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Protein binding of five platinum compounds

Comparison of two ultrafiltration systems

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Summary. Amicon Centriflo CF50A cones and Amicon MPS-1 micropartition systems with YMT filters were compared for the preparation of ultrafiltrates of plasma samples containing cisplatin, spiroplatin, JM-40, carboplatin or iproplatin. The MPS-1 system equipped with YMT membranes showed less adsorption of the platinum compounds than CF50A cones and allowed more rapid processing of smaller plasma volumes. In vitro binding to human plasma proteins measured with YMT filters after 24 h of incubation was 94%, 89%, 83%, 31% and 0 for cisplatin, spiroplatin, JM-40, carboplatin and iproplatin, respectively. These values corresponded with the initial half-lives in plasma and the decomposition half-lives of intact drug in plasma ultrafiltrate as measured by HPLC. It is suggested that the degree of protein binding is related to the stability of the leaving groups.

Introduction

Cisplatin [*cis*-diamminedichloroplatinum(II), CDDP] is a widely used anticancer drug for the treatment of solid tumors [2]. Its side effects [13] resulted in the synthesis of many analogues in attempts to obtain compounds with improved antitumor activity and reduced toxicity. Of these, spiroplatin (aqua(1,1-bis(aminomethyl)cyclohexane)sulfatoplatinum(II), TNO-6], JM-40 [ethylenediamminemalonatoplatinum(II)], carboplatin [diammine(1,1-cyclobutanedicarboxylato)platinum(II), CBDCA, JM-8], and iproplatin [*cis*-dichloro-*trans*-dihydroxy-*cis*-bis(iso-propylamine)platinum(IV), CHIP JM-9] have been investigated in phase I clinical studies [6]. The structural formulas of these compounds are shown in Fig. 1.

Platinum compounds are irreversibly bound to plasma proteins [10], which means that only unbound drug may be regarded as therapeutically active. Therefore, pharmacokinetics and drug monitoring should be performed in plasma ultrafiltrates. Up to now Amicon CF50A cones have been widely used for these studies. The newly developed Amicon MPS-1 micropartition system has the advantage that smaller blood volumes can be processed in a shorter time, while a selection of filter types is available. The purpose of this study was to compare the MPS-1 system equipped with YMT filters against CF50A cones for adsorption characteristics and to compare the in vitro protein binding of the five platinum compounds with the most appropriate system.

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Materials and methods

Materials

Platinum compounds were kindly supplied by various sources: CDDP and JM-40 by Bristol Myers, Weesp, The Netherlands, TNO-6 by Dr H. Meinema, TNO, Utrecht, The Netherlands, CBDCA by Dr K. J. Harrap, Institute of Cancer Research, Sutton UK and CHIP by Johnson Matthey, Sonning Common, Reading, UK. Amicon Centriflo ultrafiltration systems with CF50A membrane cones and MPS-1 micropartition systems with YMT filters were obtained from Amicon, Oosterhout, The Netherlands. A plasma pool was constituted from heparinized plasma of ten healthy adult volunteers. The total protein level of this pool was 58 g/l. Aqueous stock solutions of the platinum (Pt) compounds were freshly prepared for each experiment. CDDP and CHIP were dissolved in 0.15 *M* NaCl (400 μg Pt/ml), JM-40 and CBDCA in water (400 μg Pt/ml), and TNO-6 in water (200 μg Pt/ml). MSE minor cen-

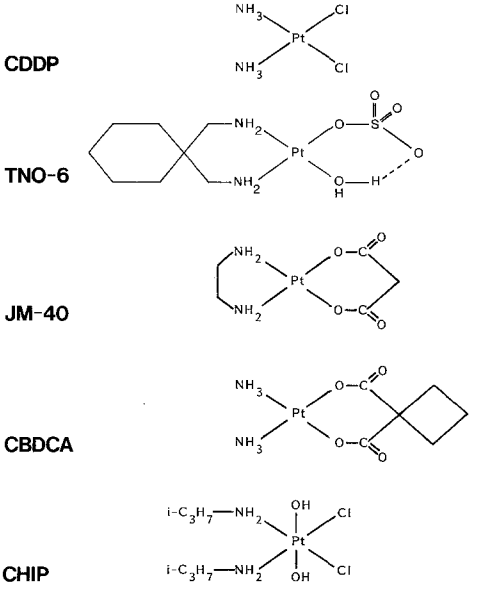

Fig. 1. Structural formulas of the five platinum compounds

trifuges (Beun de Ronde, Amsterdam, The Netherlands) equipped with swinging-head and fixed-angle rotors were used for the cones and the MPS-1 systems, respectively.

Methods

Preparation of filters. CF50A cones were soaked in distilled water for at least 1 h. Before use excess water was removed by centrifugation for 30 s. YMT filters were used without any pretreatment.

Adsorption from aqueous solutions. Aqueous solutions containing 1 μg Pt/ml were prepared by diluting the stock solutions with the appropriate solvent. Aliquots of 4 ml and 1 ml of each solution, both fractionated and unfractionated, were filtered through CF50A and YMT filters, respectively. Platinum concentrations in the initial solutions and filtrates were determined by AAS.

Adsorption from plasma. Small volumes of the aqueous stock solutions were added to aliquots of the plasma pool to obtain a concentration of 10 μg Pt/ml. Each solution was gently shaken in the dark at 37 °C for 24 h. Two portions of 4 ml were ultrafiltered in CF50A cones at 1000 *g*. Ultrafiltrate fractions of 200 μl were successively collected. Two portions of 1 ml were ultrafiltered in the MPS-1 micropartition system with YMT filters at 2000 *g*. The ultrafiltrate was collected in fractions of 150 μl. In both experiments, all fractions were collected in separate receivers during predetermined centrifuging times. The platinum concentration in each fraction was determined by AAS. All experiments were done in quadruplicate.

Protein binding. Small amounts of the aqueous stock solutions of the platinum compounds were added to aliquots of the plasma pool to obtain a concentration of 10 μg Pt/ml. Two solutions of each compound were incubated in the dark at 37 °C in a shaking waterbath for 24 h. At set time intervals samples of 1 ml were withdrawn from the solution and ultrafiltered with the MPS-1 system provided with YMT filters. The platinum concentration of the first 150 μl ultrafiltrate obtained from each plasma sample was determined by AAS and corrected for adsorption to the filters.

Platinum analysis. Plasma ultrafiltrate samples were diluted with a solution of 2 *M* HCl in 0.6 *M* NaCl in a ratio of 9 : 1 (v/v). Standard solutions of each platinum compound in plasma ultrafiltrate, with a concentration of 1, 2, and 3 μg Pt/ml, were treated in the same way. Each sample was analyzed in duplicate, and 20 μl of each sample was introduced into a Perkin-Elmer model 5000 atomic absorption spectrophotometer (Perkin-Elmer, Gouda, The Netherlands) equipped with an HGA-500 graphite furnace and an AS-40 autosampler. A four-stage heating program was used, consisting of drying at 110 °C for 25 s, ashing at 1400 °C for 30 s, atomizing at 2650 °C for 3 s using maximum power, and conditioning at 2550 °C for 5 s. Ramps were used between the steps. The inert gas was nitrogen.

Results

The adsorption differences between CF50A cones and YMT filters of platinum compounds from aqueous solutions as measured in the complete filtrates are shown in Table 1. CBDCA and JM-40 were hardly adsorbed to CF50A cones, while TNO-6 was adsorbed to a very high extent. In general, YMT filters adsorbed a higher amount of the platinum compounds than CF50A cones, with the exception of TNO-6. Fractionated filtration of aqueous solutions showed that adsorption was absent in the final filtrates of 500 μl and 2 ml with YMT and CF50A cones, respectively. TNO-6, however, was still adsorbed to a large and variable extent.

Table 1. Adsorption differences between CF50A cones and YMT filters of platinum compounds in aqueous solutions and human plasma

Compound	Recovery in aqueous solns (% original conc.)		Conc. ratio YMT/CF50A in final fraction, UF_p[a]
	CF50A	YMT	
CDDP	95	89	1.07
TNO-6	16	62	1.05
JM-40	99	91	1.07
CBDCA	100	92	1.04
CHIP	96	96	1.12

[a] UF_p, plasma ultrafiltrate

Filtration of human plasma by both filter types revealed that less than 0.2% of protein crossed the membranes. Recoveries of platinum present in subsequent fractions of plasma ultrafiltrate obtained with CF50A cones and YMT filters are shown in Fig. 2, in which it has been assumed that platinum concentrations in the final fractions obtained with YMT filters represent 100% recovery of the free platinum present in plasma samples after 24 h of incubation. The concentrations in the initial fractions were substantially different between the two filter types. In general a plateau value was reached after ultrafiltration of 300 μl with YMT filters and 600 μl with CF50A cones. The concentration in the final fraction obtained with the YMT filter was always higher than that in the final fraction obtained with CF50A cones. The ratio of these concentrations for each platinum compound is given in Table 1. For TNO-6 there was no longer a striking difference between the two filters, although the plateau value was only slowly reached when CF50A cones were used.

The recovery of platinum in the first 150 μl obtained with YMT filters is shown in Table 2. The reproducibility of the amount recovered was high, which means that plateau concentrations can be reliably calculated from the concentration measured in the first fraction.

Binding of the platinum compounds to human plasma proteins as measured by filtration through YMT filters is shown in Fig. 3. The main kinetic parameters calculated from these measurements, together with decomposition half-lives determined by HPLC analysis of the parent platinum compounds in plasma ultrafiltrates, are summarized in Table 3. A wide variation in the rate and the extent of protein binding between the platinum compounds was observed. Overall, the sequence of the platinum compounds according to increasing initial half-life corresponded with that according to the time interval during which pseudo first-order binding rates were observed as well as with the percentage of free platinum after 24 h and the half-life of decomposition in ultrafiltrate. TNO-6 was the only exception, with the highest initial reaction rate but a free frac-

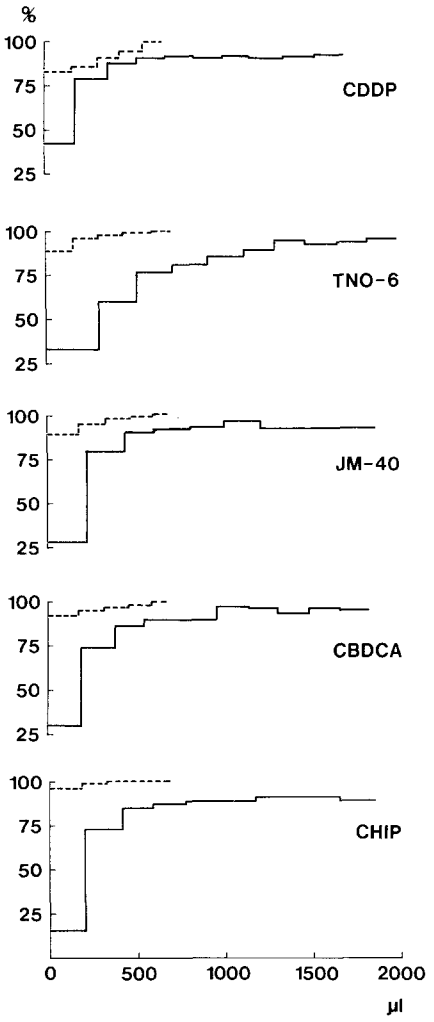

Fig. 2. Recovery of platinum in plasma ultrafiltrate fractions obtained with YMT (filters) (. . .) and CF50A cones (———) expressed as percentage of the concentration present in the final fraction obtained with the YMT filter

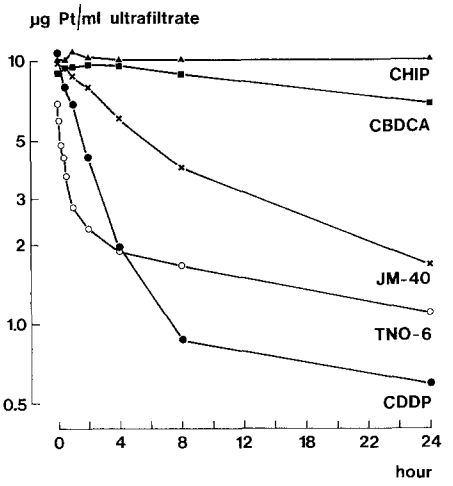

Fig. 3. Binding of platinum compounds to human plasma proteins in vitro

Table 2. Recoveries of platinum in the first 150 µl plasma UF with YMT filters

Compound	Mean recovery[a]	CV[b] (%)	*n*
CDDP	88	1.9	6
TNO-6	91	2.3	8
JM-40	91	4.4	4
CBDCA	93	5.1	5
CHIP	96	2.1	4

[a] Percentage of plateau value
[b] CV, coefficient of variation

tion of 11% at 24 h after incubation, which was twice that for CDDP. The sequence of the compounds with regard to their initial reaction rate with plasma proteins: CBDCA < JM-40 < CCDP < TNO-6, appeared to be inversely related to the sequence in the stability of the leaving groups: malonate > chloride > sulfate. CHIP could not be incorporated in this sequence because of its quadrivalent platinum.

Discussion

The plateau concentration reached in plasma ultrafiltrate with YMT filters was assumed to represent 100% recovery of ultrafilterable platinum. Although it remains an assumption, this is based on the 100% recovery of platinum in the final fraction(s) of filtered aqueous solutions and the possibility of bypassing clotted proteins on the filter to some extent. In contrast to aqueous solutions, ultrafiltration of platinum compounds from human plasma by means of CF50A cones systematically results in lower platinum concentrations in the ultrafiltrate than are obtained with YMT filters. This observation can be explained by the rotors to be used for centrifugation, a swing-out rotor for CF50A cones and a fixed-angle rotor for the MPS-I sys-

Table 3. In vitro kinetic data of five platinum compounds in plasma and plasma ultrafiltrate

Compound	Protein binding in vitro			Decomposition in UF	
	Initial $t_{1/2}$ (h)	Time interval (h)	% Free after 24 h	$t_{1/2}$[a]	Lit. reference
CDDP	1.7	0–4	6	2.3 h	[11]
TNO-6	0.5	0–0.5	11	15 min	–[b]
JM-40	5.9	0–8	17	10 h	[14]
CBDCA	48.6	24	69	20 days	[4]
CHIP	∞	24	100	∞	[9]

[a] At room temperature
[b] Disappearance of the aquasulfato compound (personal, unpublished, results)

tem. The result is that ultrafilterable platinum species always have to pass a concentrated protein solution before they reach the filter, while in the case of YMT filters the protein-enriched portion in the filter can be bypassed to some extent. The low concentration in the initial fraction of ultrafiltrate obtained with CF50A filters can be explained by the water content of the filters as a result of the pretreatment. With the exception of TNO-6, reliable ultrafiltrate platinum concentrations can only be measured after discarding the first 600 µl ultrafiltrate. In the case of YMT filters, the first portion of ultrafiltrate already contains a high and reproducible fraction of the platinum concentration. This means that YMT filters can be used in two ways: either by using the first 150 µl and correcting the concentration for the concentration ratio between the initial and final ultrafiltrate portions, or by using the ultrafiltrate fraction after discarding the first 300 µl (except for CDDP). The advantage of the first procedure is that protein binding can be rapidly, and therefore reliably, measured in the case of fast protein-binding reaction rates such as are encountered for TNO-6 and CDDP during the first hours after administration of the platinum compounds in vivo and in vitro. Another advantage of YMT filters is that less than 1 ml plasma can be processed, as against at least 4 ml with CF50A cones. This means that blood sample volumes of patients and animals can be substantially reduced. The discrepancy in the adsorption of TNO-6 from aqueous solutions and human plasma may be explained by the presence of highly reactive leaving groups in aqueous solutions of TNO-6, such as SO_4^{2-}, OH^-, and H_2O [1, 3], whereas 24 h after addition to human plasma only less reactive compounds can be expected [5]. This may also explain why TNO-6 binds to a lower extent to plasma proteins than CDDP after 24 h of incubation.

As shown in Table 3 the half-life of decomposition in plasma ultrafiltrate increases in the sequence TNO-6 < CDDP < JM-40 < CBDCA < CHIP. The decomposition rate corresponds with the stability of the platinum-ligands bonds [1] as far as it concerns bivalent platinum compounds. This (in)stability is also reflected by the presence of large amounts of aquated species in TNO-6 solutions [3], smaller amounts in CDDP solutions [11], and their absence in solutions of the other three compounds [4, 9, 14]. Half-lives of decomposition corresponded with initial half-lives of protein binding, suggesting a similar reaction mechanism. As for CDDP [12], it is supposed that aquation of the parent platinum compound is the first and rate-determining step, mainly occurring during the initial time interval as a pseudo first-order reaction. The initial half-life of TNO-6 and the corresponding initial time interval are short, because aquated species are already available [3] for direct interaction with plasma proteins. This short initial half-life is also responsible for the low initial free platinum concentration, owing to protein binding during the ultrafiltration procedure. After the initial time interval deviations from the first-order reaction are observed, which can be explained by assuming interactions with alternative binding sites or slower reactions between proteins and reaction products originating from concomitant reactions between the platinum compounds and the nucleophils in plasma ultrafiltrate. Although Momburg et al. [7] deny such secondary reactions, it should be realized that their experimental conditions most probably were not in agreement with their presuppositions, i.e., it cannot be expected that malonates (JM-5, JM-8, JM-74) decompose in plasma ultrafiltrate at −17 °C over 24 h and it seems likely that JM-20 reacts with phosphate ions [8], after which an interaction with proteins comparable to that observed after preincubation with plasma ultrafiltrate can be expected. To confirm the suggested structure-activity relationship further studies are needed, including a larger number of platinum compounds with the same amine ligands.

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