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Short communication

DNA binding of iproplatin and its divalent metabolite *cis*-dichloro-*bis*-isopropylamine platinum(II)

L. Pendyala[1], A. V. Arakali[1], P. Sansone[1], J. W. Cowens[1,2], and P. J. Creaven[1]

[1]Division of Clinical Pharmacology and Therapeutics, Department of Medicine and [2] Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, NY 14263, USA

Summary. The quadrivalent second-generation platinum complex iproplatin and an in vivo divalent metabolite of iproplatin, *cis*-dichloro-*bis*-isopropylamine platinum (CIP) were tested for binding to DNA in vitro. DNA binding was determined according to radioactivity measured using [^{14}C]-iproplatin and [^{14}C]-CIP and also by platinum content. Results indicate that (a) iproplatin shows negligible binding to DNA, (b) CIP binds to DNA in a time-dependent fashion, and (c) the isopropylamine ligand is intact when CIP is bound to DNA. Glutathione (GSH) inhibits the binding of CIP to DNA, possibly by inhibiting binding to DNA of the aquated form of CIP.

Introduction

Iproplatin is a second-generation platinum complex that shows clinical activity against small-cell carcinoma of the lung [14], carcinoma of the ovary [5], breast carcinoma [13], and squamous-cell carcinoma of the head and neck [1].

Among the platinum complexes that have been tested clinically, iproplatin is structurally unique in that it is a quadrivalent complex. Unlike the divalent platinum anticancer agents that are square planar complexes, iproplatin has an octahedral configuration, with two hydroxy ligands projecting at 90° above and below the square plane.

In previous studies using electron impact mass spectrometry and [^{195}Pt]-NMR (nuclear magnetic resonance) spectroscopy, we have identified *cis*-dichloro-*bis*-isopropylamine platinum(II) (CIP), the corresponding divalent species, as a major human metabolite of iproplatin [16, 17]. HPLC studies of cell extracts and plasma have also indicated the presence of CIP as a metabolite [16, 18].

Offprint requests to: L. Pendyala

The fact that CIP itself is cytotoxic in experimental systems [4, 6] and that the hydroxy groups in iproplatin could well impair DNA binding of this complex by steric hindrance [19] raised the possibility that the DNA-binding species is the reduced metabolite CIP rather than the parent complex iproplatin. To explore this possibility, we measured the relative DNA-binding abilities of iproplatin and CIP. We report the results of these studies, which have been presented elsewhere in preliminary form [15].

Materials and methods

Calf-thymus DNA and glutathione (GSH) were purchased from Sigma Chemical Company. Analytical grade iproplatin and CIP were a gift from Bristol-Myers Co. Iproplatin labelled with carbon 14 (sp. act., 24.6 mCi/mmol) was synthesized at Roswell Park Memorial Institute according to a proprietary procedure supplied by Johnson Matthey Inc. (Specialties Division, Wayne, Pa.) from 2-[^{14}C]-aminopropane (sp. act. 12.3 mCi/mmol; New England Nuclear, Boston, Mass.) and K_2PtCl_4 [17]. The synthesized [^{14}C]-iproplatin was 99% pure as determined by HPLC [17]. With continued storage at –20° C, a small amount of [^{14}C]-CIP (which is a precursor in the synthesis of labelled iproplatin) was found to accumulate in the preparation, presumably a product of radiolysis. Immediately, before their use, the labelled iproplatin and CIP were purified by HPLC on a Vydac C18 semi-preparative column (15–20 μm; Waters Associates, Milford, Mass.) with water to acetonitrile gradients. The purity of each was checked by HPLC [18] using analytical standards as markers.

The DNA-binding studies were typically carried out by the incubation of 10 μg/ml platinum complexes and 1 mg/ml DNA in 5 mM phosphate buffer (pH 7.5) at 37° C for up to 28 h [2]. Duplicate samples were periodically removed and DNA was precipitated by the addition of ice-could 10% trichloroacetic acid (TCA) to the samples, which were allowed to stand on ice for 30 min. The precipitated DNA was washed four times with ice-cold 10% TCA and twice with 100% ethanol [12]. The washed DNA precipitate was then hydrolyzed in 6% perchloric acid at 90° C for 1 h; one aliquot was used for radioactivity counting (Searle Mark III scintillation counter; Des Plaines, Ill.); one, for platinum measurement by flameless atomic absorption spectrophotometry (FAAS; Instrumentation Laboratories, Wilmington, Mass); and one, for determining the amount of DNA by the measurement of UV absorbance at 265 nm (Cary 219 UV spectrophotometer; Varian Instruments, Palo Alto, Calif.).

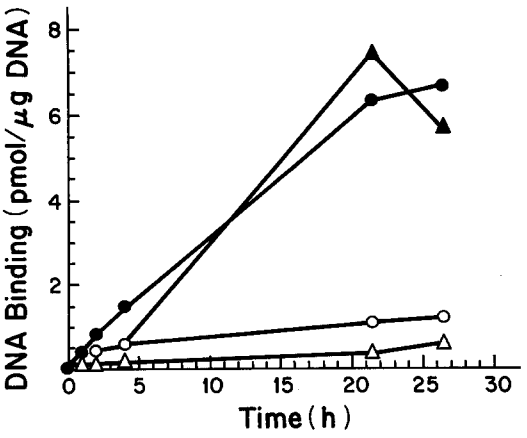

Fig. 1. Binding of iproplatin and CIP to DNA in an incubation comprising 10 μg/ml platinum complexes tagged with carbon 14 and 1 mg/ml DNA in 5 mM phosphate buffer (pH 7.5) at 37° C. Periodically samples were removed, DNA was precipitated, and determinations of elemental Pt bound to DNA as measured by FAAS and of radioactivity as measured by scintillation counting were carried out. *Open circles and open triangles*, iproplatin; *closed circles and closed triangles*, CIP. *Circles* show drug binding according to radioactivity and *triangles* indicate that according to Pt measurement

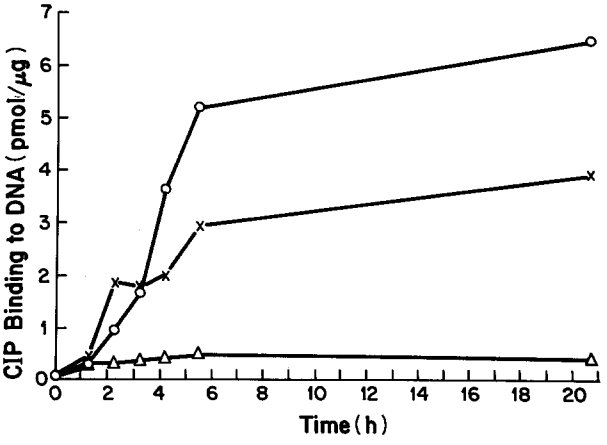

Fig. 2. Effect of GSH (2 and 5 mM) on the binding of 10 μg/ml CIP and 1 mg/ml DNA. The experimental conditions are the same as for Fig. 1. Measurements are based on FAAS. *Open circles*, control with no GSH; *crosses*, 2 mM GSH in the CIP/DNA reaction mixture; *triangles*, 5 mM GSH

Results

Binding of iproplatin and CIP to calf-thymus DNA

Incubation of DNA with iproplatin for up to 30 h led to negligible binding; incubation with CIP led to a time-dependent increase in binding for up to 20 h (Fig. 1). Based on the amount of drug bound to DNA, we estimate that approximately 30% of the CIP had reacted with DNA within this period. The results of platinum measurement by FAAS and of the radioactivity measurement by scintillation counting were essentially identical.

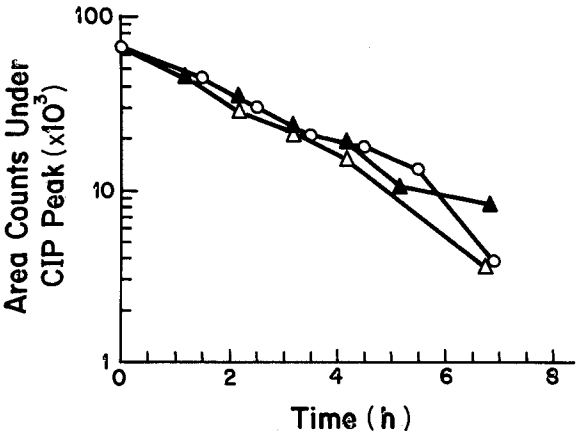

Fig. 3. Reactivity of 10 μg/ml CIP with 2 and 5 mM GSH when incubated in 5 mM phosphate buffer (pH 7.5) at 37° C. Unreacted CIP was measured by HPLC. *Open circles*, controls with no GSH; *open and closed triangles*, 2 and 5 mM GSH along with CIP, respectively

Effect of GSH on binding of CIP to calf-thymus DNA

The addition of GSH to the CIP/DNA incubation mixture inhibited the binding of CIP to DNA (Fig. 2). A concentration of 2 mM produced partial inhibition, and 5 mM achieved nearly total inhibition of the binding. When the reduction of iproplatin by GSH was tested in a 6 h incubation, 2 mM GSH had no effect, whereas 5 mM GSH reduced about 80% of the drug (data not shown). These results suggest that GSH may mediate the reaction of iproplatin with DNA at GSH concentrations of >2 mM.

Reactivity of CIP with GSH

To determine whether the observed inhibition of CIP binding to DNA by GSH resulted from a direct reaction of GSH and CIP, the reactivity of CIP with GSH was tested. The experiments were carried out under conditions identical to those used in the DNA-binding experiments, but without DNA in the incubation mixture. The progress of the reaction was followed by HPLC determination of the area under the unchanged CIP peak. The results of these experiments (Fig. 3) indicate that (a) the concentration of CIP declines with time and (b) both 2 and 5 mM GSH have no effect on this process. The decline in the concentration of unchanged CIP in these experiments was identical to that observed when CIP is incubated directly in water (data not shown).

Discussion

In patients receiving iproplatin, CIP is detected in the plasma and urine, indicating that the quadrivalent complex is reduced to the divalent form in vivo [16, 17]. It has been

postulated that this reduction is a prerequisite for the reactivity of the quadrivalent Pt complexes [7–9]. This has been shown to be the case for tetraplatin [10]. Blatter et al. [3] showed that iproplatin and another Pt(IV) complex, *cis*-dichloro-*trans*-dihydroxy-*cis*-diammine platinum, did not alter the electrophoretic mobilities of PM2 DNA, whereas the corresponding divalent forms did. These investigators postulated that the reduced forms of the quadrivalent complexes actually react with DNA. The present studies confirm this hypothesis and also demonstrate that the isopropylamine ligand (which carried the radiolabel in this study) remains intact during this binding.

As expected from its reactivity with other Pt(II) complexes [2, 10, 11], GSH inhibits the binding of CIP to DNA, but not by complexing directly with the drug. The rate of decline of CIP in aqueous media is not affected by the addition of GSH; this decline in the concentration of CIP in aqueous media suggests that the drug undergoes aquation reaction(s). If the aquated species is the form that reacts with DNA, GSH could prevent DNA binding of CIP by reacting with this aquated species. It is interesting that the rate of decline in the concentration of CIP in aqueous media is very similar to its rate of binding to plasma proteins in vitro [17]. This suggests that the rate-limiting step in the reactivity of CIP may be the exchange of chloride ligands for water. These studies confirm the role of CIP as a cytotoxic metabolite of iproplatin.

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