SHORT COMMUNICATION

Cisplatin and platinum pharmacokinetics during hyperthermic isolated limb perfusion for human tumours of the extremities

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Isolated limb perfusion (ILP) with chemotherapeutic agents was first introduced by Creech et al. in 1958. Stehlin (1969) added hyperthermia to this technique and made hyperthermic isolated limb perfusion (HILP) an interesting method in the treatment of malignancies of the extremities.

Advantages of ILP are the fact that the tumour is locally treated with high concentrations of the chemotherapeutic drug, whereas only low drug concentrations are reached in the systemic circulation. This is attained by minor or no systemic side-effects. One case has been described in which ILP was performed because of a contra-indicative renal impairment in the treatment with cisplatin (CDDP) (Roseman et al., 1985). Beneficial effects of ILP are anticipated in the treatment of poor vascularised tumours and with drugs having a high total body clearance. The application of ILP is limited to drugs which do not need metabolic activation. The use of an extracorporeal circulation allows hyperthermic treatment. With CDDP this can be of advantage as hyperthermia may enhance its cytotoxicity (Fisher & Hahn, 1982; Hahn, 1979; Herman, 1983; Wallner et al., 1986; Alberts et al., 1980; Meyn et al., 1980). Enhanced blood flow, due to vasodilation (Hahn, 1979; Song et al., 1980), enhanced cellular drug uptake (Herman, 1983; Alberts et al., 1980), tissue extraction (Riviere et al., 1986), DNA cross-linking (Meyn et al., 1980; Herman et al., 1989) and decreased DNA repair (Wallner et al., 1986) are postulated to explain the phenomenon of hyperthermic potentiation. Some selectivity of treatment might hereby be introduced, because malignant cells have shown to be more sensitive to heat than normal cells (Herman, 1983; Herman et al., 1989; Giovanna et al., 1973; Giovanna et al., 1976; Kase & Hahn, 1975; Cavaliere et al., 1967). ILP with chemotherapeutic agents can allow limb saving procedures (Hoekstra et al., 1987; Roseman, 1987).

The technique is improved during the last decade. More physiological perfusions are performed taking into account such variables as the patients blood pressure and perfusion pressure (Fontjne et al., 1985, Van Os et al., 1985; De Vries et al., 1988; Van Os et al., 1989).

Although several studies on HILP with CDDP were reported in the recent years, little is known about the pharmacokinetics of the drug in these circumstances.

In this study the pharmacokinetics of total platinum (Pt), ultrafiltrated platinum (Pt) and CDDP in the perfusate are presented during HILP for human tumours of the limb. Nine patients, six with intrathax metastases of melanoma, one metastasised extremity osteosarcoma of the femur and two with recurrent malignant fibrous histiocytoma (one of the soft tissues and one of the femur) were treated with HILP with CDDP. None of the patients had received systemic CDDP previously. The patients with recurrent melanoma of the lower extremity were treated previously with one or two HILP treatments with melphanal or melphanal and dacarbazine. Patients characteristics are summarised in Table I. The study was approved by the local medical ethical committee of the University Hospital Groningen. All patients gave informed consent.

Prior to the perfusion treatment patients received systemic i.v. hydration with 2–3 l normal saline in 24 h to prevent relative hypovolemia during surgery. During the perfusion diuresis was monitored to assure diuresis over 50 ml h⁻¹ in all patients. All limb perfusions were performed as described before (Hoekstra et al., 1987). The limb was perfused with 350 ml 5% dextan 40 in glucose 5% (Isodex, Pharmacia AB, Sweden), 250 ml plasma, 250 ml red blood cells, 30 ml 8.4% NaHCO₃, 0.5 ml 5000 IU ml⁻¹ heparin (Thromboliquine, Organon B.V., Oss, The Netherlands) and 200 to 800 ml CDDP 0.5 mg ml⁻¹ (depending on dose) (Platinol, Bristol Myers SAE, Spain) at subcutaneous and muscle temperatures of 39–40°C with the aid of a pump oxygenator.

CDDP dose, administered as a part of a phase I dose finding study, was 20 mg l⁻¹ extremity volume in four patients, 25 mg l⁻¹ in three patients and 30 mg ml⁻¹ in two patients. Limb toxicity was scored according to Wieberdink et al. (1982). CDDP was added to the circulating perfusate in 10 min. At the end of the CDDP infusion, the first 10 ml perfusate sample was collected (t = 0 min) in a heparin coated glass tube. Additional samples were collected at 10 min intervals (t = 10, 20, 30, 40, 50 and 60 min) and transported on ice immediately. Samples were centrifuged for 10 min at 2000 g and the cell fraction was removed; in the supernatant tPt was measured. For determination of tPt concentrations, 1 ml supernatant samples were ultrafiltrated (2000 g; 60 min) with Amicon Centifree microparticulation systems provided with YMT membranes (Amicon, Oosterhout, The Netherlands). Tissue biopsies were taken for tPt determination in six patients at the end of the HILP, when the normal limb circulation was restored. At the end of the procedure (t = 60 + min) the limb was washed out with 1000 ml Isodex (5% dextan 40 in dextrose 5%) and with 250 ml plasma and 250 ml red blood cells respectively. Thereafter, the systemic circulation was restored.

Leakage, from the perfused limb to the patients circulation was determined during the perfusion with ¹⁵³I-albumin and ⁹⁹mTc-albumin. A small dose (about 10 μCi) of ¹⁵³I-albumin is injected in the body circuit and exactly ten times the small dose is injected in the external circulation. The ⁹⁹mTc-albumin is injected in the body circulation only; its activity is recorded for the detection of dilution by infusion and shift of the detector sensitivity by displacement of the detector. A scintillation counter, placed over the heart, detects the amount of ⁹⁹mTc- and ¹⁵³I-labelled albumin in the body circulation. In the two patients with popliteal perfusion leakage was not monitored with isotopes. Leakage was also determined by measuring patients tPt plasma concentration at the
Concentrations $tPt$ and $fPt$ in the perfusate were determined by flameless atomic absorption spectrophotometry (FAAS). Absorption was measured at 265.9 nm with a spectral bandwidth of 0.5 nm and deuterium background signal correction. Perfusion $tPt$ samples were diluted with three volumes 23-laurylthioheptane 0.3% w/v (Brij 35 Solution, Sigma Chemicals Company, St Louis, USA) and were analysed without further pretreatment. The method has a detection limit of 0.1 mg Pt$^{-1}$ and a standard deviation of 7.7% at a concentration of 12 mg Pt$^{-1}$. Tissue $tPt$ concentrations were determined with FAAS using standard addition method with Pt chloride. Tissue was weighed and dissolved in 65% nitric acid under heating to 80–90°C and diluted to 2.5 ml with demineralised water. Each sample was measured in duplicate. Plasma $tPt$ concentrations were measured by FAAS after diluting the 100 μl sample with 300 μl Brij 35. CDDP concentrations were measured by HPLC, equipped with an anion exchange Nucleosil 5 SS column (Bouma & Uges, 1980), and UV detection (230 nm). Eluens consisted of methanol:sodium acetate 0.1 M (65:35 v/v%) with sufficient acetic acid to pH = 5.0. A standard solution of 20.0 mg CDDP in 1.0 l NaCl 0.9% was used. Ultrafiltrate perfusate samples of 20 μl were directly injected in the HPLC column. This method has a detection limit of 0.3 mg$^{-1}$ and an intra assay standard deviation of 1.9% at a concentration of 20.0 mg CDDP$^{-1}$. The calibration curve (0–20 mg CDDP$^{-1}$) was found linear $(r = 0.999)$; the inter assay standard deviation was 2.9%. Each sample was measured in duplicate.

For $tPt$, $fPt$ and CDDP perfusate elimination kinetics, data were subjected to logarithmic regression analysis (concentration = A.e$^{kt}$). The areas under the concentration vs time curves (AUC) were calculated using the model independent trapezoidal rule (Rowland & Tozer, 1980) and covers the perfusion period $(t = 0–60$ min). Data were analysed by the two sided Student’s t-test. Only P-values <0.05 were considered significant.

The local grade of toxicity is summarised in Table II. The grade of toxicity was not correlated to the dosage (in mg$^{-1}$ limb volume) but to the total amount of delivered CDDP, AUC of $tPt$ and Cmax. Limb toxicity consisted of a moderate to severe limb oedema and a motor-sensory neuropathy, documented with electro-myography in five patients. Electromyography showed denervation potentials with disturbances in the motor and sensory conduction velocities of the peroneal and sural nerve of the affected limb.

In all patients $tPt$ concentrations were determined, whereas $fPt$ and CDDP concentrations were determined only in patients 7–9. The data from patients 8 and 9 should be considered separately, as patient 8 had a much smaller limb volume (due to popliteal perfusion) compared to patient 9. After 60 min perfusion a decrease of 54.3 ± 12.4% $(n = 9), 79.9 ± 14.7% (n = 3)$ and $79.2 ± 4.2% (n = 3)$ (mean ± s.d.) of concentrations of $tPt$, $fPt$ and CDDP in the perfusate was observed, indicating considerable extraction. The perfusate concentrations versus time show good linear correlation $(r = 0.95 ± 0.07$) when represented semi-logarithmically and therefore first order kinetics was assumed $(C = A.e^{kt})$. Pharmacokinetic parameters of all patients are summarised in Table II. The mean $t$ for $tPt$ (51.2 ± 13.0 min) was found to be higher $(P < 0.005)$ than for $tPt$ and CDDP (28.3 ± 11.7 min and 27.3 ± 3.2 min, respectively), whereas the latter two are not significantly different. The AUCs, representing tissue drug exposure, are also depicted in Table II. Systemic plasma concentrations of $tPt$, determined at the end of the perfusion, before restoration of the normal circulation were found to be relatively low $(<0.5–21.4 \mu M)$. The mean leakage of the limb to the patients circulation as determined with radioactive albumin was 3.3% (range 0–9.5%). During 7 days after the perfusion, systemic plasma $tPt$ concentrations of patient 8 (popliteal perfusion) were $<0.5 \mu M$ and of patient 7 (iliacal perfusion), it dropped from 12.4 to 3.5–4.4 μM.

Figure 1 shows the mean fraction $fPt$: $tPt$ during the perfusion. It is decreased to 40 ± 8% $(n = 3)$ after 60 min perfusion $(P < 0.01)$. The mean fraction $fPt$ was 71 ± 22% during the entire perfusion.

Concentrations in tissue biopsies taken from the tumour and surrounding tissues are summarised in Table III. A wide variation in concentrations within patients and tissues is observed, but especially high $tPt$ concentrations are found in the skin.

Using the normal i.v. route of CDDP administration therapeutic $tPt$ concentrations of 0.5–5 mg$^{-1}$ (2.5–25 μM) are reached during the first 48 h after bolus dose or during 5 days of continuous infusion (Guillo et al., 1980; Gormley et al., 1979; Vermarkten et al., 1982; Bues-Charbit, et al., 1987). This study shows that much higher concentrations can be reached with HILP. During the whole perfusion period high $tPt$ concentrations can be reached which would be unacceptably toxic at systemic use. Despite the same dosage applied per liter limb volume, we found much smaller perfusate drug concentrations in patient 8 compared to patient 9. This is attributable to the small limb volume in patient 8 with a popliteal perfusion. In these circumstances there is a greater relative dilution of CDDP in the perfusate. One should take this into account when extremely large or small limbs are perfused. This finding underscores the relevance of the assessment of the actual exchangeable blood volume as described by Lejeune et al. (Lejeune & Ghanem, 1987) and thus calculating the drug dose necessary to get the desired drug concentrations in isolated perfusion. Furthermore, this fact makes the correlation of toxicity and pharmacokinetic parameters in our study difficult. In the past the dosage of chemotherapeutics used in ILP was based on body weight. Since the studies of Weiberdink and Fontijn, today calculation of dosage is based on the volume of the perfused limb. However, for pharmacokinetic purposes dose calculation according to Lejeune should be considered preferable as in our study CDDP toxicity was correlated to the total dose delivered instead of to the dose per liter limb volume. From our toxicity data we conclude that the total dose of CDDP should not exceed 275 mg. This is in accordance to the

### Table I Patient characteristics

| Patient | Age (years) | Tumour          | Limb volume CDDP dose (mg $^{-1}$) | CDDP dose (mg) | Location of perfusion |
|---------|------------|-----------------|-----------------------------------|----------------|-----------------------|
| 1       | 21         | Osteosarcoma    | 13.0                              | 20             | 260                   | Iliacal              |
| 2       | 66         | Melanoma        | 7.5                               | 20             | 150                   | Popliteal            |
| 3       | 56         | Melanoma        | 8.8                               | 20             | 180                   | Iliacal              |
| 4       | 66         | Melanoma        | 8.0                               | 20             | 160                   | Femoral              |
| 5       | 70         | Fibrous histio-cytoma (bone) | 11.0   | 25             | 275                   | Iliacal              |
| 6       | 56         | Melanoma        | 15.6                              | 25             | 390                   | Femoral              |
| 7       | 72         | Melanoma        | 12.0                              | 25             | 300                   | Iliacal              |
| 8       | 58         | Fibrous histio-cytoma (soft tissue) | 3.3     | 30             | 100                   | Popliteal            |
| 9       | 59         | Melanoma        | 13.0                              | 30             | 400                   | Iliacal              |
Table II Pharmacokinetic parameters of tPt in the perfusate and of fPt and CDDP in ultrafiltrated perfusate during 60 min HILP with 20, 25 or 30 mg CDDP l⁻¹ extremity, assuming first order kinetics, leakage data and toxicity

| Patient | Δt (min) | Cmax (µM) | C₁-90 (µM) | AUC (µM.min) | Systemic tPt concentration (µM) | Maximal leakage radiolabelled albumin (%) | Local toxicity grade |
|---------|----------|-----------|------------|--------------|-------------------------------|------------------------------------------|---------------------|
| **Total Pt** | | | | | | | |
| 1 | 65 | 249 | 174 | 11035 | 13.3 | 4.2 | III |
| 2 | 41 | 287 | 113 | 9480 | 3.6 | nd | III |
| 3 | 42 | 318 | 123 | 12095 | 6.1 | 0.0 | III |
| 4 | 54 | 286 | 128 | 12020 | <0.5 | 3.0 | III |
| 5 | 53 | 349 | 164 | 13485 | 6.6 | 2.1 | III |
| 6 | 67 | 507 | 272 | 25065 | <0.5 | 0.0 | IV |
| 7 | 37 | 410 | 145 | 15255 | 21.4 | 9.5 | IV |
| 8 | 37 | 386 | 107 | 10195 | <0.5 | nd | II |
| 9 | 69 | 594 | 318 | 26190 | 1.0 | 4.2 | IV |
| **Ultrafilterable Pt** | | | | | | | |
| 7 | 18 | 525 | 10518 | | | | |
| 8 | 26 | 338 | 7085 | | | | |
| 9 | 41 | 570 | 21870 | | | | |
| **Ultrafilterable CDDP** | | | | | | | |
| 7 | 25 | 246 | 7121 | | | | |
| 8 | 26 | 260 | 5530 | | | | |
| 9 | 31 | 309 | 10195 | | | | |

Toxicity (according to Wieberdink) grade I: no subjective or objective evidence of reaction, grade II: slight erythema and/or oedema, grade III: considerable erythema and/or oedema with some blistering; slightly disturbed motility permissible, grade IV: extensive epidermolysis and/or obvious damage to the deep tissues, causing definite functional disturbances, threatening or manifest compartmental syndromes, grade V: reaction which may necessitate amputation. nd = not determined, in patients with popliteal perfusion.

Figure 1 Mean fraction fPt:tPt in the perfusate during 60 min HILP.

findings of Coit et al. (1991) and Di Filippo et al. (1989) who defined a maximum tolerated dose of 150 mg CDDP m⁻² and 3.2 mg CDDP kg⁻¹ body weight, respectively.

Our albumin leakage data and systemic tPt concentrations show that HILP was performed with relatively low leakage to the patients systemic circulation. However, higher than neglectable systemic tPt concentrations were found in some patients. Because of the high doses applied at HILP, hydration of the patients remains necessary to avoid systemic toxicity. No systemic side-effects, but temporary or definitive local motoric and sensoric symptoms in the perfused limb were observed.

Others found leakage of 2.5% leading to systemic tPt concentrations of 2.6–3.1 µM Pt during 60 min perfusion and 3.6–5.1 µM Pt during 5 postoperative days (Di Filippo et al., 1989). Pommier et al. (1988) found a mean systemic tPt level of 2.5 µM Pt at 5 min after the start of the perfusion and 3.9 µM Pt after 60 min perfusion in patients with malignant melanoma. They monitored leakage only by measuring systemic tPt concentrations.

The mean Δt for fPt and CDDP were found to be smaller than for tPt. This is probably due to the fact that the free Pt species leave the vascular compartment and cross plasma membranes more readily compared to protein-bound species. A very high fraction fPt was found. At normal i.v. adminis-

Table III Tissue drug concentrations (nmol tPt g⁻¹ tissue) in tumour and adjacent tissue after 60 min HILP

| Patient | Skin | Fat | Muscle | Tumour | Concentration tPt in perfusate at t = 60 min (µM) |
|---------|------|-----|--------|--------|---------------------------------|
| 1 | 97.4 | nd | 20.5 | 87.1 | 174 |
| 2 | 66.6 | nd | 23.6 | 116.4 | 113 |
| 3 | 113.8 | nd | 1.0 | 32.3 | 123 |
| 4 | nd | nd | nd | 128 | |
| 5 | 179.4 | 133.3 | 174.3 | 21.0 | 164 |
| 6 | 109.2* | 13.3* | 22.6* | 15.9* | |
| 7 | nd | nd | nd | 272 | |
| 8 | 116.4 | nd | 21.0 | 74.3 | 145 |
| 9 | 106.1 | nd | 78.4 | 98.9 | 107 |
| 9 | nd | nd | nd | 57.9* | 318 |

*14 days post perfusion. **8 days post perfusion. nd: not determined.
tration of CDDP the fraction Pt is about 0.05–0.10 (Verma-ken et al., 1982; Bues-Charrub et al., 1987; Dominici et al., 1989; Forastiere et al., 1988). The four- to twenty-fold increase of the free fraction in this study is most probably attributable to the low protein content of the perfusate. This is an advantage as free drug concentrations are more closely related to drug activity and thus probably anti-tumour effect. At the start of the perfusion all Pt was unbound whereas a gradual increase of protein binding was observed during perfusion time. This may be explained by the fact that the parent compound (CDDP) itself does not bind to protein whereas its hydrolytic products do (LeRoy et al., 1979). These products are formed in aqueous solutions with a half-life of 6–8 h at 25°C and probably even faster at temperatures applied at HILP (LeRoy et al., 1979).

In literature data about tissue Pt exposure at HILP are scarce. Di Filippo et al. (1989) found about the same AUCs for tPt after approximately the same dosage CDDP at 60 min HILP as reported here, however, data on intact CDDP and elimination rate constants are absent in their study.

Although perfusate concentrations varied minimally among individuals, tissue concentrations in normal as well as malignant tissue were shown to vary in a wide range. Di Filippo et al. (1989) have found similar results. Bielack et al. (1989), recently found a large intra-tumour variation of Pt distribution in patients with osteosarcoma treated with intra-arterial or i.v. infusion of CDDP. Some variation might be explained by differences in tissue water content, as samples were not dried before tPt determination. The high Pt accumulation in skin supports the application of HILP in melanoma. It might be more realistic to express tissue concentrations per g protein or DNA content instead of per tissue weight or to determine the amount of Pt-DNA adducts formed (Terheggen et al., 1988).

In conclusion, with HILP substantial drug extraction occurs, with relatively low leakage to the patients systemic circulation. With the described method it is possible to reach a considerable higher fraction of free drug compared to normal i.v. CDDP administration without systemic toxicity. ILP offers the opportunity to modify the perfusate composition such that it favourably influences CDDP kinetics.

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