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Effect of Mannitol and Plasma on the Cytotoxicity of Cisplatin*

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Abstract—*A soft agar cell culture for human granulocyte precursor cells (CFU-c) was used to assess the cytotoxic effect of cisplatin before and after preincubating the drug with either mannitol and/or fresh human plasma. Consistent growth inhibition of CFU-c was found with survival of 87, 64 and 34% at cisplatin concentrations of* 10^{-7} M, 10^{-6} M *and* 10^{-5} M *respectively. Dose-related survival was not changed by preincubating cisplatin with mannitol in molar ratios of* 1:100 *and* 1:1000 *for 4 and 24 hr. Preincubating* 10^{-5} M *cisplatin with human plasma increased CFU-c survival corresponding to a loss of activity of 92 and 98% after 4- and 24-hr preincubations respectively. No significant difference in survival of CFU-c was noted whether bone marrow cells were exposed to cisplatin in human plasma or in the ultrafiltrate of human plasma. Mannitol did not change the decrease in cisplatin activity due to plasma protein binding. These data indicate that the cytotoxic effect of cisplatin can be reduced by exposure to human plasma but not to mannitol.*

INTRODUCTION

CISPLATIN, an active, divalent, square planar coordination complex of platinum, has recently become one of the major new anticancer drugs. One of the most important dose-limiting side-effects in man is renal toxicity [1–5]. Myelosuppression has been of minor concern with conventional dose regimens; however, the WBC nadir was shown to reach 35% of pretreatment values when higher dose levels of cisplatin were used [5, 6]. In order to reduce renal toxicity the combination of forced diuresis by hydration, diuretic drugs and osmotic facilitated diuresis with mannitol have been applied [7–9]. According to Eshaque *et al.* [10], cisplatin forms stable 2:1 complexes with D-mannitol. Although this complex formation was identified only after 48–72 hr, the authors could not exclude the possibility that early complex formation did actually occur. In addition, Litterst *et al.* [11, 12] showed a progressive decrease in the recovery of platinum in the ultrafiltrate of dog plasma incubated for various times in the presence of cisplatin and then subjected to membrane ultrafiltration. These data suggest that the cytotoxic potential of cisplatin may be reduced if exposed to mannitol and/or human plasma. As *in vitro* studies showed a definite colony-suppressing effect of cisplatin on lymphoma cells [13] and granulocyte precursor cells [14] we used the semi-solid agar culture system to assess the potential reductive effects of mannitol and human plasma on cisplatin-induced cytotoxicity.

MATERIALS AND METHODS

Chemicals and media

Cisplatin was supplied by the National Cancer Institute (Bethesda, MD). Fresh drug solution (dissolved in 0.9% saline) was prepared for each experiment. Alpha medium was purchased from Flow laboratories, Rockville, MD. Fetal calf serum (FCS), dialyzed and non-dialyzed, was purchased from Grand Island Biological, NY, phytohemagglutinin was purchased from Wellcome Research Laboratories, Beckenham, U.K.,

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and preservative-free heparin was purchased from Connaught Medical Research Laboratories, Toronto, Ontario, Canada. For centrifugal ultrafiltration we used Centriflo CF-50A conical filters (Amicon Corp., Lexington, MA). Filters were stored in ethanol/water (10/90 by vol. solution before use). Human plasma was obtained from the Blood Center in Kansas City, KS. Plasma was placed in Centriflo filters and centrifuged at 1000 *g* (the maximum recommended acceleration) for 15 min. The ultrafiltrate was transparent and contained less than 2% of plasma protein as determined by the Biuret method [15].

Bone marrow

Bone marrow aspirates for this study were obtained from hematologically normal adults undergoing staging for untreated solid tumors (normal marrow). After informed consent was obtained, 2 ml of aspirate were taken from the posterior iliac crest. This was added to 0.2 ml of alpha medium containing 200 units of preservative-free heparin. The buffy coat was obtained by centrifugation (10 min at 1000 r.p.m.) and washed with alpha medium containing 10% FCS in the manner described previously [16].

Culture system

The agar culture technique [17] was used with minor modifications to assay human granulocyte precursor cells (CFU-c) after exposure to cisplatin. In brief, the culture system consisted of a single layer of 0.3% agar in a 35-mm plastic Petri dish containing 5×10^5 nucleated cells and alpha medium with 15% FCS and 15% LCM. The latter is prepared by incubation of normal human peripheral leukocytes with 1% phytohemagglutinin over a 7-day period. Cultures were incubated for 2 weeks at 37°C in an atmosphere of 7% CO_2/air. Colonies of 50 or more cells were counted using an inverted microscope. The median number of colonies in the control plates was 74 (42–186). Nucleated human bone marrow cells, 5×10^5, suspended in 0.5 ml alpha medium with 10% dialyzed FCS, were exposed to various concentrations of cisplatin (range 10^{-8}–10^{-4} M) for 1 hr at 37°C. The cells were washed with alpha medium containing 10% FCS by centrifugation at 4°C. The control cultures were treated in an identical fashion but without drug (cisplatin). In addition, cisplatin at concentrations of 10^{-5} M and 10^{-6} M was preincubated with human plasma and/or mannitol (10^{-3} M) for 1, 4 and 24 hr respectively at room temperature. Human bone marrow cells were then exposed for 1 hr to the ultrafiltrate and full plasma of these various cisplatin–plasma combinations. Quadruplicate dishes were set up for each drug concentration in each experiment. The mean number of colonies of the drug-exposed groups was expressed as a percentage of the control culture.

RESULTS

Human bone marrow cells were exposed to graded concentrations of cisplatin and assessed for surviving CFU-c. The results are expressed as the percentage of surviving CFU-c (Fig. 1). Growth inhibition of 50% was achieved with 2.8×10^{-6} M cisplatin (0.84 μg/ml). Mannitol up to 5×10^{-3} M alone did not inhibit the growth of CFU-c. Having cisplatin in the dark at room temperature up to 24 hr (alone or with mannitol) before exposing to the bone marrow cells did not change the cytotoxic effect of 10^{-5} M and 10^{-6} M cisplatin compared to the results shown by Fig. 1. However, preincubation of cisplatin with human plasma increased the surviving fraction of CFU-c with increasing preincubation time (Fig. 2). The surviving fraction was 100% of the control within 4 hr preincubation of plasma with 10^{-6} M cisplatin. The presence of mannitol (10^{-3} M) during the preincubation period of cisplatin and plasma did not change the surviving fraction of CFU-c (Fig. 2). The ultrafiltrate of the plasma (with cisplatin preincubated) showed the same surviving fraction of CFU-c as full plasma, namely 84 and 103% for 10^{-5} M cisplatin for 4 and

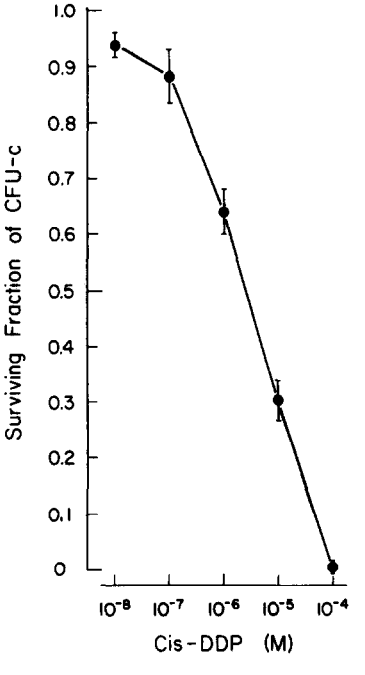

Fig. 1. Sensitivity of normal human bone marrow cells to cis-*diamminedichloroplatinum (II) in culture. Cells were exposed to graded concentrations of the drug for* 1 hr *at* 37°C, *washed twice and assayed for the surviving fraction of CFU-c. Each point represents 6 or more experiments consisting of quadruplicate plates. Bars, S.E.M.*

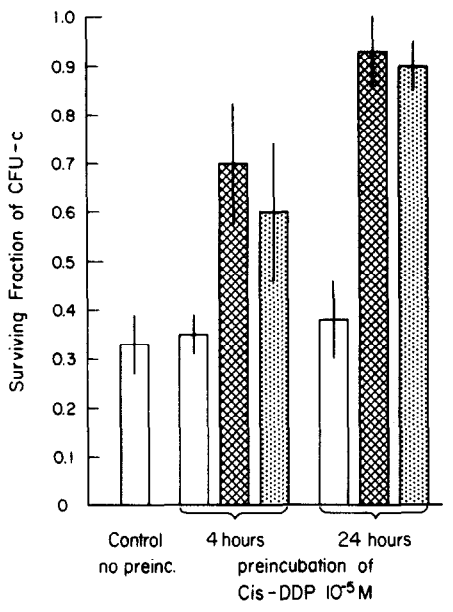

Fig. 2. Effect of human plasma and mannitol on the cytotoxicity of cis-*DDP* (10^{-5} M). cis-*DDP was preincubated in the presence of human plasma (cross-hatched bars) or human plasma and mannitol* (10^{-3} M) *(stippled bars). The reaction vessel (plastic tube) was left in the dark at room temperature for* 4 *and* 24 hr *respectively before testing the drug effect on CFU-c.* cis-*DDP was not preincubated with HP or HP + MA for the control (empty bar). Mean ± S.E.M. for quadruplicate dishes are shown.*

24 hr respectively. For 10^{-6} M cisplatin there was also no difference in surviving CFU-c whether full plasma or ultrafiltrate of preincubated plasma (with cisplatin preincubated) was used.

DISCUSSION

Cisplatin has shown significant antitumor activity in many animal tumors [18, 19] as well as in human tumors *in vitro* [13, 20] and *in vivo* [21]. Cisplatin shares non-cycle specificity of poly-functional alkylating agents [22]. On this basis a high-dose pulse of cisplatin may be more effective than low-dose continuous administration. Mannitol-induced diuresis was used in order to ameliorate the renal toxicity of high-dose cisplatin [9]. We used the soft agar cell culture to assess the cytotoxicity of cisplatin on human CFU-c. CFU-c could consistently be suppressed by 50% with 2.0×10^{-6} M cisplatin. Similar *in vitro* survival data of murine hemopoietic precursor cells after *in vitro* exposure to cisplatin were found by others [23]. Stable D-mannitol-cisplatin complexes unable to inhibit tumor cell growth have been reported [11]. However, we found that preincubation with mannitol did not afford any protection to the cytotoxic effect of cisplatin on human CFU-c *in vitro*. Litterst *et al.* [11] was able to recover 41 and 8.5% of platinum in the ultrafiltrate of dog plasma incubated at 37°C for 4 and 24 hr respectively. Using the same preincubation periods, we exposed human CFU-c for 1 hr to the recovered cisplatin in the ultrafiltrate. CFU-c survival increased to 70 and 94% of control values, corresponding to a 92 and 98% loss of activity of 10^{-5} M cisplatin after 4 and 24 hr of preincubation respectively. In line with our data Bannister *et al.* [15] found, using the X-ray fluorescence method, that the initial cisplatin concentration of 3×10^{-5} M was below the limits of detection (242 μg/l = 8×10^{-7} M) after 7 hr of incubation with fresh human plasma at 37°C *in vitro*. Exposing CFU-c to full plasma instead of ultrafiltrate did result in similar survival data of CFU-c. This suggests that the reduced cytotoxicity is related to reduced activity of cisplatin, resulting from a loss of drug, presumably through protein-binding mechanisms. Mannitol did not interfere with this process.

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