyldithiocarbamate (DDTC) and thiosulphate (TS). Salmon sperm DNA (0.5 mg/mL) was incubated with 25 μg/mL (83 μM) cisplatin for 1 hr in 50 mM phosphate buffer, pH 7.2 at 37° in the absence or presence of modulating agent. DDTC and TS were potent inhibitors of the platination of the DNA (95 and 89%, respectively, with 4.2 mM of modulating agent). The WR-compounds were also remarkably active in the inhibition of DNA platination. Prevention of adduct formation in the presence of 4.2 mM WR-compound decreased in the order WR1065 (74%) > WR33278 (63%) > WR2721 (51%). The prevention of CDDP–DNA adduct formation by WR1065 was strongly concentration-dependent up to 4.2 mM but at higher concentrations this protection hardly increased at all. In the presence of the modulating agents, increased levels of CDDP monofunctionally bound to a guanine residue were observed with a simultaneous decrease in the relative abundance of bifunctional adducts. All modulators were also able to reverse part of the CDDP–DNA adducts formed. After a 2-hr incubation of already platinated salmon sperm DNA with 4.2 mM of modulating agent, the removal of Pt from DNA amounted to about 43% with DDTC, 28% with WR1065 and 13–14% with TS, WR2721 and WR33278. Even CDDP bifunctionally bound to two adjacent guanines in the same DNA strand, which is considered to be a very stable adduct, was partly reversed. Our observations suggest that WR2721, especially when administered prior to or concomitantly with CDDP, can be expected to protect those tissues from CDDP-induced damage to DNA that are able to efficiently dephosphorylate WR2721 followed by uptake of the thiol metabolite WR1065. This stresses the importance of a selective formation and uptake of WR1065 by non-tumour tissues for the successful use of WR2721 as a protective agent in combination with platinum-based cancer chemotherapy.

Cis-diamminedichloroplatinum(II) (cisplatin, CDDP‡) is an active cytostatic agent, used in the treatment of several types of solid tumour. The antitumour activity is assumed to result from its binding to DNA [1]. Toxic side effects (nephrotoxicity, neurotoxicity and bone marrow toxicity) limit the therapeutic potency of this drug [2]. CDDP-induced myelotoxicity is probably due to damage to the DNA [3] and also of its nephrotoxicity, damage to DNA has been reported as a probable cause [4]. Much effort has been put into reducing these toxic side effects by the administration of so-called modulating (or rescue) agents, usually sulfur-containing compounds with a high affinity for Pt(II) [5]. However, these modulating agents should not reduce the antitumour activity of CDDP. TS is a strong nucleophile which is concentrated in the kidney where it is supposed to protect against CDDP-induced damage. However, TS also inactivates active Pt-species in the circulation, thus interfering with the antitumour activity of CDDP. Therefore, TS is mainly applied in "two-route" combination chemotherapy where CDDP is locally (e.g. i.p.) administered to the tumour while i.v. administered TS protects the kidneys [6]. DDTC is a strong nucleophile which is able to reduce the toxic side effects of CDDP, when administered 2–3 hr after CDDP, without interfering with antitumour activity [7–11]. It was hypothesized that DDTC is able to reverse toxic CDDP–protein interactions while CDDP–DNA adducts, responsible for the antitumour activity, are not reversed. Observations that DDTC *in vitro* could reactivate CDDP-inactivated renal enzymes but could not reverse CDDP–DNA

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‡ Abbreviations: CDDP, cisplatin, *cis*-diammine-dichloroplatinum(II); DDTC, diethyldithiocarbamate; TS, thiosulphate; Pt-GG, bifunctional adduct of CDDP with two adjacent guanines in the same DNA strand; Pt-AG, bifunctional adduct of CDDP with guanine and adenine in the base sequence pApG in a DNA strand; G-Pt-G, bifunctional adduct of CDDP with either two non-adjacent guanines in the same strand or with two guanines in the opposite DNA strand; Pt-G, CDDP monofunctionally bound to a guanine residue.

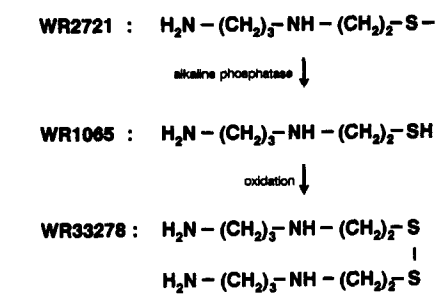

Fig. 1. Structural formula of WR2721 and its main metabolites.

interactions support this hypothesis [10]. However, the clinical use of DDTC is hampered by a reversible but very severe toxicity to the central nervous system [11]. WR2721, a prodrug of the radioprotective thiol-compound WR1065, protects mice against the nephrotoxic and myelotoxic action of CDDP without an adverse effect on the antitumour activity [12, 13]. Early clinical trials suggest the same selective protection of non-tumour tissues [14]. WR2721 is given 30 min before CDDP. It is preferentially metabolized into and taken up as the active thiol-metabolite WR1065 by non-tumour tissues due to their high alkaline phosphatase activity and high pH as compared to poorly vascularized, hypoxic tumour tissue [15–17]. Inside the cell, WR1065 is oxidized to the symmetrical disulphide WR33278 and several mixed disulphides with endogenous thiols [15]. The molecular structures of WR2721 and its metabolites WR1065 and WR33278 are presented in Fig. 1. For a better understanding of the mechanism of action of these modulating agents we investigated their ability to prevent the formation of CDDP–DNA adducts as well as their ability to reverse these CDDP–DNA interactions.

MATERIALS AND METHODS

Chemicals. CDDP was obtained from the Bristol Myers Co. (Syracuse, NY, U.S.A.). WR2721 and WR1065 were obtained from US Bioscience (West Conshohocken, PA, U.S.A.). WR33278 was prepared by bubbling moisturized air for 24 hr through a solution of WR1065 in 10 mM phosphate buffer at pH 7.4. Completion of the reaction was confirmed by electrochemical measurement with a +0.4 to −1.6 V sampled direct current scan using a PAR303 static mercury drop electrode with a PAR174 potentiostat (EG&G instruments, NJ, U.S.A.) and a BD100 strip chart recorder (Kipp & Zonen, Delft, The Netherlands). The mercury–thiol complex oxidation wave (−0.38 V vs Ag/AgCl) was replaced by a disulfide reduction wave (−0.55 V vs Ag/AgCl). All other chemicals used were of analytical grade.

DNA incubations. Salmon sperm DNA (0.5 mg/mL) (Millipore Corp., Bedford, MA, U.S.A.) was incubated with 25 μg/mL (83 μM) CDDP for 1 hr in 50 mM phosphate buffer, pH 7.2 at 37°. The reaction was stopped with the addition of ammonium bicarbonate to a concentration of 100 mM, followed by an overnight dialysis against 100 mM ammonium bicarbonate at 37°, as described elsewhere [18]. The influence of modulating agents on the formation of the CDDP–DNA adducts was investigated by the incubation of DNA with CDDP in the presence of modulating agent ("co-incubation"). To establish the stability of the CDDP–DNA adducts in the presence of modulating agents, immediately after incubation of the DNA with CDDP, the platinated DNA was collected by precipitation with two volumes of ethanol (−20°) after addition of 0.1 volumes of 3 M sodium acetate/1 mM EDTA (pH 5.5). The DNA was collected by centrifugation, washed with 80% ethanol (−20°) and redissolved in the same phosphate buffer with modulating agent added. Then the samples were incubated for 2 hr at 37° ("post-incubations"). After that, the reaction was stopped as described above. All modulating agents were used at 4.2 mM, a 50-fold molar excess to CDDP (83 μM). For the intracellularly active thiol-metabolite WR1065, the effect on CDDP–DNA adduct formation and reversal was also investigated at molar ratios of 10, 100 and 500. The incubations to determine the prevention of formation and the reversal of CDDP–DNA adducts by co- and post-incubations with the modulating agents were performed in triplicate.

CDDP–DNA adduct quantification. Platinated DNA was enzymatically digested to the unmodified mononucleotides and the four platinum-containing (di)nucleotides Pt-GG, Pt-AG, G-Pt-G and Pt-G. Then, the digests were separated on a mono Q column (Pharmacia) and the Pt in the collected fractions was quantified, in duplicate, by graphite furnace atomic absorption spectrometry according to the method of Fichtinger-Schepman [19]. The amounts of DNA were determined from the UV absorbance [19]. Total DNA-bound Pt was determined, in duplicate, by graphite furnace atomic absorption spectrometry before the separation of the digestion products of the DNAs.

RESULTS

To study the influence of modulating agents during the incubation of CDDP with DNA, the platination of salmon sperm DNA in the absence of modulating agent (control) was first determined. After a 1-hr incubation at 37° with 10 μg/mL (33 μM) CDDP, the level was 14 pmol Pt/μg DNA. The distribution of the different adducts was Pt-GG, 63%; Pt-AG, 15%; G-Pt-G, 12%; and Pt-G, 10%. This is in good agreement with earlier findings [19]. Platination was originally performed in 10 mM phosphate buffer. However, when the DNA was incubated in the presence of the modulating agents WR1065 and WR33278, part of the DNA precipitated. This could be prevented by increasing the phosphate buffer concentration to 50 mM. Because the platination of DNA is lower at this higher phosphate buffer concentration [18], the CDDP concentration had to be increased to 83 μM to obtain a similar degree of platination as obtained with 33 μM CDDP in 10 mM phosphate buffer.

Table 1. Total DNA-bound Pt and the sum of the four adducts after chromatography of the digested DNA samples

Sample	Co-incubations		Post-incubations	
	DNA-bound Pt	Σ adducts*	DNA-bound Pt	Σ adducts*
Control	14.4 ± 0.6 (100)	11.6 ± 0.2 (100)	17.4 ± 2.1 (100)	10.2 ± 0.1 (100)
$WR1065_{500}$†	1.2 ± 0.3 (8)	0.4 ± 0.0 (3)	9.4 ± 0.7 (54)	5.9 ± 0.1 (58)
$WR1065_{100}$	2.0 ± 0.3 (14)	1.2 ± 0.1 (10)	11.5 ± 1.4 (66)	6.2 ± 0.0 (60)
$WR1065_{50}$	3.8 ± 0.6 (26)	2.3 ± 0.1 (20)	11.8 ± 0.8 (72)	6.7 ± 0.4 (65)
$WR1065_{10}$	12.8 ± 1.4 (55)	6.5 ± 0.3 (56)	14.3 ± 1.1 (86)	8.0 ± 0.1 (78)
$WR33278_{50}$	6.0 ± 1.9 (36)	3.4 ± 0.1 (29)	15.1 ± 2.2 (87)	8.7 ± 0.1 (85)
$WR2721_{50}$	7.2 ± 0.9 (49)	4.6 ± 0.1 (40)	15.0 ± 2.2 (86)	9.5 ± 0.6 (93)
TS_{50}	1.6 ± 0.3 (11)	0.8 ± 0.0 (7)	15.1 ± 1.9 (87)	7.6 ± 0.8 (75)
$DDTC_{50}$	ND‡	0.4 ± 0.0 (3)	11.9 ± 1.9 (68)§	5.78 ± 0.2 (57)

Data given in pmol Pt/μg DNA (means ± SD); in brackets, mean % compared to control in each experiment.

* Single experiment.

† Subscripts indicate the molar ratio to the initial CDDP concentration of 83 μM.

‡ Not determined because the formed $Pt(DDTC)_2$ complex precipitated together with the DNA during the alcohol precipitation.

§ Some of the $Pt(DDTC)_2$ complex probably precipitated together with the DNA during the alcohol precipitation.

Compared to the control, DNA platination was considerably reduced in the presence of the modulating agents (co-incubations, Table 1 and Fig. 2a). DDTC was the most active followed by TS > WR1065 > WR33278 > WR2721. As shown for WR1065, the prevention of DNA platination increased, in a non-linear fashion, with increasing concentrations of the agent (Fig. 2b). The relative occurrences of the four CDDP–adducts in the DNA digests were calculated and are given in Fig. 3a. As can be seen in Table 1, the total of these adducts, determined in a single experiment, is somewhat lower than the total DNA-bound Pt. However, the reduction of total adducts (as % of control) by co- and post-incubations with the modulating agents is in good agreement with the data on total DNA-bound Pt. For each of the modulating agents, an increase is observed in the percentage of the Pt-G adduct, a small increase in that of the Pt-AG adduct and a decrease in the percentage of the Pt-GG and G-Pt-G adducts (Fig. 3a). It can be seen that the increase in Pt-G and the decrease in Pt-GG are concentration-dependent as determined for WR1065 (Fig. 3b). This dependence on WR1065 concentration is less obvious for the small but consistent increase/decrease in the relative abundance of the Pt-AG adduct/G-Pt-G adduct, respectively (Fig. 3b).

All modulating agents were also able to reverse part of the CDDP–DNA adducts formed (post-incubations, Table 1, Fig. 4a). DDTC showed the greatest effect. Of the WR-compounds, WR1065 had the greatest effect and was even more effective than TS. As described above for the prevention of adduct formation, the reversal of DNA platination also increased non-linearly with increasing concentrations of WR1065 (Fig. 4b). In contrast to the co-incubations, shifts in the relative abundance of the various CDDP–DNA adducts were hardly observed as compared to the control (compare Fig.

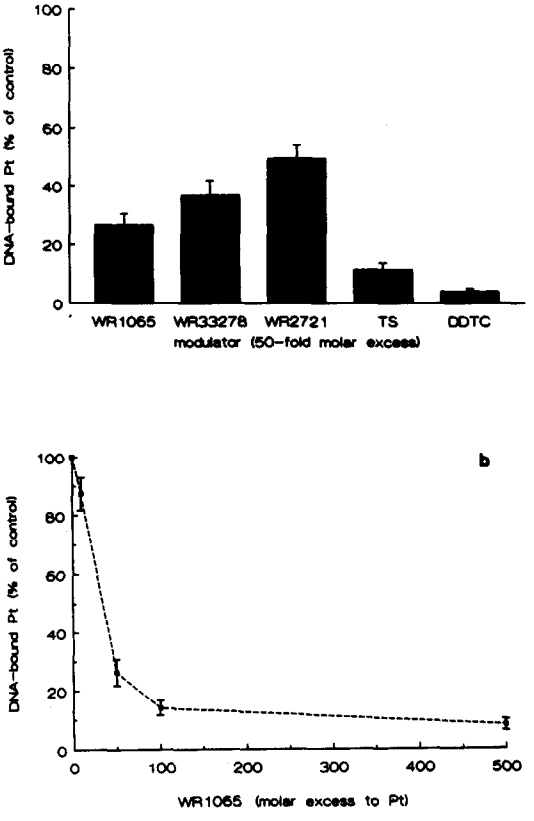

Fig. 2. Effects of co-incubation of several modulating agents on the platination of salmon sperm DNA. Salmon sperm DNA and CDDP (83 μM) were incubated for 1 hr at 37° with the modulating agents at a concentration of 4.2 mM (a) and with WR1065 at several molar ratios to CDDP (b). The data are means (± SD) of three separate experiments. For DDTC the sum of the four CDDP–DNA adducts is given (see Table 1).

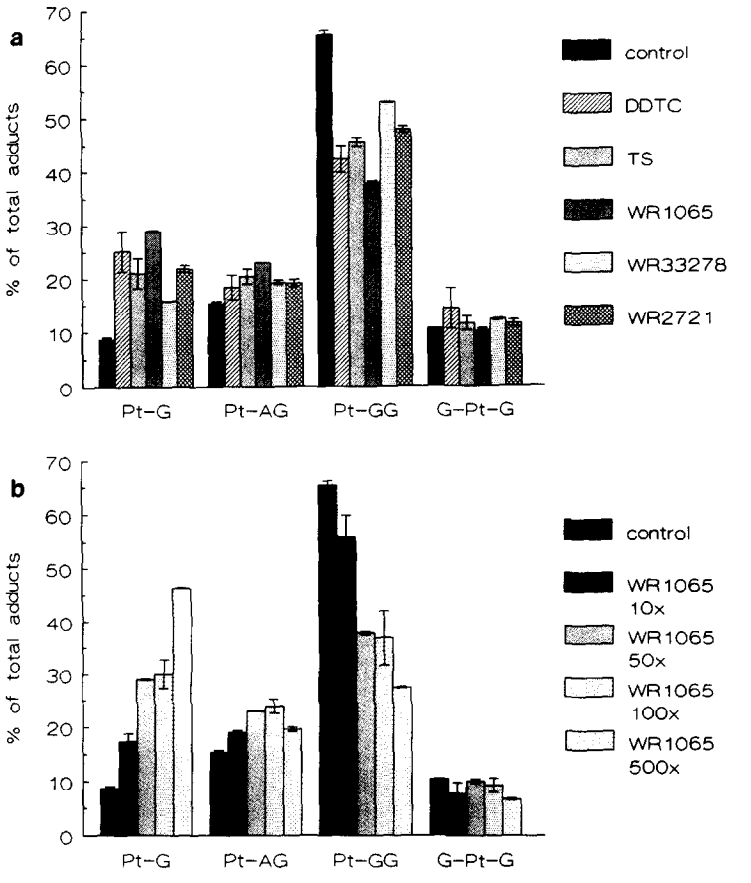

Fig. 3. The relative occurrences of the various CDDP–DNA adducts upon co-incubation (1 hr at 37°) of salmon sperm DNA and CDDP (83 μM) with the modulating agents at a concentration of 4.2 mM (a) and with WR1065 at several molar ratios to the initial CDDP concentration (b).

5a with Fig. 3a). However, with increasing concentrations of WR1065 the shifts in the relative occurrences of the various adducts are, although less pronounced, qualitatively similar to those found for the co-incubations (compare Fig. 5b with Fig. 3b).

DISCUSSION

CDDP most probably exerts its antitumour effect through binding to DNA [1] and also its toxic side effects may be (partly) the result of DNA platination [3, 4]. Modulating agents are intended to selectively protect non-tumour tissues against drug-induced toxicity [5]. Therefore, studies on the effects of these agents on the formation and stability of CDDP–DNA interactions are crucial for a proper understanding and design of clinical treatment regimens.

All the modulating agents tested in this study interfere strongly with the formation of CDDP–DNA adducts (Fig. 2). This will be the result of a direct inactivation of CDDP (before hydrolysis can occur) and inactivation of the reactive aqua species (before it can bind to DNA). Using the second-order reaction rate constants for the direct inactivation of CDDP by 4.2 mM of modulating agent [20, 21], it can be calculated that in our *in vitro* model system this reaction will account for a decrease in DNA platination of 32, 31, 3, 2 and 0.4% for DDTC, TS, WR1065, WR2721 and WR33278, respectively. Thus, the remaining decrease in DNA platination is suggested to be due to the inactivation of the aqua species of CDDP by 4.2 mM of modulating agent [65, 58, 71, 49 and 63% for DDTC, TS, WR1065, WR2721 and WR33278, respectively (Table 1)]. This implies a very efficient inactivation of the aqua products by the WR-compounds, comparable to that induced by the strong nucleophiles DDTC and TS. WR1065 and WR33278 are protonated at physiological pH, they were shown to concentrate around DNA due to ionic interactions with the negatively charged phosphate "backbone" of the DNA [22]. Indeed, a large effect on DNA structure (a decrease in supercoiling) was observed for WR1065 [23]. Thus, the so-called counterion condensation of WR1065 and WR33278 in the vicinity of the DNA could account for an increased inactivation of intact and/or hydrolysed CDDP near the DNA. In particular, the "*in-situ* quenching" of the reactive hydrolysed CDDP species is a plausible explanation for the large decrease observed in the DNA platination which is believed to proceed through primary

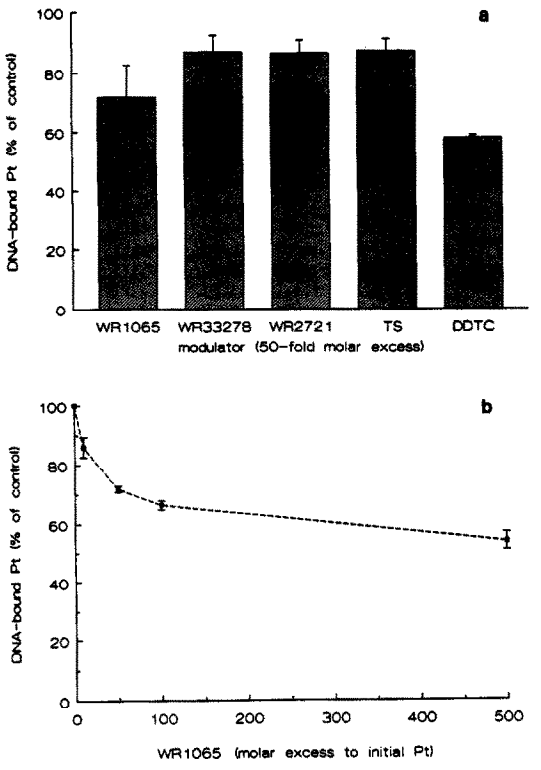

Fig. 4. Effects of post-incubations of the modulating agents on the platination of salmon sperm DNA. The DNA was isolated after a 1-hr incubation with 83 μM CDDP at 37°, redissolved and subsequently incubated for 2 hr at 37° with the modulating agents at a concentration of 4.2 mM (a) and with WR1065 at several molar ratios to CDDP (b). The data are means (± SD) of three separate experiments. For DDTC the sum of the four CDDP–DNA adducts is given.

hydrolysis of CDDP [1]. Also, WR2721 had a large effect on DNA platination. Because of its negatively charged phosphate group, WR2721 is not expected to concentrate around the DNA [23]. However, after protonation, the amino group of the molecule may be directed towards the DNA thus allowing counterion condensation to occur. This explanation is highly speculative and, considering the fact that WR2721 itself does not enter the cell [15–17], of minor importance for the effectiveness of WR2721 as a modulating agent in platinum-based chemotherapy.

Only a minor part of the decrease in DNA platination observed in the co-incubations will be due to reversal of CDDP–DNA adducts because the reversal is much less effective than the prevention of platination. The decreases in DNA-bound Pt after post-incubations with the modulating agents demonstrate the actual release of Pt from the DNA (Fig. 4 and Table 1). This finding is in contrast to previous observations with DDTC by Bodenner *et al.* [10] who did not observe any release of Pt from CDDP-treated DNA during post-incubation with DDTC. The reason for this discrepancy may be that in their study the reaction of DNA with CDDP went to completion, whereas we incubated the DNA with CDDP for only 1 hr.

The increase in the relative amounts of Pt-G during co-incubations with the modulating agents is probably due to trapping (quenching) of newly formed monoadducts by the modulating agent before they can rearrange to form bifunctional adducts. It is also possible that CDDP or its hydrolysed species react mono-functionally with the modulating agent followed by a reaction of this product with a guanine in the DNA after hydrolysis of the second chlorine ligand. An increase in the relative occurrence of the mono-functionally bound Pt-G must lead to decreases in the percentages of other bifunctionally bound adducts. Such a decrease is seen for the Pt-GG adduct (see Fig. 3). Since bifunctional adducts are much more cytotoxic than monofunctional adducts [1], the shift towards greater amounts of mono-functional adducts will result in less cytotoxicity. Increases in the relative abundance of the Pt-G adduct after post-incubations with the various modulating agents are barely seen (Fig. 5a). However, the increase in the relative abundance of the Pt-G adduct with higher concentrations of WR1065 (Fig. 5b) proves that part of the Pt-G present at the start of the post-incubation will be quenched by the modulating agent. Also, bifunctional CDDP–DNA adducts may have reacted with WR1065 to form a monofunctional Pt-G adduct. The next step will be that another molecule of modulating agent binds to the Pt-G adduct, resulting in the release of Pt from the DNA. This is supported by the fact that the decrease in bifunctional Pt-DNA adducts is larger than the increase in Pt-G adducts. The decrease in the relative amounts of the bifunctional adducts was small or absent for the Pt-AG adduct compared to the Pt-GG adduct. This may suggest that the reversal of the Pt-GG adduct is somewhat favoured over the reversal of the Pt-AG and G-Pt-G adducts.

It can be concluded that WR2721 and its main metabolites WR1065 and WR33278, as well as TS and DDTC, cause a decrease in the platination of salmon sperm DNA *in vitro* when present concomitantly with CDDP. Part of the already formed CDDP–DNA adducts is reversed during post-incubation with these modulating agents but this decrease in adduct levels is small compared to those obtained during co-incubations.

Considering the WR-compounds, the observed effects are the largest for WR1065 which is most probably the active metabolite. In the clinic, where WR2721 is administered prior to CDDP, steady-state levels of 0.1 mM WR2721 were observed in the plasma [15]. Tissue kinetics have not been performed in humans. However, in WR2721-treated mice, WR1065 rapidly entered the tissues. WR1065 levels of several millimolar were observed in various non-tumour tissues, exceeding the concentration of WR2721 in the plasma by an order of magnitude [15, 17]. Therefore, a significant prevention of CDDP-induced damage to the DNA may be expected in tissues that are able to accumulate WR1065 efficiently. TS is administered concomitantly with CDDP. Protective plasma levels of 1 mM were achieved [6]. Tissue levels of TS have not been

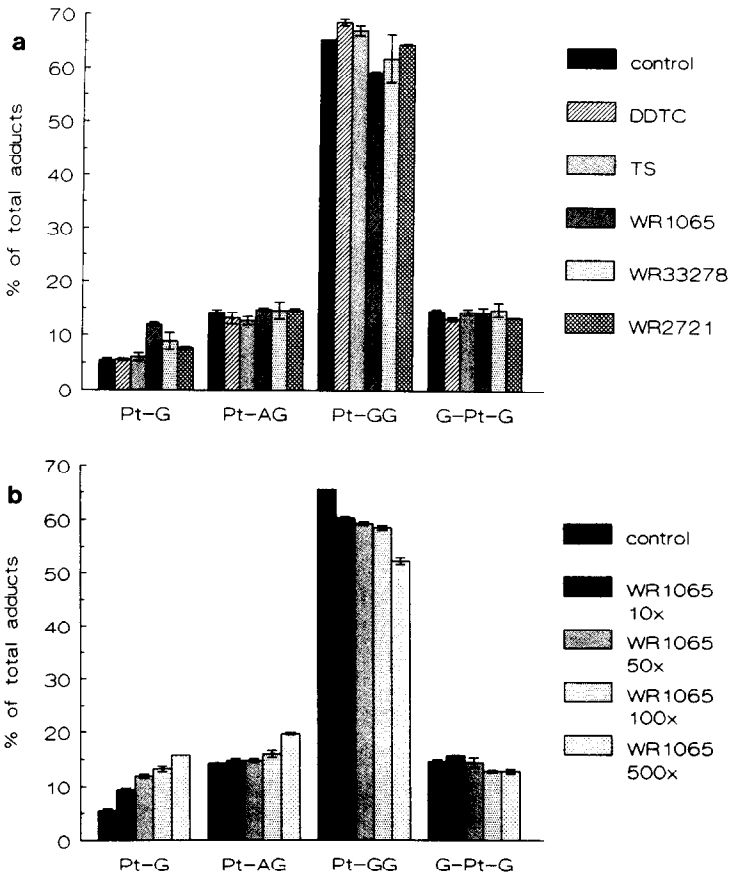

Fig. 5. The relative occurrences of the various CDDP–DNA adducts after post-incubation (2 hr at 37°) of CDDP-treated salmon sperm DNA with the modulating agents at a concentration of 4.2 mM (a) and with WR1065 at several molar ratios to the initial CDDP concentration (b).

measured. Protection against DNA damage in normal and tumour tissues will depend on the ability of these tissues to accumulate TS. DDTC is administered after CDDP. Protective plasma levels of 0.4 mM were achieved [11]. Tissue levels were not determined. In mice, a preferential uptake of radioactivity after the administration of [^{35}S]DDTC was observed for kidney > bone marrow, lung > tumour [23]. The delayed administration of DDTC implies that part of the damage by CDDP will already have occurred and will only be slightly reversed. Damage by CDDP still present at the time of DDTC administration will be partly prevented. Of course, this will be highly dependent upon the ability of the tissue to accumulate DDTC. Although care must be taken when extrapolating the results from our *in vitro* model system to the *in vivo* situation, it is expected that a strong prevention of damage to DNA caused by cisplatin may be expected when tissues have efficiently accumulated WR1065, TS or DDTC. Reversal of CDDP–DNA adducts by these modulating agents is expected to offer less protection. However, the finding that conformational changes in the DNA induced by WR1065 might increase enzymatic DNA repair [24] might provide a rationale for applying WR2721 after CDDP administration also.

In conclusion, WR2721 administered prior to platinum cytostatic agents is expected to offer protection to tissues which are able to accumulate WR1065 efficiently. Therefore, the selective accumulation of WR1065 by non-tumour tissues is crucial for the successful use of WR2721 in platinum chemotherapy.

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REFERENCES

1. Reedijk J, The mechanism of action of platinum antitumour drugs. *Pure Appl Chem* **59**: 181–192, 1987.
2. Roberts JJ, van der Vijgh WJF, Vermorken JB and Douple EB, Cisplatin. In: *Cancer Chemotherapy Annual* (Eds. Pinedo HM and Chabner BA), pp. 118–132. Elsevier, Amsterdam, 1984.
3. Tay LK, Bregman CL, Masters BA and Williams PD, Effects of *cis*-diamminedichloroplatinum(II) on rabbit

kidney *in vivo* and on rabbit renal proximal tubule cells in culture. *Cancer Res* **48**: 2538–2543, 1988.
4. Prajda N, Kralovanszky J, Gal F, Kiss F and Kerpel-Fronius S, Evaluation of side effects of platinum complexes (CDDP, CBDCA, CHIP) on rat bone marrow. *In Vivo* **3**: 267–270, 1989.
5. Borch RF and Markman M, Biochemical modulation of cisplatin toxicity. *Pharmac Ther* **41**: 371–380, 1989.
6. Goel R, Cleary SM, Horton C, Kirmani S, Abramson IS, Kelly C and Howell SB, Effect of sodium thiosulphate on the pharmacokinetics and toxicity of cisplatin. *J Natl Cancer Inst* **81**: 1552–1560, 1989.
7. Borch RF and Pleasants ME, Inhibition of *cis*-platinum nephrotoxicity by diethyldithiocarbamate in a rat model. *Proc Natl Acad Sci USA* **76**: 6611–6614, 1979.
8. Borch RF, Katz JC, Lieder PH and Pleasants ME, Effect of diethyldithiocarbamate on tumour response to *cis*-platinum in a rat model. *Proc Natl Acad Sci USA* **77**: 5441–5444, 1980.
9. Jones MM, Basinger MA, Mitchell WM and Bradley CA, Inhibition of *cis*-diamminedichloroplatinum(II)-induced renal toxicity in the rat. *Cancer Chemother Pharmacol* **17**: 38–42, 1986.
10. Bodenner DL, Dedon PC, Keng PC and Borch RF, Effect of dithiocarbamate on *cis*-diamminedichloroplatinum(II)-induced cytotoxicity, DNA cross-linking, and γ-glutamyl transpeptidase inhibition. *Cancer Res* **46**: 2745–2750, 1986.
11. Quazi R, Chang AYC, Borch RF, Montine T, Dedon PC, Loughner J and Bennett JM, Phase I clinical and pharmacokinetic study of diethyldithiocarbamate as a chemoprotector from toxic effects of cisplatin. *J Natl Cancer Inst* **80**: 1486–1488, 1988.
12. Yuhas JM, Spellman JM, Jordan SW, Pardini MC, Afzal SMJ and Culo F, Treatment of tumours with the combination of WR-2721 and *cis*-diamminedichloroplatinum(II) or cyclophosphamide. *Br J Cancer* **42**: 574–585, 1980.
13. Wasserman TH, Phillips TL, Ross G and Kane LJ, Differential protection against cytotoxic chemotherapeutic effects on bone marrow CFUs by WR-2721. *Cancer Clin Trials* **4**: 3–6, 1981.
14. Glover D, Fox KR, Weiler C, Kligerman MM, Turrisi A and Glick JH, Clinical trials of WR-2721 prior to alkylating agent chemotherapy and radiotherapy. *Pharmac Ther* **39**: 3–7, 1988.
15. Shaw LM, Glover D, Turrisi A, Brown DQ, Bonner HS, Norfleet AL, Weiler C, Glick JH and Kligerman MM, WR2721 pharmacokinetics. *Pharmac Ther* **39**: 195–201, 1988.
16. Calabro-Jones PM, Aguilera JA, Ward JF, Smoluk GD and Faheyr RC, Uptake of WR 2721 derivatives by cells in culture: identification of the transported form of the drug. *Cancer Res* **48**: 3634–3640, 1988.
17. Utley JF, Seaver N, Newton GL and Fahey RC, Pharmacokinetics of WR-1065 in mouse tissue following treatment with WR-2721. *Int J Radiat Oncol Biol Phys* **10**: 1525–1528, 1985.
18. Fichtinger-Schepman AMJ, van der Veer JL, Lohman PHM and Reedijk J, A simple method for the inactivation of monofunctionally DNA-bound *cis*-diamminedichloroplatinum(II). *J Inorg Biochem* **21**: 103–112, 1984.
19. Fichtinger-Schepman AMJ, van der Veer JL, den Hartog JHJ, Lohman PHM and Reedijk J, Adducts of the antitumour drug *cis*-diamminedichloroplatinum(II) with DNA: formation, identification and quantitation. *Biochemistry* **24**: 707–713, 1985.
20. Dedon PC and Borch RF, Characterization of the reactions of platinum antitumour agents with biologic and nonbiologic sulfur-containing nucleophiles. *Biochem Pharmacol* **36**: 1955–1964, 1987.
21. Treskes M, Howerda U, Klein I, Pinedo HM and van der Vijgh WJF, The chemical reactivity of the modulating agent WR2721 (ethiofos) and its main metabolites with the antitumour agents cisplatin and carboplatin. *Biochem Pharmacol* **42**: 2125–2130, 1991.
22. Smoluk GD, Fahey RC and Ward JF, Equilibrium dialysis studies of the binding of radioprotector compounds to DNA. *Radiat Res* **107**: 194–204, 1986.
23. Evans RG, Engel CR, Wheatley CA, Nielsen JR and Ciborowski LJ, An *in vivo* study of the radioprotective effect of diethyldithiocarbamate (DDC). *Int J Radiat Oncol Biol Phys* **9**: 1635–1640, 1983.
24. Vaughan ATM, Grdina DJ, Meechan PJ, Milner AE and Gordon DJ, Conformational changes in chromatin structure induced by the radioprotective aminothiol WR 1065. *Br J Cancer* **60**: 893–896, 1989.