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# Glutathione-mediated activation of anticancer platinum(IV) complexes

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The cancer chemotherapeutic activity of *cis*-diamminedichloroplatinum(II) (*cis*-DDP*) is now well recognized, with the drug being used against a broad spectrum of tumors including those of the testicles, ovaries, bladder, head and neck. The clinical application is limited by a variety of toxicities, of which nephrotoxicity is the most serious, but ototoxicity, myelotoxicity, peripheral neuropathy and nausea and vomiting also occur. A search for better drugs has led to the development of several promising new platinum complexes. Carboplatin, for example, is not significantly nephrotoxic [1] but is cross-resistant in all cells with acquired resistance to *cis*-DDP [2, 3]. On the other hand, 1,2-diaminocyclohexaneplatinum (DACH-Pt) complexes are both non-nephrotoxic [4, 5] and demonstrate activity against a number of resistant cell lines [2, 3, 6, 7]. DACH-Pt-$Cl_2$ is practically insoluble and more soluble analogues have been synthesized recently. One such compound is tetraplatin (DACH-Pt-$Cl_4$) which differs from previous drugs in being a Pt(IV) complex. The activity of this complex is presently being studied intensively [8–10]. Several Pt(IV) complexes have been studied previously for their mechanism of action [11–14]. These complexes are characterized by much slower ligand exchange than Pt(II) complexes which has led to the hypothesis that Pt(IV) drugs may need activation by reduction before eliciting their antitumor activity. Ascorbic acid can cause this reduction *in vitro* [11]. Ascorbic acid also enhances the reaction of Pt(IV) complexes with GMP [15]. The role of reduction in the reaction of Pt(IV) complexes with DNA is the subject of this communication.

## *Materials and methods*

The drugs were obtained from the following sources: *cis*-DDP (NSC 119875), Bristol Laboratories, Syracuse, NY; DACH-Pt-$SO_4$, Dr. Khokhar, M.D. Anderson Hospital and Tumor Institute, Houston, TX; and tetraplatin (NSC 363812), Developmental Therapeutics Program, Division of Cancer Treatment, NCI. Salmon testes DNA and glutathione were purchased from the Sigma Chemical Co., St. Louis, MO.

The murine leukemia cell lines studied were L1210/0 (sensitive), L1210/DDP (resistant to *cis*-DDP) and L1210/DACH (resistant to DACH-Pt-$SO_4$). The development of these resistant cell lines and the procedures for maintenance and growth inhibition studies have been reported previously [16, 17]. The results are expressed as $IC_{50}$, defined as the drug concentration that caused 50% inhibition of cell growth relative to untreated control cells as measured after a 3-day growth period in the presence or absence of drug.

Reactions with DNA were performed by incubating 100 $\mu$g of DNA with 1.5 $\mu$g tetraplatin (approximately 1 Pt : 100 nucleotides) in 20 mM $NaClO_4$, pH 5.5, for 0–24 hr at 37°. These are standard conditions used in previous studies [18, 19] and permit maximum sensitivity while retaining a low drug input that minimizes effects from neighboring adducts. Glutathione was added as required. The DNA was precipitated with ethanol and dissolved in 0.1 N HCl at 100° for 15 min. The platinum content was assayed by atomic absorption on a Perkin Elmer model 2380 spectrophotometer equipped with a graphite furnace and model HGA 400 programmer.

* Abbreviations: *cis*-DDP, *cis*-diamminedichloroplatinum(II); and DACH-Pt, 1,2-diaminocyclohexaneplatinum.

## *Results and discussion*

These studies were initiated by the question of whether murine leukemia L1210 cells that had been made resistant to DACH-Pt-$SO_4$ were also resistant to tetraplatin. The results presented in Table 1 clearly demonstrate that L1210/DACH cells were resistant but L1210/DDP cells were sensitive to tetraplatin. Of interest is the observation that, in the sensitive L1210/0 cell line, the concentration of drug required was very similar for each drug tested. However, when incubated with DNA for 24 hr, tetraplatin showed minimal reaction (Fig. 1), whereas *cis*-DDP reacts completely in 4 nr [18] and DACH-Pt-$SO_4$ reacted completely in less than 1 hr (data not shown). Therefore, for tetraplatin to be as toxic as DACH-Pt-$SO_4$ would require a facile, non-rate-limiting reduction either in the culture medium or within the cell. That this reduction can occur within the cell was demonstrated by incubating L1210/0 cells with tetraplatin for 2 hr in Hanks' balanced salt solution (HBSS) without serum, McCoy's medium without serum and McCoy's medium with serum. The cells were rinsed and growth inhibition was assessed over 3 days. The $IC_{50}$ values under these conditions were 0.45, 0.9 and 1.8 $\mu$M respectively. It is evident that the complete media are more likely to inactivate, rather than activate, tetraplatin. No reduction of tetraplatin will occur in HBSS.

The most abundant reducing agent in a cell is glutathione which usually exists in millimolar concentrations [19]. DNA, tetraplatin and various concentrations of glutathione, were therefore, incubated together for up to 24 hr. At an approximately equimolar concentration of glutathione and tetraplatin (the reaction contained about 17 $\mu$M tetraplatin), almost total reaction with DNA was observed. At high concentrations of glutathione (1 and 10 mM), inhibition of reaction was observed because of complexing of glutathione with the reduced drug. These concentrations of glutathione cause the same degree of inhibition of reaction between DNA and *cis*-DDP [20]. To determine whether the gluthathione-mediated reduction was fast compared to the reaction with DNA, tetraplatin was incubated with equimolar glutathione for 4 hr before DNA was added. This did not change the rate of reaction of drug with DNA (data not shown), demonstrating that reduction was not rate-limiting; rather, the rate of reaction with DNA was controlled by the dissociation of the two remaining chloride ligands as observed with *cis*-DDP [18].

These results suggest that, once a Pt(IV) complex enters a cell, it will be immediately reduced to a Pt(II) complex simply by reaction with glutathione or other intracellular reducing agents. In the case of tetraplatin, it would then react in exactly the same manner as DACH-Pt(II) complexes. We have demonstrated previously that DACH-Pt-$SO_4$ and *cis*-DDP produce adducts at similar sites in DNA as assayed by inhibition of DNA polymerase activity on a platinated template [2]. These adducts are predominantly intrastrand cross-links between neighboring bases in DNA, specifically GG and AG sequences (reviewed in Ref. 21). In preliminary studies, we have shown that DACH-Pt-$SO_4$ and tetraplatin produced the same adducts in DNA. Therefore, the remaining question relates to why L1210 cell lines can be developed that exhibit different patterns of resistance to these various platinum complexes. In part, this is due to differences in drug accumulation [22]. In addition, L1210/DDP but not L1210/DACH cells have slightly elevated glutathione levels [22]. However, the intracellular glutathione concentrations are in a vast excess over the intracellular drug concentration,

Table 1. Inhibition of growth of L1210 cell lines by various platinum complexes

| Drug treatment | $IC_{50}$ (μM) | | |
|---|---|---|---|
| | L1210/0 | L1210/DDP | L1210/DACH |
| *cis*-DDP | 0.33 | 25.4 (77) | 0.96 (2.9) |
| DACH-Pt-$SO_4$ | 0.44 | 2.7 (6.2) | 14.0 (32) |
| Tetraplatin | 0.33 | 1.3 (4) | 8.3 (25) |

Each value is the mean of at least two experiments with three determinations made at each concentration in each experiment. The values in parentheses represent fold resistance compared to L1210/0 cells.

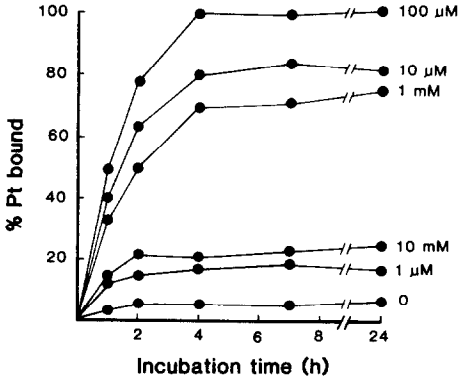


Fig. 1. Effect of glutathione on the reaction of tetraplatin with DNA. DNA (100 μg) and tetraplatin (1.5 μg) (final concentration 17 μM) were incubated in the presence of the indicated concentrations of glutathione for 0–24 hr. Bound Pt was then analyzed by atomic absorption spectrometry.

and slight changes would not be expected to have a major effect on toxicity. A significant part of the resistance in L1210/DDP cells is also mediated by differences in DNA repair proceses [23].

In summary, Pt(IV) complexes have been shown to be activated by glutathione in a manner that leads to their reaction with DNA. It would appear that Pt(IV) complexes cannot survive at this oxidation state in a cell and would immediately undergo reduction to the active form.

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