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# Cytotoxic Effects of Long-term Circulating Ultrafiltrable Platinum Species and Limited Efficacy of Haemodialysis in Clearing Them

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**We applied haemodialysis to clear platinum (Pt) circulating species following renal insufficiency due to an accidental cisplatin overdosage (205 mg/m² instead of 100 mg/m²). Serum samples were repeatedly obtained during this clinical episode from day 5 up to day 30 after cisplatin dosing. A serum aliquot taken at day 22 after cisplatin administration was tested to assess the possible cytotoxicity exhibited by the circulating Pt species on a head and neck tumour cell line. The profile of ultrafiltrable (UF) Pt during successive haemodialysis cycles was striking. After each haemodialysis cycle, a marked decrease in UF Pt, occurred but was followed by more or less pronounced rebounds. Cisplatin concentration–cytotoxic effect curves obtained *in vitro* from patient serum before cisplatin administration and healthy control serum exhibited very similar concentration effect profiles. In contrast, the patient serum taken at day 22 after cisplatin administration resulted in marked cytotoxic effects, which were much greater than those which could have been anticipated considering the Pt concentration of this serum sample. The present report underlines the limited usefulness of haemodialysis for rescuing cisplatin treated patients, exhibiting unanticipated postinfusion renal failure with overexposure to the drug. The *in vitro* investigations suggest that pharmacological effects of Pt derivatives may not only be attributable to short-term effects of the drug diffusion into tissues, but also to more delayed effects from Pt circulating species.**
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## INTRODUCTION

THE MOST common side-effects of cisplatin include nephrotoxicity and ototoxicity, gastrointestinal disturbances and bone marrow suppression. There is clinical evidence that the therapeutic efficacy of cisplatin increases with increasing dose [1], but that higher doses of cisplatin also raise the vulnerability of the kidney to toxic lesions by the drug [2]. Means of preventing dose limiting cisplatin-induced side effects (renal toxicity and emesis) include intravenous (i.v.) hydration with hypertonic saline and repetitive dosing with parenteral antiemetics. In addition, several studies suggest that the prophylactic use of sodium thiosulphate attenuates the renal toxicity of cisplatin after high-dose treatment by the intraperitoneal (i.p.) route [3, 4].

Although it is clear that measures of prevention exist for managing the potential renal toxic effects of cisplatin, little is known about how to manage accidental overexposure to abnormally elevated circulating cisplatin concentrations due to unanticipated renal failure. We applied haemodialysis to clear circulating cisplatin species following renal insufficiency due to an accidental cisplatin overdosage. Clinical and pharmacological data are presented.

*Case report*

The female patient was 54 years old when she presented for locoregional and metastatic progression of a lung adenocarcinoma surgically treated 3 years before. A multiregimen chemotherapy was used which should include administration of cisplatin 100 mg/m² on day 1 and etoposide 120 mg/m²/day on days 1, 2 and 3. In fact, due to a retrospectively identified mistake, the patient actually received 300 mg of cisplatin, that is 205 mg/m², instead of 100 mg/m². Cisplatin administration was preceded by vigorous hyperhydration with 2 l of saline including 2 g/l KCl and 2 g/l $MgCl_2$. After cisplatin injection (5 h at 1 mg/min i.v.), hyperhydration was maintained with 1 l of 5% glucose solution including 2 g KCl, 1g $CaCl_2$ and 2 g $MgCl_2$. Renal function was normal before starting treatment (blood creatinaemia at 79.6 μmol/l). Immediate clinical tolerance to treatment was good. At day 5 renal insufficiency was detected with blood creatinine rising to 398 μmol/l. Two days later, haemodialysis was initiated, including 7 cycles delivered up to day 20. During this time, renal insufficiency persisted with diuresis maintained and blood creatinine at 327 μmol/l. The patient developed severe leucopenia (grade 4 on day 12) and thrombocytopenia (on

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day 13), both reversible on days 15 and 18, respectively. Starting on day 3 and followed up to day 30, a cisplatin pharmacokinetic survey was begun. Aliquots of serum were kept for testing the *in vitro* cytotoxicity of the cisplatin-related circulating species.

## MATERIALS AND METHODS

*Cisplatin plasma determinations*

Serum samples were repeatedly obtained during this clinical episode from day 5 up to day 30 after cisplatin dosing. Blood samples (maximum volume of 3 ml not available in all cases) obtained in ethylenediaminetetraacetic acid (EDTA) tubes were immediately placed in a water bath containing ice for transportation to the laboratory (within 10–15 min), then centrifuged at 4°C. A 500 μl aliquot of the resulting plasma was centrifuged for 30 min at 2000 *g* and 4°C in a Centrifree micropartition unit (Amicon, Denvers, Massachusetts, U.S.A.). The resulting ultrafiltrate fractions were used to measure filterable platinum (Pt). Total Pt was measured in the whole plasma fraction. Samples were stored at −20°C until analysis.

*Platinum* (Pt) *assay*

Quantitative analysis of Pt in samples was performed by atomic absorption spectrophotometry (AAS) using a Perkin-Elmer model 3030 atomic absorption spectrophotometer with background correction by the Zeeman effect for trace analysis. Ultrafiltrates were measured without dilution; before analysis, plasma was diluted to 1/2 using a 0.2% $HNO_3$ solution containing 0.01% Triton X-100. The injected volume was 20 μl. A standard curve (0.162, 0.325 and 0.65 μg Pt/ml) was automatically plotted by an autosampler using a spiked blank plasma specimen that had previously been diluted to 1/2 (0.65 μg Pt/ml). Analysis included the following steps: drying at 110°C for 40 s, ashing at 1400°C for 20 s and atomisation at 2650°C with a 3 s stop flow; and tube cleaning at 2650°C for 4 s. The limit of sensitivity (twice the background noise) was 5 ng/ml for plasma and 2.5 ng/ml for ultrafiltrates. Reproducibility was calculated from 25 successive series of analyses; it was 8.5%, 7.6% and 6.5% for Pt concentrations of 0.162, 0.325 and 0.65 μg/ml, respectively.

*Haemodialysis*

The duration of each haemodialysis cycle was 4 h. The haemofiltration apparatus (Hospal 2400s type, Rhone Poulenc, France) was equipped with a polyacrylonitrile membrane (1 $m^2$). The substitution fluid contained 142 mEq of $Na^+$, 1 mEq of $Mg^{++}$, 110 mEq of $Cl^-$, 3.2 mEq of $Ca^{++}$, 3.0 mEq of $K^+$, 30 mEq of $CH_3COO^-$ and 3 mM of dextrose. The blood flow rate was 200 ml/min and the ultrafiltrate flow 500 ml/min.

In vitro *cytotoxicity analysis*

A serum aliquot taken on day 22 (D22) after cisplatin administration, had a sufficient volume (3 ml) and was thus tested to assess the possible cytotoxicity exhibited by the long-term circulating cisplatin species. The serum concentration in total Pt was 0.31 μg/ml, and 0.024 μg/ml in ultrafiltrable Pt. The human tumour cell line (CAL 27) used for *in vitro* tests was isolated at our Institute from a patient with a squamous cell carcinoma of the head and neck [5]. Cells were routinely cultured in a humidified incubator (Sanyo) at 37°C with an atmosphere of 8% $CO_2$ in air. Cells were grown in DMEM medium supplemented with 10% serum, penicillin (50000 IU/l), streptomycin (86 μmol/l) and L-glutamine (2 mM).

Four different experiments were run in parallel according to the type of serum tested. There was fetal bovine serum (FBS), serum from a healthy control, serum from the patient obtained before cisplatin administration and serum from the patient obtained at day 22 after cisplatin administration. Cells were exposed for 120 h to various cisplatin concentrations ($5.10^{-2}$ to 5 μg/ml total cisplatin) added to the culture medium. Cells were grown in 96-well microtitration plates. The cytotoxic effects of cisplatin were assessed by the MTT semi-automated test [6] in 96-well incubating plates. Results were expressed as the relative value of absorbance compared with controls without cisplatin. Absorbance was set at 540 nm and measured on a Titertek twin-reader. Each experimental point was evaluated in sextuplicate. For all experiments, the coefficients of variation ranged between 3 and 10%.

## RESULTS

Both total (T) and ultrafiltrable (UF) plasma concentrations of Pt-related species during the clinical episode are shown in Figure 1. On day 5, after cisplatin dosing and when renal failure was diagnosed, the T and UF plasma concentrations of Pt were 1.413 and 0.118 mg/ml respectively. The repeated use of haemodialysis led to a progressive decrease of T Pt reaching 0.335 μg/ml on day 28. Similarly, the UF plasma concentrations of Pt declined to 0.024 on the same day. The UF plasma concentration time profile of Pt during successive haemodialysis cycles is striking. Each haemodialysis cycle resulted in a marked decrease in Pt, but the declines were followed by more or less pronounced rebounds. This phenomenon was observed after every haemodialysis cycle as, for instance, at day 7 where the UF Pt concentration before haemodialysis was 0.28 μg/ml declining to 0.025 μ/ml after haemodialysis and then increasing to 0.100 μg/ml 2 days later.

Typical concentration–cytotoxic effect curves are shown in Figure 2. Curves obtained using human serum (patient serum before cisplatin administration and healthy control serum) exhibited very similar concentration effect profiles. The presence of FBS instead of human serum in the culture medium induced slightly more pronounced cytotoxic effects for identical cisplatinum concentrations tested. In contrast, the incubation of CAL 27 cells with the patient serum at day 22 resulted in very marked cytotoxic effects compared with the other experimental conditions tested. Surprisingly, the cytotoxic effects observed with the patient serum at day 22 were much higher than those which could have been anticipated when taking into account the

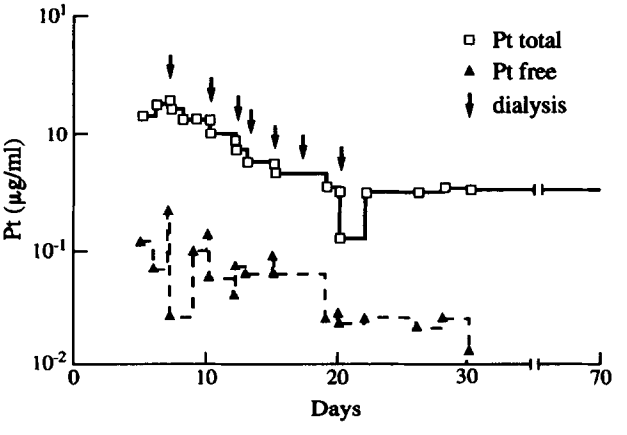


**Figure 1. Profile of serum concentrations of Pt-related species during the clinical episode.**

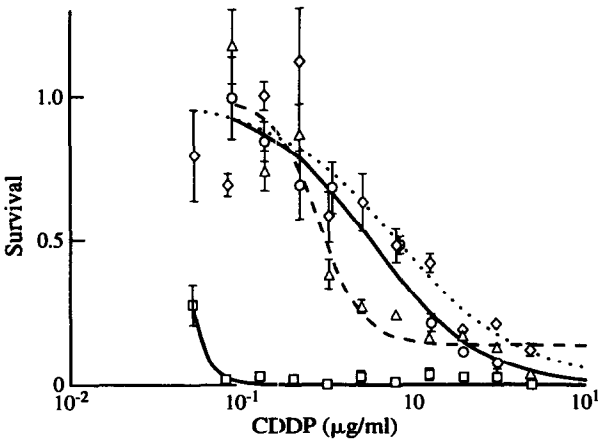


**Figure 2. Cisplatin concentration–cytotoxic response curves with CAL 27 cells. □ patient serum after cisplatin treatment (day 22); ○ patient serum before cisplatin treatment; ◇ healthy control serum; △ fetal bovine serum.**

Pt concentration of this serum sample (0.031 μg/ml in T Pt, i.e. 0.047 μg/ml cisplatin in the culture medium).

## DISCUSSION

The use of haemodialysis has been proposed and successfully validated for allowing cisplatin chemotherapy in patients exhibiting acute renal failure before treatment [7, 8]. In the present case, it was decided to initiate haemodialysis to rapidly clear unexpectedly elevated concentrations of Pt-related species resulting from an accidental drug overdose inducing renal failure. At day 5, there was biological evidence for renal insufficiency; the blood concentrations in T and UF Pt were 1.413 and 0.118 μg/ml, respectively. These concentrations remained unchanged for two subsequent days (Figure 1). It must be emphasised that UF Pt concentrations usually fall below the 0.1 μ/ml range 4–6 h after a conventional 100 mg/m$^2$ dose [9]. Surprisingly, haemodialysis had only a modest effect on clearing UF Pt in the present case. This was explained by rebound phenomena observed shortly after the cessation of each haemodialysis cycle (Figure 1). Such rebounds have been reported previously in a similar case of post Pt infusion renal failure, in a 27 year old male with testicular carcinoma treated as part of a phase II trial including bone marrow transplant with ifosfamide, carboplatin and etoposide [10]. Dialyses were repeated daily over 4 days, and at each stage plasma UF Pt decreased substantially, but returned to predialysis levels within a few hours. In the present case the five haemodialysis cycles applied over a period of 10 days resulted in a general UF Pt decrease from 0.2 to 0.02 μg/ml, giving an apparent half-life of 3 days. This is unsatisfactory when compared with the half-life of Pt in a patient with normal renal function, which is close to 0.4 h [9]. Sharma and Edwards [11] have studied the subcellular distribution of Pt after systemic administration in laboratory animals. They found, at 24 h following cisplatin injection, that about 50% of cytosolic Pt was present as low molecular weight (<1000) potentially diffusible species. Like others [12], we also recently described a biphasic Pt cellular efflux pattern after exposure of human tumour cells to cisplatin *in vitro* [13]. It can thus be proposed that the rebounds in UF Pt concentrations were due to a release of Pt-unidentified species from tissues.

As a complementary investigational step, the potential cytotoxicity of these released Pt species was investigated *in vitro*, using a serum sample taken well after cisplatin dosing. Very surprisingly, it was found that this serum was much more cytotoxic than equimolar cisplatin added to blank serum (Figure 2). It is possible that prolonged exposure to cisplatin, as in the case of this patient, may have saturated plasma binding of Pt resulting in a higher unbound fraction compared to fresh plasma. However, Wright and colleagues [10] made a similar observation, and found that the postinfusion cytotoxic ultrafiltrable Pt species were not the unchanged drug itself. More recently, Melvick and colleagues [14] studied the biological consequence of cisplatin binding to serum protein as well as to cellular components. Based on the cytotoxic effects on human NHIK 3025 cells, their data indicate that cisplatin can be released in an active form from the cells. In addition, Hegedüs and associates [15] studied the reactivity of cisplatin bound to human plasma proteins, and found that these complexes were still able to react with strong nucleophiles, thus questioning the dogma that only UF cisplatin is potentially active *in vivo*.

In conclusion, the present report underlines the limited usefulness of haemodialysis for rescuing cisplatin treated patients exhibiting unanticipated postinfusion renal failure following overexposure to the drug. In addition, the results of our complementary *in vitro* investigations concur with those of others [10, 14, 15], in that the pharmacological effects of Pt derivatives may not only be attributable to short-term effects of the drug's diffusion into tissues, but also to more delayed effects from Pt circulating species. The identification of these Pt circulating species may be warranted. These observations should be considered when discussing the still debated schedules for Pt derivative administration [16].


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# Prognostic Factors for Local Control, Regional Control and Survival in Oropharyngeal Squamous Cell Carcinoma

**C.A. Regueiro, G. Aragón, I. Millán, F.J. Valcárcel, A. de la Torre and R. Magallón**

**We have performed univariate and multivariate analysis to identify the clinical and treatment-related prognostic factors in a series of 254 patients with newly diagnosed, histologically proven, oropharyngeal squamous cell carcinoma treated with radical radiation therapy. The probabilities of local control, regional control, disease-free survival (DFS) and adjusted survival (AS) were calculated using the Kaplan–Meier method and differences between curves were evaluated by the Mantel–Cox test. The obtained significant variables in the univariate analysis were analysed using the Cox proportional hazards model. In the Cox multivariate analysis, four variables significantly influenced local control probability in the following order: tumour diameter, N stage, alcohol intake and weight loss. N stage significantly influenced the probability of regional control. Five variables influenced both DFS and AS: N stage, tumour diameter, weight loss, alcohol intake and tumour origin within the posterior oropharyngeal wall.**

**Key words: oropharynx, carcinoma, prognostic factors, alcohol, weight loss**
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## INTRODUCTION

THE EXTENT of the primary tumour and the absence, presence and extent of lymph node metastases are considered the most important factors determining prognosis of oropharyngeal squamous cell carcinoma. Treatment selection is usually based on these factors. Other factors may also influence the outcome of patients with oropharyngeal carcinoma. The influence of primary site [1], histological grade [2], patients gender [3], performance score [4, 5] and haemoglobin value [3] have been reported.

An analysis of the influence of clinical and therapeutical factors on prognosis should evaluate their possible impact on local control and regional control, as well as on tumour lethality, since locoregional relapses represent the most frequent cause of failure, and current treatment modalities treat locoregional disease.

This study attempts to identify, by means of a multivariate analysis, the clinical and treatment-related factors significantly associated with outcome in a series of patients with oropharyngeal carcinoma treated with curative intent at one department of radiotherapy.

## PATIENTS AND METHODS

### *Patients*

Between July 1964 and December 1989, 344 patients with histologically proven diagnosis of squamous cell carcinoma of the oropharynx were admitted at the Department of Radiation Oncology of Clinica Puerta de Hierro (Madrid, Spain). Those patients with newly diagnosed carcinomas and without previous irradiation of the oropharynx, whose radical radiation therapy

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