Phase I Clinical and Pharmacokinetic Study of Titanocene Dichloride in Adults with Advanced Solid Tumors

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ABSTRACT

This Phase I dose-escalation clinical trial of a lyophilized formulation of titanocene dichloride (MKT4) was conducted to determine the maximum tolerated dose, the dose-limiting toxicity (DLT), and pharmacokinetics of titanium (Ti) after a single i.v. infusion of MKT4. Forty patients with refractory solid malignancies were treated with a total of 78 courses. Using a modified Fibonacci scheme, 15 mg/m2 initial doses of titanocene dichloride were increased in cohorts of three patients up to level 11 (560 mg/m2) if DLT was not observed. The maximum tolerated dose was 315 mg/m2, and nephrotoxicity was DLT. Two minor responses (bladder carcinoma and non-small cell lung cancer) were observed.

The pharmacokinetics of plasma Ti were assessed in 14 treatment courses by atomic absorption spectroscopy. The ratio for the area under the curve in plasma and whole blood was 1.2. The following pharmacokinetic parameters were determined for plasma, as calculated in a two-compartment model: biological half-life \( t_{1/2p} \) in plasma was 22.8 ± 11.2 h (\( x_a \pm \text{pseudo-SD} \)), peak plasma concentration \( c_{\text{max}} \) ~30 \( \mu \text{g/ml} \) at a dose of 420 mg/m2, distribution volume \( V_u = 5.34 \pm 2.1 \text{ L} \) (\( x_u \pm \text{SD} \)), and a total clearance \( Cl_{\text{total}} = 2.58 \pm 1.23 \text{ ml/min} \) (\( x_a \pm \text{SD} \)). There was a linear correlation between the area under the curve of Ti in plasma and the titanocene dichloride dose administered with a correlation coefficient \( r^2 \) of 0.8856. Plasma protein binding of Ti was in the 70–80% range. Between 3% and 16% of the total amount of Ti administered were renally excreted during the first 36 h.

The recommended dose for Phase II evaluation is 240 mg/m2 given every 3 weeks with i.v. hydration to reduce renal toxicity.

INTRODUCTION

Since the introduction of the metal complex cisplatin as one of the most active and broad spectrum drugs available to treat epithelial malignancies, efforts have been undertaken to identify new organometallic compounds with antiproliferative properties and better overall tolerability. In the group of transition metal compounds, numerous complexes were found to exhibit strong antitumor activity (1, 2). These agents contain a transition metal as their central atom, bound to two planar cyclopentadienyl rings and two cis-bound ligands, mostly halides (Fig. 1). In this class of complexes, vanadocene dichloride was the most active agent against different tumor cell lines in vitro (1, 2).

Titanocene dichloride has been shown to possess antitumor activity in doxorubicin- and cisplatin-resistant human ovarian carcinoma cell lines in vitro without cross-resistance (3–5). In vivo, titanocene dichloride induced the most pronounced growth inhibition of experimental ascites tumors (e.g., Ehrlich ascites tumor, sarcoma 180) and solid animal tumors (e.g., B16 melanoma, colon 38 carcinoma, Lewis lung carcinoma; Refs. 1 and 2). In xenograft models of human cancer cell lines in athymic nude mice, titanocene dichloride affected a >50% growth suppression (1–3, 6). The biological activity of titanocene dichloride was superior to that achieved with equitoxic doses of cisplatin, 5-fluorouracil, and cyclophosphamide (1–3, 7).

Hepatotoxicity was the DLT2 after a single i.p. injection of titanocene dichloride at doses of 40 mg/kg (ED90) and 60 mg/kg (LD10) in athymic mice. There were also pronounced elevations in the serum concentration of cortisol and glucagon. In contrast to cisplatin, titanocene dichloride did not cause nephrotoxicity after i.p. injection (8). However, i.v. bolus administration of sublethal doses led to nephrotoxicity in addition to hepatotoxic effects (9). Myelotoxicity was very mild with a slight and transient decrease of the platelet counts 8 days after administration, whereas the numbers of leukocytes and erythrocytes were apparently not influenced (10). The evaluation of the subchronic toxicity of titanocene dichloride given i.v. to rats in doses of 1.5, 3.0, and 6.0 mg/kg/day for 4 weeks revealed the following effects: venous wall irritation; weight loss; decreased reticulocyte count; elevated serum bilirubin and cholesterin;

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2 The abbreviations used are: DLT, dose-limiting toxicity; MKT4, lyophilized formulation of titanocene dichloride; MTD, maximum tolerated dose; Ti, titanium; AUC, area under the curve; NAG, N-acetyl-\( \beta \)-d-glucosaminidase.
Eligibility. Eligible for the study were patients with histological proof of malignancy for which conventional treatment had failed or was not available. Measurable disease was not required but, when present, was evaluated before dosing and every 22 days. Other eligibility criteria were: age between 18 and 75 years; Karnofsky-performance index $\geq 70\%$; life expectancy $\geq 3$ months; no chemotherapy or radiotherapy within the 4 weeks preceding the study; written informed consent; adequate bone marrow (leukocyte, $>3,500/mm^3$; platelet count, $>100,000/mm^3$); and renal (serum creatinine, $<1.5$ mg/100 ml) and hepatic (serum bilirubin, $<1.5$ mg/100 ml; alanine aminotransferase and aspartate aminotransferase less than two times the upper limit of normal values, unless secondary to metastatic liver disease) function. Exclusion criteria were: acute infectious disease, left ventricular hypertrophy, persistent toxicity of prior therapy (except alopecia or actinic dermatitis), and refusal of informed consent. The patients were hospitalized for treatment.

Study Design. The study was performed according to German drug regulations and good clinical practice guidelines. The protocol was approved by the ethical boards of the universities of Berlin, Essen, and Hannover.

Baseline values before treatment comprised complete blood cell count with differential and reticulocytes, sodium, potassium, calcium, creatinine, creatinine clearance, urea, uric acid, cholinesterase, alanine aminotransferase, aspartate aminotransferase, total bilirubin, alkaline phosphatase, $\gamma$-GT, lactic dehydrogenase, serum glucose, triglycerides, thyroid hormones, and plasmatic coagulation parameters. Complete physical examination and history, chest X-ray, electrocardiogram, and tumor measurements as appropriate were performed in addition. After infusion, patients had weekly assessments including all laboratory tests mentioned above. Physical examination, evaluation of performance status and drug-related toxicity according to the WHO criteria were assessed before each treatment course. Response evaluation was performed according to the WHO criteria in patients with measurable disease after each treatment cycle.

Drug Administration. MKT4, a lyophilized formulation of titanocene dichloride, was supplied by medac GmbH in 25-mg vials. Immediately before infusion, it was reconstituted in 25 ml of 50 mM malic acid to a final concentration of 1 mg of titanocene dichloride/ml of malic acid ($pH = 3.5$). After reaching the 420 mg/m$^2$ dose level, the increased infusion volume necessitated an increase of the titanocene dichloride concentration. Three patients then received 75-mg vials. The content was reconstituted in 37.5 ml of 25 mM malic acid to a final concentration of 2 mg of titanocene dichloride/ml of malic acid ($pH = 3.0$). The MKT4 infusion was given light-protected via a central i.v. line over 30 min (15–180 mg/m$^2$) or over 60 min (180–560 mg/m$^2$). Blood pressure, pulse, and temperature were recorded before therapy and 1, 4, 8, and 24 h after therapy. Serum glucose monitoring was performed every 6 h up to 24 h after MKT4 infusion. Subsequent courses were repeated every 21 days. A delay of 1 week was permitted. Individual therapy was continued until there was objective evidence of disease progression, nephrotoxicity WHO grade 2, other nonhematological toxicity WHO grade 3, hematological toxicity WHO grade 4, or a $>20\%$ treatment-related decline of the Karnofsky performance index. Treatment was also discontinued at the discretion of the treating physician or according to the patient’s decision. Antiemetics were only used if there was nausea/vomiting during the previous treatment course.

Dose-Escalation Procedures. The starting dose of titanocene dichloride was 15 mg/m$^2$. The dose increase was based on a modified Fibonacci scheme and proceeded as follows: 30, 50, 75, 105, 135, 180, 240, 315, 420, and 560 mg/m$^2$. A minimum of three patients were entered at each dose level. There was no dose escalation in individual patients. MTD was defined as the titanocene dichloride dose leading to DLT in at least two of six patients. DLT was defined as nephrotoxicity grade 2, other nonhematological toxicity grade 3, or hematolog-
and results are given as arithmetic means.

... with a detection limit of 0.02 μg of Ti/ml and a quantitation length of 319.9 nm. The assay was calibrated in a range up to 0.4 μg of Ti/ml, linked to a HGA 400 graphite furnace at a wave-

... drying, as done with the Ti standard solution in the correspond-

... in a paralle procedure. Flameless atomic absorption spectroscopy assay. Briefly, the samples were transferred to phosphate-buffered polypropylene tubes, and frozen at -20°C until they were analyzed.

Table 1  Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entered</td>
<td>40</td>
</tr>
<tr>
<td>Evaluable</td>
<td>39</td>
</tr>
<tr>
<td>Male/female</td>
<td>12/28</td>
</tr>
<tr>
<td>Median age in yrs (range)</td>
<td>52 (19–75)</td>
</tr>
<tr>
<td>Karnofsky performance index</td>
<td>100%</td>
</tr>
<tr>
<td>70–90%</td>
<td>25</td>
</tr>
<tr>
<td>Previous chemotherapy</td>
<td>39</td>
</tr>
<tr>
<td>Tumor type</td>
<td></td>
</tr>
<tr>
<td>Colorectal carcinoma</td>
<td>10</td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>5</td>
</tr>
<tr>
<td>Lung cancer</td>
<td></td>
</tr>
<tr>
<td>NSCLC*</td>
<td>3</td>
</tr>
<tr>
<td>SCLC</td>
<td>1</td>
</tr>
<tr>
<td>Soft tissue sarcoma</td>
<td>4</td>
</tr>
<tr>
<td>Cancer of the retrolingual region</td>
<td>2</td>
</tr>
<tr>
<td>Testicular carcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Bile duct carcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Others</td>
<td>9</td>
</tr>
</tbody>
</table>

* NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.

Curvature fitting was performed with the validated TopFit 2.0 pharmacokinetic and pharmacodynamic analysis system for the PC (14) using a two-compartment model because at dose level 135 mg/m² a one-compartment model and two-compartment model were compared, and due to statistical reasons, the two-

... node affected, three patients each of nephrototoxicity grade 4, further evaluation of toxicity at this level was performed by treating three additional patients.

**Pharmacokinetics.** The pharmacokinetics of total Ti in plasma and urine were analyzed for 14 courses in 10 patients starting at the dose level of 135 mg/m² MKT4.

Peripheral blood (2 × 5 ml) was collected in EDTA-coated tubes immediately before drug administration as well as at 15, 30, 45, and 60 min after starting infusion. Specimens were also collected at 1, 2, 3, 4, 6, 8, 24, and 48 h measured from the start of the infusion. One sample each of whole blood and plasma (after centrifugation at 1500 × g for 10 min) was frozen at −20°C for subsequent analysis. Urine was collected every 6 h over 24 h after starting infusion and every 12 h over 48 h after dosing. Samples of 10 ml were taken from each urine specimen, transferred to phosphate-buffered polypropylene tubes, and frozen at −20°C until they were analyzed.

Total Ti was determined in samples of whole blood, plasma, plasma ultrafiltrate, and urine using a validated flameless atomic absorption spectroscopy assay. Briefly, the samples were decomposed with nitric acid and perchloric acid after drying, as done with the Ti standard solution in the corresponding matrix in a parallel procedure. Flameless atomic absorption spectroscopy assay was performed in a 2380 Perkin-Elmer instrument linked to a HGA 400 graphite furnace at a wavelength of 319.9 nm. The assay was calibrated in a range up to 0.4 μg of Ti/ml. Under these conditions, >94% of Ti was recovered with a detection limit of 0.02 μg of Ti/ml and a quantitation limit of 0.05 μg of Ti/ml. Analyses were performed in triplicate, and results are given as arithmetic means.
the mean number of courses/patient was two. Thirteen patients received one treatment course; one of these patients (dose level 420 mg/m²) died of unexpected rapid tumor progression 5 days after treatment and, thus, cannot be evaluated. Of the other patients, 20 received two treatment courses, 4 received three courses, 2 received four courses, and 1 received five courses. The dose escalation is shown in Table 2.

Thirty-seven patients were treated with a 1-mg/ml-concentrated solution of titanocene dichloride in malic acid buffer up to the level of 560 mg/m². The infusion time was extended, and the concentration increased with further dose and infusion volume increases. Subsequently, the next three patients (38–40) were infused at dose level 315 mg/m² with a concentrated solution of 2 mg/ml titanocene dichloride. To prevent nephrotoxicity, prehydration with 1 liter of isotonic saline over 2 h and posthydration with 2 liters of isotonic saline over 4 h was administered to these patients.

Thirty-five patients were withdrawn from the study with progressive disease. In three of those patients, renal toxicity was an additional reason for treatment discontinuing. One patient (dose level 30 mg/m²) decided to discontinue the study after five treatment courses at his own request, despite stable disease. One patient treated with a 2-mg/ml concentration of 315 mg/m² titanocene dichloride was taken out of the study because of renal toxicity (WHO grade 3). There were three deaths on study. A patient with hepatocellular carcinoma died 20 days after one course at the 15 mg/m² dose level due to gastrointestinal bleeding in conjunction with tumor infiltration into the bowel, as confirmed by autopsy. Another patient suffering from testicular cancer with multiple lung metastases died of respiratory failure 10 days after one treatment course at the 420 mg/m² dose level. Pulmonary embolism was suspected, but no further diagnostic evaluations were performed in this patient. The third patient who had biliary cancer with multiple liver metastases developed fatal hepatic and renal failure 10 days after an infusion of 420 mg/m² titanocene dichloride. The early deaths of the first two patients were clearly related to rapid tumor progression. In the third patient, an additional influence of the study drug could not be excluded. Thus, possibly one death was at least partially drug-related due to grade 4 hepatotoxicity.

**Toxicity.** Dose-dependent gastrointestinal toxicity (loss of appetite, nausea, and vomiting) was observed in 12 of 39 assessable patients. Antiemetic therapy with 8 mg of ondansetron in three patients (240, 420, and 560 mg/m²) seemed to reduce these side effects, whereas in one patient at the 420 mg/m² dose level vomiting reached WHO grade 3 despite premedication.

Seventeen assessable patients complained of a metallic taste during or shortly after the MKT4 infusion, which disappeared within 24 h in all cases. A dose-dependent incidence and severity of this side effect became obvious at the 105 mg/m² dose level.

Transient asymptomatic hypoglycemia (serum glucose <3.5 mmol/l) was observed within 6–10 h after MKT4 infusion in five patients treated at lower dose levels (up to 180 mg/m²) and in one patient treated at the 315 mg/m² dose level. Three diabetic patients (50, 105, and 315 mg/m²) with hyperglycemia (serum glucose >7.5 mmol/l) evidenced normalization of serum glucose, rendering insulin therapy unnecessary for a limited period of time.

Dose-dependent renal toxicity (elevated serum creatinine) was seen in 13 assessable patients starting at the 50 mg/m² level. At 420 mg/m², renal toxicity was observed in all five assessable patients (one patient with WHO grade 1, three patients with WHO grade 2, and one patient with WHO grade 3). At 315 mg/m², nephrotoxicity (WHO grade 1) was seen in one of three patients receiving titanocene dichloride at a 1-mg/ml concentration. However, when the concentration of the solution was raised to 2 mg/ml, all three patients treated at this level experienced renal impairment (a WHO grade 3 creatinine increase and uremic symptoms, requiring temporary hemodialysis in one patient) despite prophylactic hydration.

At the 420 mg/m² dose level, each patient showed a WHO grade 1 or 2 transaminases increase. A patient with progressive cholangiocarcinoma had a WHO grade 2 increase of transaminases and WHO grade 4 bilirubin (the fatal outcome of this
Fig. 2  Nephrotoxicity of titanocene dichloride analyzed in three patients at the 315 mg/m² dose level (2 mg/ml titanocene dichloride concentration; i.v. hydration). A, urinary protein (mg/g creatinine). B, serum urea (mmol/l). C, serum creatinine (μmol/l). D, Alpha,-Microglobulin in urine (mg/g creatinine). E, NAG in urine (units/g creatinine).

One patient was treated at a dose level of 560 mg/m². Due to the large infusion volume in this patient, the prescribed infusion time of 1 h was exceeded by more than 3.5 h. A WHO grade 1 creatinine increase, WHO grade 3 nausea and vomiting, and metallic taste were observed. The patient received antiemetic therapy with ondansetron.

Infrequent side effects were: thoracic pain during the titanocene dichloride infusion (one patient at the 30 mg/m² dose level and one patient at the 105 mg/m² dose level); WHO grade 1 temporary hypertension (one patient treated with 2-mg/ml-concentrated 315 mg/m² titanocene dichloride); and WHO grade 2 constipation (one patient at the 315 mg/m² dose level). No other toxicities were observed, and especially there was no evidence of hematological toxicity, cardiotoxicity, or alopecia.

The side effects experienced by all patients during all therapy courses are listed in Table 3.

Renal Toxicity Studies. Proteinuria as a nonspecific parameter of renal dysfunction was observed in all patients, the maximum elevation reaching 155 times normal values at 315 mg/m² with i.v. hydration 1 week after treatment (Fig. 2A).
Phase I Study of Titanocene Dichloride

Serum urea and creatinine, as well as high molecular-weight proteins and albumin in urine, were studied as parameters of glomerular function. Creatinine and urea levels were elevated 1 week after treatment in all patients and returned to normal in one patient (Fig. 2, B and C). The electrophoretic separation of urinary proteins revealed the development of a mixed type (glomerular and tubular) proteinuria with increases in the fractions of high and low molecular-weight proteins and albumin. Alpha1-Microglobulin, NAG, and glucose excretion were investigated as parameters of proximal tubular function. Increased urinary excretion rates of alpha1-microglobulin were already recorded within a few hours after infusion and were elevated during the entire observation period (Fig. 2D). NAG, an enzyme relatively specific to the proximal tubule, showed an increase within a day and a maximum 72 h after infusion. NAG increases could only be reversed during the observation period in one patient (Fig. 2E). Glucosuria was observed in one patient 1 day and in the other two patients 1 week after MKT4 infusion (data not shown). Urine analyses obtained from one patient in the second course confirmed the nephrotoxic effects of MKT4 listed above.

**Pharmacological Studies.** The pharmacokinetics of total Ti were concomitantly determined in plasma and whole blood in six comparative analyses in the first four patients treated with titanocene dichloride doses between 135 and 240 mg/m² (Table 4). A constant ratio of 1:2 was obtained for AUC0-∞ in plasma and whole blood in four of six patients, and analysis was then carried out in plasma alone.

The pharmacokinetics of total Ti were analyzed in plasma and urine for 14 cycles in 10 patients. The parameters for the plasma kinetics of Ti calculated in a two-compartment model are given in Table 4. The biological half-life t1/2 was in plasma was thus 22.8 ± 11.2 h (harmonic mean xh ± pseudo-SD), and the peak plasma concentration cmax was 30 µg/ml at a dose of 420 mg/m². The distribution volume Vd was 5.34 ± 2.1 L (arithmetic mean x ± SD), and the total clearance Cl total was 2.58 ± 1.23 ml/min.

There was a good correlation between the concentration-time curves of Ti in plasma and the MKT4 dose administered (Fig. 3). This was confirmed when the Ti AUC0-∞ in plasma was plotted against the titanocene dichloride dose (Fig. 4) and resulted in linear regression analysis with a 0.8856 correlation coefficient.

Comparing the AUC0-∞ of total Ti in plasma and ultrafiltrate reveals a plasma protein binding of Ti in the range of 70–80%. The Ti kinetics in plasma and ultrafiltrate in a patient during the first 420 mg/m²-treatment is shown in Fig. 5.

The determination of the AUC0-∞ of total Ti in urine showed that between 3% and 16% of the total Ti administered was renally excreted during the first 36 h after administration.

**Antitumor Activity.** Objective remissions according to WHO criteria were not observed. However, in eight patients the disease was stable for up to 5 months. Thirty-two patients had progressive disease. In one patient with metastatic bladder cancer treated with 30 mg/m² titanocene dichloride, the malignant pleural effusion, resistant to several treatment approaches before inclusion into the study, did not recur during the entire 5-month treatment period. Another patient with rapidly progressive non-small cell lung cancer showed a ~25% regression of mediastinal lymph node metastases after two 50 mg/m²-treatment courses.

**DISCUSSION**

Because titanocene dichloride has a promising spectrum of antineoplastic activity in vitro and in vivo, it has now been evaluated in a clinical Phase I trial. In contrast to the preclinical findings, in which hepatic toxicity was observed (8), nephrotoxicity proved to be dose-limiting in this study. A detailed investigation of the mechanisms responsible for the renal disturbance in patients treated at the 315 mg/m²-dose level with titanocene dichloride revealed both glomerular and, more strikingly, proximal-tubular impairment. A similar pattern of toxicity has been reported for cisplatin (17). These effects were still detectable 3 weeks after MKT4 infusion and seemed to be dependent on the total dose. On the basis of the results of this

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**Table 4** Plasma kinetics of Ti after i.v. MKT4 infusion

<table>
<thead>
<tr>
<th>Patient</th>
<th>MKT4 dose (mg/m²)</th>
<th>Terminal t1/2 (h)</th>
<th>AUC0-∞ (µg/ml)</th>
<th>Ti cmax (µg/ml)</th>
<th>Vd (liter)</th>
<th>Cl total (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W.V.</td>
<td>135</td>
<td>52.7</td>
<td>297</td>
<td>7.7</td>
<td>6.43</td>
<td>1.45</td>
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<tr>
<td>A.W./I</td>
<td>180</td>
<td>12.0</td>
<td>141</td>
<td>13.5</td>
<td>4.06</td>
<td>4.09</td>
</tr>
<tr>
<td>A.W./2</td>
<td>180</td>
<td>25.8</td>
<td>289</td>
<td>14.5</td>
<td>4.33</td>
<td>1.99</td>
</tr>
<tr>
<td>F.K.</td>
<td>180</td>
<td>20.1</td>
<td>114</td>
<td>10.2</td>
<td>8.5</td>
<td>5.06</td>
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<tr>
<td>J.S./I</td>
<td>240</td>
<td>27.9</td>
<td>285</td>
<td>11.7</td>
<td>6.03</td>
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<td>J.S./2</td>
<td>240</td>
<td>56.3</td>
<td>362</td>
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<td>9.28</td>
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<tr>
<td>A.E.</td>
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<td>11.4</td>
<td>182</td>
<td>20.0</td>
<td>3.89</td>
<td>4.21</td>
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<td>M.T.</td>
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<td>287</td>
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<td>2.67</td>
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<td>M.K.</td>
<td>315</td>
<td>49.6</td>
<td>558</td>
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<td>1.81</td>
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<tr>
<td>R.S.</td>
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<td>28.3</td>
<td>717</td>
<td>21.6</td>
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<td>1.41</td>
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<tr>
<td>P.M./I</td>
<td>420</td>
<td>21.3</td>
<td>908</td>
<td>31.3</td>
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<td>1.48</td>
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<tr>
<td>P.M./2</td>
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<td>22.2</td>
<td>699</td>
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<td>3.69</td>
<td>1.92</td>
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<tr>
<td>M.B./I</td>
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<td>304</td>
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<td>5.9</td>
</tr>
<tr>
<td>M.B./II</td>
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<td>7.4</td>
<td>219</td>
<td>23.4</td>
<td>4.51</td>
<td>8.19</td>
</tr>
</tbody>
</table>

a: Arithmetic mean x ± SD; n = 12 (patient M.B. not included), except for b.
b: Harmonic mean xh ± pseudo-SD.

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ride and related metabolites with the analytical methods of vanadium salts (18). Also, the interaction of titanocene dichloride with the insulin receptor, as it was shown to be induced by transient hypoglycemia, was mainly observed in patients at lower dose levels. The mechanism responsible for this effect could be phosphorylation of the insulin receptor, as it was shown to be induced by vanadium salts (18). Also, the interaction of titanocene dichloride and related metabolites with the analytical methods of serum glucose has not been excluded. One patient who experienced hypoglycemia during both treatment courses had normal insulin concentration measured at the same time as serum glucose.

Determining the clinical pharmacokinetics of titanocene dichloride was one of the objectives of the present Phase I study. However, up to the 135 mg/m² level, titanocene dichloride could not be clearly identified in plasma or urine by high-performance liquid chromatography with spectrophotometric detection at 250 nm used according to in vitro investigations of titanocene dichloride solutions. Thus, it is necessary to develop effective methods for ex vivo stabilization in whole blood as well as further sensitization of titanocene dichloride detection. In consequence, we determined total Ti as the method applied in the early pharmacokinetic investigations of cisplatin to determine total platinum in plasma by atomic absorption spectroscopy (19) before development of intact cisplatin detection methods (20). Thus, only 14 pharmacokinetic investigations in 10 patients treated with a dose range of 135–560 mg/m² titanocene dichloride could be performed. A ratio of 1:2 for AUC(Ti) in plasma and whole blood was found. The biological half-life $t_{1/2,\text{B}}$ in plasma was $22.8 \pm 11.2$ h ($\mu_b \pm$ pseudo-SD) with a peak plasma concentration $c_{\text{max}}$ of $\sim 30 \mu g/ml$ at a dose of $420 \text{ mg/m}^2$, a distribution volume $V$ of $5.34 \pm 2.11$ (lx ± SD) and a total clearance $C_l$ of $2.58 \pm 1.23 \text{ ml/min}$ (lx ± SD). There was a correlation between the AUC(Ti) of Ti in plasma and the MKT4 dose administered with a correlation coefficient $r^2$ of 0.8856, suggesting linear pharmacokinetics in the dose range investigated. Thus, the pharmacokinetics of Ti are in good agreement with those of platinum (20), with the exception of significant differences in the distribution volume $V$. Considering the high (70–80%) plasma protein binding of Ti, comparing the attainable nontoxic Ti concentrations in plasma with cytotoxic concentrations in vitro is interesting for the potential clinical efficacy of titanocene dichloride. Studies by Harstrick et al. (4) and Christodoulou et al. (5) showed that the IC$_{50}$ for titanocene ranges from 25–100 μM. The peak plasma concentration $c_{\text{max}}$ of Ti determined in this study is $\sim 30 \mu g/ml$ at a dose of $420 \text{ mg/m}^2$, which corre-

![Fig. 3](image-url) Representative plasma-concentration-time course of total Ti after i.v. dosing of 240 mg/m² titanocene dichloride.

![Fig. 4](image-url) Correlation between the MKT4 dose and the AUC(Ti) of Ti in plasma (linear regression, correlation coefficient $r^2 = 0.8856$).

![Fig. 5](image-url) Comparison of the pharmacokinetics of Ti in plasma and in ultrafiltrate in a patient at the 420 mg/m² dose level.
sponds to 157 μg/ml or 720 μM titanocene dichloride. An 80% plasma protein binding yields an equivalent concentration of free titanocene (~144 μM). This is higher than the IC50 determined in vitro and could, therefore, be sufficient for a clinical cytotoxic effect of titanocene dichloride.

In summary, 240 mg/m² is the recommended dose for further Phase II studies when a 1-mg/ml concentration of titanocene dichloride is administered as a 60-min infusion every 3 weeks. A concomitant hydration therapy may reduce the gastrointestinal side effects, but one has still to be aware of the possible cumulative nephrotoxicity. A Phase I trial exploring multiple dose schedules should be performed, because preclinical evaluations revealed greater efficacy and tolerability in animals treated every 3rd day, instead of with a single bolus injection (9, 21).

REFERENCES


Phase I clinical and pharmacokinetic study of titanocene dichloride in adults with advanced solid tumors.


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