EARLY BIOTRANSFORMATIONS OF OXALIPLATIN AFTER ITS INTRAVENOUS ADMINISTRATION TO CANCER PATIENTS

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ABSTRACT:
This article deals with the fate of oxaliplatin 1 and 3 h after its i.v. administration (130 mg/m²) to three patients. Its binding to plasma proteins and penetration into red blood cells were monitored by chromatography on-line with inductively coupled plasma mass spectrometry. Oxaliplatin biotransformations in plasma ultrafiltrate (PUF) and in urine were studied by chromatography coupled to inductively coupled plasma mass spectrometry or to electrospray ionization mass spectrometry. Oxaliplatin biotransformations in plasma ultrafiltrate were found. The peaks at 200 and 160 kDa corresponding to albumin contained 40% of the Pt found. The peak at 60 kDa corresponding to low-molecular weight species contained 40% of the Pt bound; the peak at 60 kDa corresponding to hemoglobin and the peak at 200 kDa corresponding to other Pt compounds, is present in plasma and urine and that Pt is bound to albumin, γ-globulins contained 40% of the Pt bound; the peak at 200 kDa corresponding to low-molecular weight species contained 40% of the Pt bound; the peak at 60 kDa corresponding to albumin contained 40% of the Pt found. The peak at 60 kDa could correspond to oxaliplatin, to its degradation products, or to adducts between Pt compounds and low-molecular weight species such as glutathione, L-methionine, and L-cysteine.

The three main platinum (Pt) compounds currently used for the treatment of patients with cancer are cisplatin, carboplatin, and oxaliplatin (OP). Most data concerning the pharmacokinetics of these drugs in patients have been obtained by determining total Pt in blood, plasma, and urine (Belt et al., 1979; Bastian, 1994; Duffull and Robinson, 1997; Gamelin et al., 1997, 1998) without discriminating between the drugs themselves, their metabolites, or their possible adducts. This is true particularly for oxaliplatin because only few works deal with its biotransformation in vitro and in vivo. Human studies have shown that within the first hours after oxaliplatin infusion, a large part of plasma oxaliplatin in plasma is bound to proteins and a significant amount is sequestered by red blood cells (RBCs) (Pendyala and Creaven, 1993; Gamelin et al., 1997, 1998). Pendyala and Creaven (1993), after addition of oxaliplatin to human plasma, detected oxaliplatin and did not observe the formation of oxaliplatin metabolites in PUF. According to Allen et al. (1999), within 2 h after infusion to patients, oxaliplatin was undetectable in PUF and in urine, but many biotransformation products, among them [Pt(dach)(OH)2]2+, were present. In contrast, according to Luo et al. (1999) after administration of oxaliplatin to rat, oxaliplatin itself, in addition to several Pt compounds, was found in PUF and in urine but not [Pt(dach)(OH)2]2+. These discrepancies concerning the presence of oxaliplatin itself and one of its degradation products, [Pt(dach)(OH)2]2+, perhaps linked to the different experimental conditions, particularly the animal species, led us to study the fate of oxaliplatin during the first hours after its i.v. administration to patients. We studied the binding of Pt to plasma proteins, penetration into RBCs, and formation of metabolites or adducts using hyphenated analytical methods such as gel chromatography or reversed phase liquid chromatography (RPLC) on-line with inductively coupled plasma mass spectrometry (ICPMS) and electrospray ionization mass spectrometry (LC-MS). The analysis was made on freshly collected samples because our preliminary studies showed that oxaliplatin was unstable in biological fluids even during storage at −20°C.

Materials and Methods

Treatment. Oxaliplatin as Eloxatine was administrated at a dose of 130 mg m−² in 500 ml of sterile 5% glucose as a 2-h infusion in association with an antiemetic drug, granisetron (Kytril, 3 mg), and a corticoid, methylprednisolone (Solumedrol, 120 mg). 5-Fluorouracil (5-FU) was administrated at a
dose of 1300 mg m\(^{-2}\) in 50 ml of 0.9% NaCl as a 4-h infusion after the end of oxaliplatin administration.

**Patients and Sampling.** The three patients, aged 75, 56, and 66 years, included in this study had histologically proven metastatic colorectal cancer requiring treatment by oxaliplatin and they gave their informed consent for sampling of blood and urine. Patient 1 had never been treated with oxaliplatin, unlike patients 2 and 3, who had already received oxaliplatin but whose last administration was at least 3 weeks earlier. Blood and urine samples were withdrawn just before oxaliplatin infusion, 1 and 3 h after the end of the infusion, times expected to be sufficient to observe early biotransformations and to eliminate a direct contamination from the infusion. Blood samples were collected into lithium-heparinized tubes and plasma was immediately separated by centrifugation at 4°C. The plasma was diluted in the mobile phase, and RBCs were hemolysated in deionized water and analyzed by gel chromatography-ICPMS. PUF was obtained by ultrafiltration of plasma using a 30-kDa molecular mass cut-off membrane (Centrífuge, Amicon Bioseparation Millipore, Beverly, MA), which was centrifuged at 1000 g at 4°C. At least 250 μl of ultrafiltrate was collected. PUF was directly analyzed by RPLC-ICPMS. Urine was filtered through a PTFE membrane disposable filter (Millipore Corporation, Bedford, MA) and diluted in the mobile phase for RPLC-ICPMS. PUF was obtained by ultrafiltration of plasma using a 30-kDa molecular mass cut-off membrane (Centrífuge, Amicon Bioseparation Millipore, Beverly, MA), which was centrifuged at 1000 g during 1 h at 4°C. At least 250 μl of ultrafiltrate was collected. PUF was directly analyzed by RPLC-ICPMS. Urine was filtered through a PTFE membrane disposable filter (Millipore Corporation, Bedford, MA) and diluted in the mobile phase for RPLC-ICPMS and LC-MS analysis.

**Chemicals.** Oxaliplatin and Pt(dach)Cl\(_2\) (DP) pure products were obtained from Debiopharm (Lausanne, Switzerland). All other chemicals were purchased from various commercial sources and used as received. Deionized water purified with a Milli-Q II Millipore system (Millipore Corporation) was used for the preparation of all solutions.

**Platinum Assay.** Total Pt in plasma, PUF, and urine was determined using a validated ICPMS method previously described in our laboratory (Allain et al., 1992) using an Elan 5000 Perkin-Elmer Sciex spectrometer (Toronto, Canada). The ICPMS parameters were those proposed by Perkin-Elmer and the Pt isotope monitored was \(^{195}\)Pt. 195.

**Gel Chromatography-UV/ICPMS.** The chromatographic system consisted of a pump model 307 equipped with an autosampler injector model 231, an injection loop of 100 μl (Gilson, Villiers le Bel, France), and a UV spectrophotometer (LDC/Milton Roy, Riviera Beach, FL). Separations of proteins with molecular masses in the range 10 to 600 kDa were performed on a Superdex 200 HR 10/30 column (Pharmacia Biotech, Uppsala, Sweden). An aqueous mobile phase containing 50 mmol l\(^{-1}\) NaH\(_2\)PO\(_4\) and 150 mmol l\(^{-1}\) NaCl was pumped through the system at 0.4 ml min\(^{-1}\). Eluent from the column was monitored by UV at 280 nm and/or with ICPMS system for the specific detection of Pt. The column was calibrated by injection of proteins with known masses (catalase, 232 kDa; ceruloplasmin, 151 kDa; albumin, 67 kDa; and cytochrome c, 12.4 kDa). Blue Dextran was used to assess the void volume.

**RPLC-ICPMS.** The LC system consisted of a quaternary pump model 4000 LC (Spectra Physics, Les Ulis, France) equipped with an autosampler injector model 231 and an injection loop of 20 μl. Separations were performed on a reversed phase column 150 × 4.6 mm, 5-μm particle size (Hichrom-CIL, Sainte Foy La Grande, France).

An aqueous mobile phase containing 15 mmol l\(^{-1}\) HCOOH in 90:10 H\(_2\)O/MeOH was used at a flow rate of 1 ml min\(^{-1}\).

**LC-MS.** The LC-MS system consisted of a triple quadrupole mass-spectrometer API 300 (Perkin-Elmer-Sciex, Toronto, Canada) equipped with a sample processor ISS 200 and an HPLC pump model 200 (Perkin-Elmer, Norwalk, CT). Ionization was operated in a positive mode at an ionspray voltage of 5200 V and an orifice voltage of 10 V. The column and the mobile phase used were the same as those adopted for RPLC-ICPMS.

### Results

**Partitioning of Platinum.** The samples taken before oxaliplatin infusion did not contain platinum in patient 1, who never received the drug; in the two others patients, who received oxaliplatin 3 weeks ago, only low amounts of total Pt were found. Table 1 shows the concentration of total Pt in plasma, PUF, and urine of the three patients. In

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pt in Plasma</th>
<th>Pt in PUF</th>
<th>Pt in Urine</th>
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</thead>
<tbody>
<tr>
<td>1 h after the end of infusion</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>2.217</td>
<td>304</td>
<td>19,777</td>
</tr>
<tr>
<td>2</td>
<td>1.975</td>
<td>184</td>
<td>23,370</td>
</tr>
<tr>
<td>3</td>
<td>1.855</td>
<td>246</td>
<td>16,773</td>
</tr>
<tr>
<td>3 h after the end of infusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.203</td>
<td>234</td>
<td>6,572</td>
</tr>
<tr>
<td>2</td>
<td>1.876</td>
<td>120</td>
<td>10,614</td>
</tr>
<tr>
<td>3</td>
<td>1.744</td>
<td>189</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA, not available.
plasma, 1 and 3 h after the end of oxaliplatin infusion, the concentration of Pt was around 2000 μg l⁻¹ for the three patients. The concentrations of Pt in PUF 1 h after the end of infusion were 304, 184, and 246 μg l⁻¹ for the three patients, respectively. Three hours after the end of infusion, the concentrations of Pt in PUF of the three patients were 234, 120, and 189 μg l⁻¹, respectively, were slightly lower than those obtained 1 h after the end of infusion. In urine, 1 h after the end of infusion, the concentrations of Pt for the three patients were 19,777, 23,370, and 16,773 μg l⁻¹, respectively, and 3 h after the end of infusion 6,572 and 10,614 μg l⁻¹ for patients 1 and 2.

**Binding of Platinum to Plasma Proteins.** The gel chromatography-ICPMS chromatograms of plasma of the three patients display, 1 and 3 h after the end of infusion, at least four Pt peaks at apparent masses of 200, 160, 60, and 2 kDa (Fig. 1). The peaks at 200 and 160 kDa, corresponding to γ-globulins, contained approximately 40% of the Pt bound; the peak at 60 kDa had the same retention time as albumin and contained about 40% of the Pt bound. The Pt species at low molecular masses, <2 kDa, represented about 15% of Pt found and could correspond to oxaliplatin itself, its degradation products, and to the formation of adducts between these Pt compounds and molecules with low molecular weight such as glutathione, l-methionine, and l-cysteine.

**Metabolic Profile of Oxaliplatin in PUF.** Figure 2 shows the RPLC-ICPMS chromatogram of a solution of oxaliplatin, 200 μg l⁻¹, diluted in 0.9% NaCl solution after 3 h of incubation at 37°C. In addition to oxaliplatin two degradation products, Pt(dach)Cl₂ and [Pt(dach)(OH₂)Cl]⁺ (MP), were found. The structure of these compounds was confirmed by LC-MS.

The RPLC-ICPMS chromatograms of PUF of the three patients obtained 1 h after the end of infusion show at least five Pt peaks: oxaliplatin, Pt(dach)Cl₂, [Pt(dach)(OH₂)Cl]⁺, and two other Pt peaks (P1) and (P2) (Fig. 3). The concentration of oxaliplatin itself represented less than 12% of the total Pt. Three hours after the end of infusion, a decrease of oxaliplatin concentration was observed for the three patients (Fig. 4). P1 has the same retention time as the methionine-Pt adducts: Pt(dach)(met), which has already been characterized by LC-MS in a previous study (Heudi et al., 1999) but the amount was not sufficient for direct identification. P2 was not identified.

**Metabolic Profile of Oxaliplatin in Urine.** The RPLC-ICPMS chromatograms of urine of three patients obtained 1 and 3 h after the end of oxaliplatin infusion show at least six Pt peaks: OP, DP, MP, and three other Pt peaks, P1, P3, and P4 (Figs. 5 and 6). The comparison of the two RPLC-ICPMS chromatograms shows that the peaks of oxaliplatin, Pt(dach)Cl₂, and [Pt(dach)(OH₂)Cl]⁺ decreased between 1 and 3 h after the end of infusion, while the one of P4 concomitantly increased. The presence of oxaliplatin in urine was confirmed by LC-MS, showing a pseudomolecular cluster ion [MH]⁺ at m/z 397 to 401 and an additional cluster ion at m/z 419 to 423 corresponding to [M+Na]⁺ (Fig. 7). The concentration of oxaliplatin in urine 1 h after the end of infusion represented about 50% of total Pt, whereas 3 h after the end of infusion, it accounted for approximately 10%. The structure of P3 was not identified, whereas P4 has the same retention time as a glutathione-Pt adduct. The structure of
this species could correspond to a bis-adduct of Pt and glutathione, 
[Pt(dach)]_2 glutathione, which had been characterized by LC-MS.

**Binding of Platinum to Proteins in RBCs.** It has been previously
demonstrated that after oxaliplatin administration, Pt accumulates in
RBCs (Gamelin et al., 1997). The gel chromatography-ICPMS chromato-
grams of RBCs hemolysates of the three patients, 1 and 3 h after the end
of infusion, were similar and only those obtained at 1 h are shown (Fig.
8). They display at least two Pt peaks at apparent masses of 60 and <2
kDa. The Pt peak at 60 kDa, corresponding to hemoglobin, contained
approximately 50% of Pt bound and the Pt peak at <2 kDa, which may
be attributed to the formation of adducts between platinum and low-
molecular-weight molecules, contained about 40% of Pt found.

**Discussion**

The pharmacokinetic studies of Pt drugs have been largely based
upon the determination of total Pt content in blood and urine (Belt et
al., 1979; Bastian, 1994; Duffull and Robinson, 1997; Gamelin et al., 1997, 1998). The method usually used for the quantification of Pt was
atomic absorption spectrometry, but now it is clear that ICPMS is a
much more sensitive method (Allain et al., 1992) that moreover can be easily coupled on-line to liquid chromatography systems. Coupling gel-chromatography to ICPMS allows the direct analysis of plasma and the identification of proteins to which Pt is bound. Coupling a reversed phase column to ICPMS permits to separate platinum drugs and to detect their possible metabolites. LC-MS brings complementary information about the structure of the products separated by chromatography. The combination of these techniques allowed us to precisely detect the early metabolism of oxaliplatin in patients.

This study demonstrates directly that in plasma of patients after oxaliplatin infusion Pt is bound to albumin and γ-globulins, confirming the in vitro data of Urien and Tillement (1995). The peak at <2 kDa containing about 15% of Pt found could correspond to oxaliplatin, Pt(dach)Cl₂, or [Pt(dach)(OH₂)Cl]⁻ and their adducts with nu-

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**Fig. 6.** RPLC-ICPMS chromatogram of urine of patients 1 and 2 obtained 3 h after the end of infusion of oxaliplatin showing at least five Pt peaks: oxaliplatin, Pt(dach)Cl₂, P1, P3, and P4.

P1 has the same retention time as the methionine-Pt adduct Pt(dach)(met), and P4 has the same retention time as the glutathione-Pt adduct [Pt(dach)]₂(glutathione). P3 was not characterized and peak 1 is a platinum impurity.

**Fig. 7.** LC-MS of urine of patient 2 obtained 3 h after the end of infusion of oxaliplatin.

The pseudomolecular cluster ion [MH]⁺ at m/z 397 to 401 corresponds to oxaliplatin and an additional cluster ion at m/z 419 to 423 corresponds to [M+Na]⁺.

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**Fig. 8.** Gel chromatography-ICPMS chromatogram of RBCs hemolysates obtained at 1 h after the end of infusion of oxaliplatin of three patients treated with a single i.v. dose of drug.

The RBCs hemolysate was diluted 10-fold with the mobile phase before injection. Two Pt peaks appear at apparent molecular masses of 60 and <2 kDa.
cleophiles species such as L-methionine, L-cysteine, or glutathione. The metabolic profiles of oxaliplatin in PUF and urine after its administration to patients show that oxaliplatin itself is present in PUF and at higher concentration in urine, as confirmed by LC-MS. However, 3 h after the end of infusion, oxaliplatin was only detected in urine and no longer in PUF. In the present study we have used fresh samples because the conditions of storage of samples influence the results. In fact, we found that oxaliplatin in PUF and in a solution containing chloride, even frozen, was not stable and moreover that changes in the binding of platinum to plasma proteins can occur during storage at −20°C (Gamelin et al., 1998). The two degradation products of oxaliplatin found in PUF and urine of patients, 1 and 3 h after the end of infusion, were Pt(dach)Cl₂ and [Pt(dach)(OH₂)Cl]⁺. The formation of these species is accompanied by the release of oxalate anion whose concentration in urine increased after oxaliplatin administration (data not shown). Concerning the presence of oxaliplatin in PUF and in urine, a few hours after its administration, our results are different from those of Allen et al. (1999), but consistent with those of Luo et al. (1999) and Pendyala and Creaven (1993). Concerning the presence of metabolites, our results are in good agreement with those of Luo et al. (1999) and Allen et al. (1999), except that these latter authors found [Pt(dach)(OH₂)Cl]⁺, which was neither observed by Luo et al. (1999) nor by us. Our results contrast with the in vitro data of Pendyala and Creaven (1993), who did not find oxaliplatin metabolites in PUF, but the analytical method they used for monitoring the biotransformation seems not sensitive enough for detecting the metabolites of oxaliplatin. In PUF and urine of the three patients, we found the methionine-platinum adduct Pt-(dach)(met), the structure of which has been already described in a previous study (Heudi et al., 1999). Moreover in urine, an adduct having the same retention as [Pt(dach)Cl₂(glutathione)]⁺ was found. These data show that L-methionine and glutathione may play an important role in the metabolism of oxaliplatin. The Pt adducts found in PUF and urine after oxaliplatin infusion can result from the interactions of oxaliplatin itself or its degradation products Pt(dach)Cl₂ and [Pt(dach)(OH₂)Cl]⁺ with nucleophile species such as L-methionine, L-cysteine, or glutathione.

In this study, the standard protocol of drugs administration, i.e., oxaliplatin infusion immediately followed by 5-FU infusion, was not modified, and an interaction between oxaliplatin and 5-FU could be suspected. However, we have studied in vitro the reaction of oxaliplatin and 5-FU and characterized one adduct only after 24 h of incubation. In PUF and urine of patients, we did not find a Pt adduct having the same retention time as the one identified in vitro. Therefore, it is unlikely that oxaliplatin and 5-FU reacted in vivo. However, we have not tested the possible interactions between 5-FU metabolites and oxaliplatin or its degradation products.

We have shown that oxaliplatin when administrated to patient is accumulated into RBCs (Gamelin et al., 1997); however, the molecules involved in the binding of Pt had not yet been identified. The present results clearly demonstrate that inside RBCs, Pt is mainly bound to the peak of about 60 kDa, corresponding to hemoglobin because it also contained a high quantity of iron and zinc. The fact that hemoglobin is the protein that binds Pt may seem obvious; nevertheless, it had to be demonstrated. For example, it has been admitted for many years that lead was bound to hemoglobin (Simons, 1986) until Bergdahl and Schütz (1996) demonstrated that, inside RBCs, lead was not bound to hemoglobin but to the enzyme 6-aminolevulinic acid dehydratase.

In conclusion, during the first hours after oxaliplatin administration to patients, in plasma, Pt is bound to albumin and to γ-globulins and in RBCs to hemoglobin and also to species of low molecular weight. In addition, oxaliplatin itself together with other Pt compounds such as [Pt(dach)(OH₂)Cl]⁺, Pt(dach)Cl₂, Pt(dach)(met), and [Pt(dach)Cl₂(glutathione)]⁺ were found in PUF or in urine.

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References


