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An analysis of potential factors allowing an individual prediction of cisplatin-induced anaemia

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Abstract

Severe cisplatin (CP)-induced anaemia significantly impairs the patient's quality of life. Prevention based on erythropoietin (EPO) administration would be cost-effective providing that individual predictive factors of anaemia are identified. The aim of the present study was to identify parameters able to predict the occurrence of CP-related anaemia. This prospective study was conducted on 40 head and neck cancer patients receiving a CP (100 mg/m^2, intravenous (i.v.) on day 1) — 5-fluorouracil (5-FU, 1 g/m^2/d×5 days by continuous infusion) induction chemotherapy. Three cycles were given at 3-weekly intervals. Platinum pharmacokinetics (total and ultrafilterable plasma platinum concentration measured 16 h after CP administration) and 5-FU pharmacokinetics (full-cycle plasma area under the curve, ($AUC_{0–105h}$) were determined at the first cycle. Anaemia was defined as the haemoglobin (Hb) loss computed between pretreatment Hb and Hb concentration measured 1–2 days before third cycle administration. The median Hb loss was 22 g/l (mean 24 ± 15, range 0–66). Significant loss of Hb (Hb loss > 30 g/l) occurred in 15 patients (38%) and 3 of them also received a blood transfusion. Patient age, 5-FU $AUC_{0–105h}$ and total platinum concentration were unrelated to Hb loss. In contrast, ultrafilterable (UF) platinum concentration was significantly correlated to Hb loss: the higher the UF platinum concentration, the greater the Hb loss ($P = 0.015$). A discriminant analysis allowed a cut-off value for UF platinum to be proposed to identify patients developing significant loss of Hb: 91% of patients exhibiting a UF platinum concentration above 50 ng/ml developed significant loss of Hb in contrast to 18% in the group of patients with a UF platinum concentration below 50 ng/ml (odds ratio (95% confidence interval, CI) of 46 (4.7–446)). In conclusion, the present platinum pharmacokinetic survey may be proposed as a valuable approach to identify patients at risk for developing severe anaemia.

Keywords: Cisplatin; Pharmacokinetics; Anaemia

1. Introduction

Cisplatin (CP)-induced anaemia is a well-known side-effect [1] which occurs in 9–40% of patients [2]. Anaemia impairs the patients' quality of life by leaving them listless and weak, hindering their ability to work and affecting their leisure activities. The standard method for alleviating these symptoms is the administration of regular blood transfusions. Although much safer than in the past, transfusions still carry numerous risks [3]. Treatment with exogenous recombinant human erythropoietin (EPO) reverses CP-associated anaemia in animal models and in clinical pilot studies [4–7]. Double-blind randomised trials have demonstrated that CP-induced anaemia was corrected by EPO administration, thus resulting in reduced blood transfusion requirements [8]. An alternative way to reduce blood transfusions in cancer patients undergoing chemotherapy may be prophylactic administration of EPO. However, EPO is very expensive and such prophylactic strategy could only become acceptable if individual predictive factors of anaemia were identified. In this study a homogeneous group of 40 head and neck cancer patients was prospectively investigated in order to try to identify parameters that might predict the occurrence of CP-related anaemia. All patients received an identical induction chemotherapy regimen consisting of CP plus 5-fluorouracil (5-FU). Demographic and biological data were analysed, along with drug pharmacokinetics.

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2. Materials and methods

2.1. Patients

Head and neck cancer patients included in this study received induction chemotherapy combining CP and 5-FU. All patients had a good performance status (below 2, WHO scale). Three cycles of chemotherapy given every 3 weeks were planned. The regimen consisted in 100 mg/m^2 CP given by intravenous infusion (1 mg/min) on day 1, followed by a 5-day continuous infusion of 1 g/m^2/d 5-FU. All patients received the hydration protocol routinely administered at our institute: 3.5 l of glucose containing 2.2 mM calcium glucuronate, 1 g/l magnesium pidolidate, 2 g/l KCl and 3 g/l NaCl. Two litres and 1.5 l were given 6 h before and 4 h after CP administration, respectively. Methylprednisolone (1 mg/kg) and HT3 antagonists were administered twice a day by intravenous infusion on day 1. Prednisolone (1 mg/kg) and metoclopramide (1 mg/kg) were given per os every day from day 2 to day 6. Patients were considered eligible for this study if they met the following criteria: haemoglobin (Hb) levels higher than 110 g/l before chemotherapy; no previous chemotherapy; no previous radiotherapy; absence of concomitant haemorrhage or haemolysis; no red blood cell (RBC) transfusions within the 4 weeks before the current chemotherapy regimen; adequate bone marrow, renal, hepatic and cardiovascular functions before chemotherapy; possibility of obtaining drug pharmacokinetic data (strictly required for CP). Initially, 56 consecutive patients were included in this prospective study. However, 16 patients were excluded for the following reasons: 4 had no platinum pharmacokinetic data available at first cycle, 2 had no Hb measurement before the third cycle, and 10 had abnormally low Hb levels before treatment (less than 110 g/l). The final analysis was thus conducted on 40 patients. All patients exhibited good performance status (WHO grade <2). Patient characteristics are given in Table 1. Since different therapies were given after completion of the third cycle of induction chemotherapy (including radiotherapy, surgery, or radiotherapy plus surgery), CP-related anaemia was analysed after two cycles of chemotherapy.

2.2. Parameters investigated

2.2.1. Biological variables

Standard chemistry tests (urea, creatinine, sodium, potassium, chlorine, total proteins) and haematology assessment (blood count) were performed during routine controls, 1–2 days before each chemotherapy cycle. The Hb loss was computed between pretreatment Hb concentration and Hb concentration measured 1–2 days before the administration of the third cycle. A significant loss of Hb (SLH) was defined as a Hb loss $\geqslant 30$ g/l.

2.2.2. Drug pharmacokinetics

Platinum and 5-FU pharmacokinetics were evaluated at first cycle of chemotherapy. For feasibility reasons, a single-sample assay was performed for platinum measurement (5 ml in EDTA tubes) during the morning of the day following CP administration, at the same time as blood samples were taken for treatment follow-up (16 h precisely after the end of CP administration, H_{16}). This single-point pharmacokinetic evaluation was essentially dictated by feasibility concerns and was based on a previous study that showed a good correlation between the area under the plasma concentration–time curve ($AUC_{0\text{-}\infty}$) of ultrafilterable (UF) platinum and platinum concentration measured the day after cisplatin administration [9]. Samples were immediately placed in a water bath containing ice for transportation to the laboratory. Tubes were centrifuged for 10 min at 4°C within 15 min of sampling. A 500 μl aliquot of the resulting plasma was centrifuged for 30 min (2000 ***g*** at 4°C) in a Centrifree micropartition unit (Amicon, Danvers, MA, USA) to obtain UF platinum. Total platinum was measured in the whole plasma fraction. Samples were stored at −20°C until analysis. Platinum analysis was performed by atomic absorption spectrophotometry (AAS) using a Perkin-Elmer (Ridgefield, CT, USA) atomic absorption spectrophotometer with background correction by the Zeeman effect. Ultrafiltrates were analysed without dilution. Plasma was diluted 1:4 in NaCl 0.9% and subsequently 1:2 in 0.2% HNO_3 solution containing 0.01% Triton X-100. The injected volume was 20 μl. A standard curve (0, 162, 325 and 650 ng/ml platinum) was automatically plotted by an autosampler. The limit of sensitivity (twice the background noise) was 5 ng/ml for plasma and 2.5 ng/ml for ultrafiltrates. Concentrations below the limit of sensitivity were considered to be equal to zero. In each series of analyses, three control samples (aliquots of

Table 1
Patient characteristics ($n=40$)

	n (%)
Sex	
Male	36 (90)
Female	4 (10)
Cancer localisation	
Hypopharynx	15 (38)
Larynx	20 (50)
Oropharynx + oral cavity	5 (13)
Age (years)	
Mean ± S.D.	57.5 ± 9.7
Median (range)	60.9 (31–71)
Haemoglobin (Hb) before treatment (g/l)	
Mean ± S.D.	135 ± 11
Median (range)	137 (111–160)

spiked samples containing known platinum concentrations) were analysed to check the assay's interday variability. From 37 consecutive analyses, the coefficients of variation were 10% for the control at 60 ng/ml, 9% for the control at 300 ng/ml and 5.2% for the control at 900 ng/ml.

A full-cycle 5-FU pharmacokinetic follow-up with individual 5-FU dose adaptation at mid-cycle was systematically performed according to our standard procedure [10,11]. Blood sampling was thus performed at 9, 24, 33, 48, 57, 81 and 105 h after the start of 5-FU infusion. Blood samples were immediately centrifuged and plasma were stored at −4° until analysis. 5-FU concentration was measured in plasma using a previously described high performance liquid chromatography (HPLC) method [10]. The area under the curve from 0 to 105 h (AUC) was calculated at each cycle using least-square methodology. The sensitivity limit was 10 ng/ml and interday reproducibility was < 8%.

2.3. Statistics

The evolution of Hb blood concentration from cycle to cycle (1 versus 2 versus 3) was tested according to the non-parametric Friedman test. Since Hb loss, pretreatment Hb, patient age, platinum concentrations and log10 (5-FU $AUC_{0–105h}$) fitted a Gaussian distribution, relationships between all studied parameters were analysed by means of parametric tests (group comparisons, univariate or multivariate linear regression). The search for a significant threshold for distinguishing patients developing a SLH from others was performed according to successive Chi-square analyses. Statistics were performed on SPSS software (Chicago, IL, USA).

Table 2
Pharmacokinetic data at cycle 1

	n	Mean	Median	S.D.	Range
Total Pt concentration at H_{16} (ng/ml)	40	2100	2090	820	470–3600
UF Pt concentration at H_{16} (ng/ml)	39[a]	39	36	22	ND–110
5-FU $AUC_{0–105h}$ (ng/ml.h)	34	31 250	30 490	12 400	12 980–81 440

ND, not detectable (i.e. < 2.5 ng/ml).
[a] One patient with unevaluable UF platinum concentration.

3. Results

Fig. 1 shows the progressive and significant decrease in haemoglobin (Hb) blood concentration from cycle to cycle (Friedman test, $P < 0.001$). At the time of third cycle administration, the median Hb loss was 22 g/l (mean 24 ± 15, range 0–66). SLH, defined as a loss ⩾30 g/l, occurred in 15 patients out of 40 (38%), and 3 of them received a blood transfusion within the 2 weeks following completion of the third cycle.

The interpatient variability of pharmacokinetics at cycle 1 is illustrated in Table 2 for total and UF platinum concentrations measured at H_{16}, as well as for 5-FU $AUC_{0–105h}$. Table 3 illustrates the relationships between all studied parameters and the CP-related Hb loss, analysed as a continuous variable. Patient age, 5-FU $AUC_{0–105h}$ and total platinum concentration were unrelated to the Hb loss. In contrast, UF platinum concentration measured at cycle 1 was significantly correlated to the Hb loss evaluated 1–2 days before the administration of the third cycle: the higher the UF platinum concentration, the greater the Hb loss (Fig. 2, $r^2 = 0.15$, $P = 0.015$). Similarly, pretreatment Hb was a

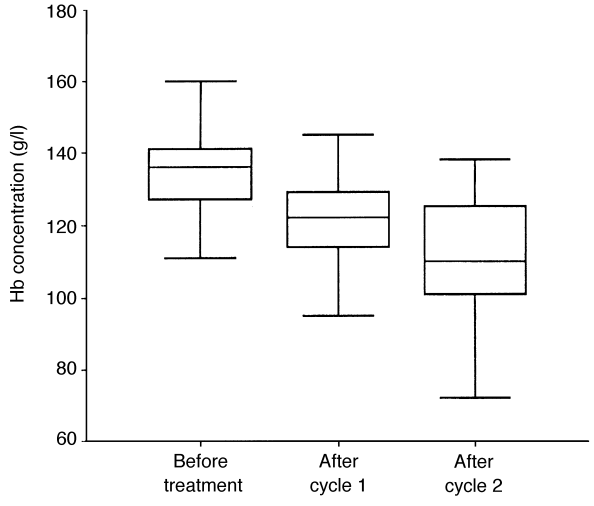

Fig. 1. Evolution of the haemoglobin (Hb) blood concentration from cycle 1 to cycle 3. Hb measurement was performed 1–2 days before each chemotherapy cycle. Each box represents the median and 95% confidence interval; the low and high horizontal bars represent the extreme values (comprised within 1.5-fold of the interquartile range).

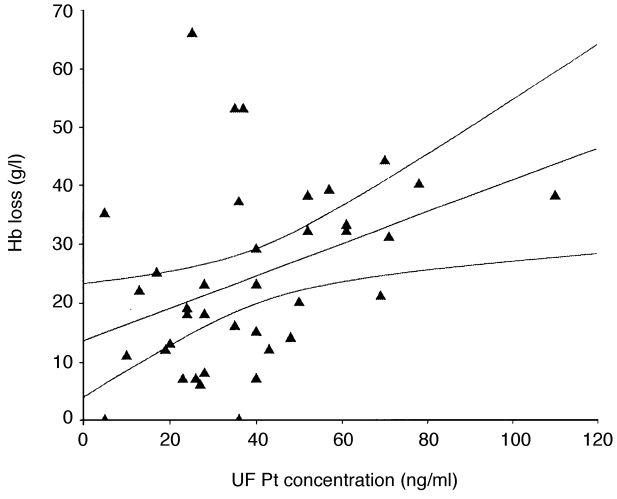

Fig. 2. Plot of the linear regression (±95% confidence interval) between the Hb loss computed between pretreatment Hb concentration and Hb concentration measured 1–2 days before the administration of the third chemotherapy cycle ($r^2 = 0.15$, $P = 0.015$).

Table 3
Relationships between Hb loss after two chemotherapy cycles and all studied parameters (univariate analyses)

Parameters	*P* value	Statistics
Age	0.15	Linear regression ($r^2 = 0.06$)
Pretreatment Hb	0.03	Positive linear regression ($r^2 = 0.12$)
Total Pt concentration[a]	0.12	Linear regression ($r^2 = 0.06$)
UF Pt concentration[a]	0.015	Positive linear regression ($r^2 = 0.15$)
Log 10 (5-FU $AUC_{0-105\ h}$)[a]	0.26	Linear regression ($r^2 = 0.04$)

[a] Evaluated at cycle 1.

significant predictor of CP-related loss of Hb (positive correlation, $r^2 = 0.12$, $P = 0.03$). In a multivariate analysis including pretreatment Hb and UF platinum concentration, UF platinum concentration was the only significant predictor of anaemia.

Fig. 3 illustrates the significant difference in UF platinum distribution between patients with SLH (mean 30 ng/ml with a 95% confidence interval (CI) of 24–37) and those without (mean 53 ng/ml with a 95% CI 40–67; Student *t*-test: $P = 0.001$). Finally, Chi-square analyses were performed in order to define the UF platinum concentration threshold best able to predict the occurrence of SLH. The best UF platinum concentration threshold was located at 50 ng/ml: 91% (10/11) of patients above the cut-off exhibited SLH whereas only 18% (5/28) of patients below developed SLH (odds ratio = 46 with a 95% CI 4.7–446, Chi-square $P < 0.001$).

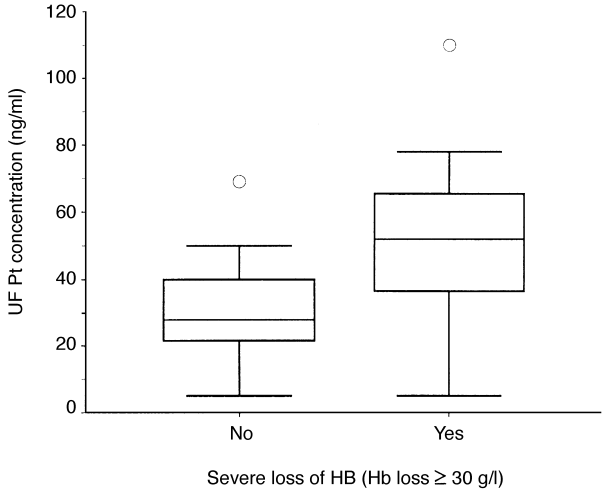

Fig. 3. Distribution of UF platinum concentrations measured 16 h after the end of the first CP administration (cycle 1) depending on the occurrence of severe loss of Hb (SLH, Hb loss ⩾30 g/l) between pretreatment measurement and Hb concentration measured before the third chemotherapy cycle. Each box represents the median and 95% confidence interval; the low and high horizontal bars represent the lowest and highest values comprised within 1.5-fold of the interquartile range; ○ outliers (i.e. outside 1.5-fold of the interquartile range).

4. Discussion

This prospective study was performed on a homogeneous group of 40 previously untreated head and neck cancer patients receiving three cycles of CP-5-FU induction chemotherapy. After two chemotherapy cycles, the median Hb loss was 22 g/l and 38% of patients had developed SLH, defined as an Hb loss ⩾30 g/l. It is likely that prophylactic EPO administration might have reduced the incidence of anaemia since EPO has been demonstrated to be effective in increasing Hb levels in patients receiving haematotoxic chemotherapy, thus decreasing transfusion requirements and improving functional status and quality of life [12,13]. However, EPO is expensive and so far has not been recognised as cost-effective in the treatment of chemotherapy-induced anaemia [14].

At least two approaches could improve the cost-effectiveness of EPO. Firstly, predictors of EPO treatment effectiveness have been identified by Cazzola and colleagues [15] who showed that a low physiological EPO blood concentration before therapy can predict the efficacy of EPO treatment. A complementary approach is to identify individual factors predicting the occurrence of severe anaemia, in order to target the group of patients for which EPO will be cost-effective. To our knowledge, such information is rare and a low baseline value of Hb seems to be the only relevant predictive factor [16]. Such a difficulty was already mentioned in the recent ASCO recommendations which were aimed at identifying individual predictors that may govern the use of haematopoïetic growth factors in general [17]. We thus investigated demographic (age), biological (pretreatment Hb concentration) and pharmacological data (CP and 5-FU pharmacokinetics). After completion of the third chemotherapy cycle, radiotherapy and/or surgery were applied in the majority of patients and 3 patients received a blood transfusion at that time. We thus analysed the influence of the first two chemotherapy cycles on the occurrence of anaemia, defined as the difference between pretreatment Hb and Hb concentration measured 1–2 days before the third cycle.

Since the present chemotherapy regimen combined CP and 5-FU, the pharmacokinetics of both drugs were analysed. Pharmacokinetic–pharmacodynamic data have clearly established that 5-FU pharmacokinetic variability may explain the variability of drug-related side-effects [18]. In contrast to specifically 5-FU-related toxicities like neutropenia and mucositis for which pharmacodynamic–pharmacokinetic relationships have been shown [18], 5-FU full-cycle AUC was not related to the occurrence of anaemia in the present study. In order to apply a strategy suitable for use on a routine basis, CP pharmacokinetics were analyzed according to a single-point method previously validated by Fournier and associates [9], who showed a significant correlation

between the area under the plasma concentration–time curve ($AUC_{0-\infty}$) of UF platinum and the platinum concentration measured the day after CP administration. In addition, we previously demonstrated the usefulness of this single-point method for predicting CP-related renal toxicity in a multivariate study including age, sex, initial creatinine clearance, cycle number and total CP dose [19]. The interest of single-point methods for platinum derivative pharmacokinetic evaluation was also recently strengthened in studies of carboplatin regimens [20]. Importantly, from the present univariate and multivariate analyses, measurement of UF platinum concentration the day after first cycle administration was the strongest predictor of anaemia occurring after two chemotherapy cycles (Table 3). Accordingly, UF platinum concentrations in patients developing SLH, defined as an Hb loss of equal to or more than 30 g/l, were significantly higher than those observed in patients who did not develop SLH (mean 53 ng/ml compared with 30 ng/ml, Fig. 3). Moreover, a discriminant analysis allowed the determination of a cut-off value for UF platinum for the identification of patients developing SLH: 91% of patients exhibiting a UF platinum concentration above 50 ng/ml developed SLH in contrast to 18% in the group of patients with a UF platinum concentration below 50 ng/ml (odds ratio equal to 46 (4.7–446)). This early and simple platinum pharmacokinetic control the day after first drug administration thus appears to be useful in targeting the group of patients who are likely to develop CP-related anaemia during treatment. It seems interesting to mention that one month of treatment with EPO costs approximatively 1500€ and one platinum pharmacokinetic control costs 15€.

One can wonder about the mechanisms responsible for the anaemia induced by this platinum overexposure. A greater suppression of the EPO response to anaemia could be advocated because of a greater exposure of the renal EPO-producing cells to the inhibiting effect of CP. However, a direct myelotoxic effect of CP is unlikely since leucopenia and thrombocytopenia are rare following CP treatment. Interestingly, CP has been shown to interact with glutathione (GSH) [21]. GSH and its related enzymatic system constitute an important cellular detoxification system [22], especially for RBCs. We previously investigated the *in vitro* biochemical effects of CP on RBC enzymatic pathways, by studying the effects of exposure duration and CP dose on GSH, oxidised GSH (GSSG), GSH peroxidase and GSH reductase [23]. This study revealed that GSH peroxidase was inhibited as a function of both exposure duration and CP dose. A similar inhibition was observed in GSH consumption and GSSG synthesis. Since CP is known to bind to GSH *in vitro* [24], the observed GSH peroxidase inhibition might be caused by an alteration of the enzyme–substrate interaction. Such enzymatic inhibition, making RBCs much more sensitive to oxidative stress, may partly explain the pathogenesis of CP-induced anaemia. The results of the present clinical study, demonstrating a role of elevated plasma UF platinum concentrations in treatment-induced anaemia, concur with the above *in vitro* investigations on RBCs [23].

In conclusion, a CP pharmacokinetic survey may be proposed as an approach in order to identify patients at risk of developing severe anaemia, and thus target the group of patients for whom prophylactic EPO administration may be cost-effective. We hope such a pharmacological strategy will be evaluated for other CP-based chemotherapy regimens.

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