Cancer Chemother Pharmacol (1987) 20: 211–212

Cancer Chemotherapy and Pharmacology

Chemical reactivity of cisplatin bound to human plasma proteins

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Summary. Human plasma was incubated with cisplatin over 24 h. Ultrafilterable platinum and platinum reactive with DDTC were determined at regular time intervals during incubation. At each time point more platinum reacted with sodium N,N^1-diethyldithiocarbamate (DDTC) than was available as ultrafilterable platinum. At 24 h 70% of total platinum (10% ultrafiltrable platinum and 60% protein-bound platinum) reacted with DDTC. This means that cisplatin bound to plasma proteins can – at least in part – still react with strong nucleophiles.

Introduction

Binding of cisplatin to plasma proteins has been studied by measuring concentrations of ultrafilterable platinum [1, 5]. Only a few papers have been published about the biological activity [4, 6], and nothing is known about the chemical reactivity of protein-bound platinum. The present experiment has been carried out to study the chemical reactivity of protein-bound platinum with sodium diethyldithiocarbamate (DDTC). DDTC was chosen because of its strong nucleophilic properties and its possible clinical application for reduction of cisplatin-induced nephrotoxicity [3].

Materials and methods

Cisplatin was kindly supplied by Bristol Myers (Weesp, The Netherlands). Sodium DDTC (analytical grade) was purchased from Merck (Amsterdam, The Netherlands). The $PtDDTC_2$ complex was synthesized by the procedure of Bannister et al. [2]. Binding of cisplatin to human plasma proteins was studied in vitro by incubating 50 μM cisplatin in plasma at 37° C for 24 h. At 0, 0.5, 1, 2, 4, 6, 8 and 24 h after the start of incubation 1-ml aliquots were taken for ultrafiltration and for reaction with sodium DDTC. Ultrafiltration was performed in Amicon MPS-1 micropartion systems (Amicon, Oosterhout, The Netherlands) containing YMT membranes (cut-off 30000 daltons) at 2000 g in a bench-top centrifuge with a fixed angle rotor [7]. The first 8-min fraction was collected for the determination of ultrafilterable platinum (sol. A). DDTC-reactive platinum was determined by adding 100 µl $2.5.10^{-2}$ M DDTC in water (which means a 500-fold excess with respect to platinum present in plasma) to a 1-ml aliquot of plasma, followed by incubation at 37° C for 4 h. The mixture was fractionated by a Sep Pak C-18 cartridge (Waters, Etten-Leur, The Netherlands): the eluate was obtained immediately after introduction of the complete reaction mixture in plasma (Sol. B) and the eluate, after purging of the cartridge with 50 ml methanol containing the reaction product $PtDDTC_2$. The methanol solution was evaporated under vacuum and the residue redissolved in 2 ml ethanol (Sol. C). The platinum compounds retained on the Sep Pak column were removed by digesting the packing material with 3 ml 65% HNO_3 at 170° C in a polytetrafluoroethylene (PTFE) bomb for 2 h. The remaining liquid was evaporated at 120° C under a stream of air after addition of 5 mg NaCl. The residue was dissolved in 1 ml 0.2 N HCl-0.15 M NaCl (Sol. D). Platinum concentrations in solutions A–D were determined by atomic absorption spectrometry as described before [8]. Standard solutions of cisplatin were prepared in the same matrix as the samples.

Results and discussion

The amounts of platinum detected in the four above-mentioned solutions are summarized in Table 1. The amounts of platinum present in solution A represent ultrafilterable platinum. The observed biphasic decrease of the platinum concentration with time corresponded with the results of previous studies [1, 2, 4, 6]. Platinum concentrations in solution B represent platinum irreversibly bound to proteins. This amount increased with time. However, if all platinum bound to plasma proteins is actually irreversibly bound then the amounts present in the solutions A and B together should total 100%. The lower values observed indicate that some of the protein-bound platinum species can be removed by the strongly nucleophile DDTC. This is in agreement with the higher platinum concentrations in solution C, representing Pt-DDTC adducts, than in solution A. Nevertheless, two peculiarities are observed: (a) the amounts present in solutions B and C together do not add up to 100%; and (b) the amounts of platinum present in solution C were expected to decrease continuously with time, but the amounts at the two latest time points were higher than the preceding values. In an attempt to find an explanation for these observations the amount of platinum retained on the column was measured at various time points. The amounts present in solution D did indeed complete the 100% platinum balance when added to the amounts

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Table 1. Platinum, expressed as percentages of total platinum (50 μM) in plasma, present in plasma ultrafiltrate (Sol.A), plasma eluate from the Sep Pak cartridge (Sol.B), methanol eluate from the Sep Pak cartridge (Sol.C) and final solution after reaction of packing material with nitric acid (Sol.D)

Time (h)	Sol.A	Sol.B	Sol.C	Sol.D
0.5	70.2 ± 1.0	5.1 ± 0.1	90.5 ± 8.6	ND
1	61.3 ± 0.8	7.4 ± 0.2	81.4 ± 7.8	13.0 ± 0.2
2	46.6 ± 0.4	10.8 ± 0.2	59.0 ± 5.0	ND
4	30.4 ± 0.2	15.6 ± 0.2	49.2 ± 4.2	40.2 ± 1.0
6	21.5 ± 0.1	19.2 ± 0.2	43.7 ± 3.6	ND
8	19.3 ± 0.1	21.1 ± 0.2	56.5 ± 4.8	12.8 ± 0.2
24	11.3 ± 0.1	28.6 ± 0.2	68.4 ± 6.2	1.5 ± 0.1

ND, not determined

present in solutions B and C. Furthermore, the observed increase and subsequent decrease in the amounts with time are complementary to the values observed in solution C. Because of their retention behaviour it is thought that the compounds present in solution D originally represented platinum bound to protein as well as DDTC. If so, it can be understood that formation of such compounds will lead to an increase of platinum in solution D with time, but also that at a certain time, when only small amounts of ultrafilterable platinum are left, replacement of DDTC by a second protein leads to a shift of platinum from solution D to solution C, as observed at 8 and 24 h after the start of incubation. It may be concluded that although dialysis experiments have suggested that non-ultrafilterable platinum species are irreversibly bound to plasma proteins [5], our observations indicate that at least some protein-bound platinum can react with strongly nucleophile substances, for example, which has implications for the fate of cisplatin in vivo and the use of rescue therapies.

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Received August 12, 1987/Accepted May 13, 1987