Nephroprotection by Theophylline in Patients with Cisplatin Chemotherapy: A Randomized, Single-Blinded, Placebo-Controlled Trial

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The aim of the present study was to assess the possible prevention of cisplatin-induced impairment of GFR by theophylline in patients with various malignancies. The trial design was parallel, randomized, single blinded, and placebo controlled. Patients received cisplatin at a dosage of 50 mg/m² either combined with etoposide, ifosfamide, and epirubicin or with paclitaxel and 5-fluorouracil/folinic acid with the usual precautions, including a standard hydration scheme before application of cisplatin in both arms. In the control arm, placebo was administered; in the verum arm, patients received theophylline in a loading dose of 4 mg/kg intravenously over 30 min before cisplatin, followed by 0.4 mg/kg per min over a minimum of 6 h, and then 350 mg three times daily orally for 4 consecutive days after completion of chemotherapy. GFR of each patient was assessed by renal clearance of inulin within 3 d before and at day 5 after cisplatin chemotherapy. Despite usual precautions, patients in the placebo group had a 21% decrease (range, 11 to 31%) of inulin clearance after a single cycle of cisplatin-containing chemotherapy (92.9 ± 3.4 versus 71.8 ± 3.5 ml/min; P < 0.01). Patients who received theophylline had no deterioration of GFR (91.5 ± 3.7 versus 90.0 ± 3.8 ml/min; P > 0.05). No adverse effects have been observed during theophylline application. Conventional precautions such as hydration and osmotic diuresis cannot prevent a significant decrease of GFR after a single cycle of cisplatin-containing chemotherapy. The prophylactic application of theophylline as an intravenous loading dose and oral maintenance regimen may preserve kidney function in terms of GFR.


Cisplatin is a potent antineoplastic drug that is usually combined with other chemotherapeutic agents in the treatment of various cancer types. Its clinical use is limited by relevant organ toxicities such as nephrotoxicity, ototoxicity, gastrointestinal toxicity, myelosuppression, and allergic reactions (1,2). A decrease of GFR up to 30% was observed after two cycles of cisplatin-based chemotherapy despite precautions such as hydration and the use of diuretics (2,3). In addition, cisplatin leads to tubular enzymuria and electrolyte disturbances. This renal damage may be associated with several patterns of histologic changes, including acute focal necrosis of the distal convoluted tubules and the collecting ducts. In rats, cisplatin-induced nephrotoxicity has its maximum on days 3 to 5 with several structural and ultrastructural changes (e.g., focal loss of brush border, cellular swelling, cytoplasmic vacuolization, condensation of nuclear chromatin).

Adenosine is involved in the regulation of renal hemodynamics, tubular function, and hormone release. In contrast to other vascular beds, adenosine induces vasoconstriction in kidney vessels, thereby coupling renal perfusion to the metabolic rate of the organ (4,5). To limit renal energy expenditure, afferent arteriolar tone and glomerular perfusion are regulated by a negative feedback mechanism (tubuloglomerular feedback), dependent on the tubular load with fluid and solutes (6,7). Substantial experimental evidence exists that adenosine is involved in signal transmission and serves as a principal mediator of tubuloglomerular feedback response (4,8,9). The renal actions of adenosine are primarily mediated via activation of the membrane-bound adenosine receptor subtype and susceptible to antagonism by theophylline (10,11). Various experimental investigations have demonstrated elevated tissue adenosine concentrations after increased metabolic load (12), renal ischemia (11), or drug-induced nephrotoxicity (13) consecutively causing a decrease of GFR.

In rats, continuously applied aminophylline treatment was able to prevent the reduction of GFR induced by cisplatin (14). Therefore, this study examined the potential nephroprotective effect of theophylline at established pharmacologic doses during cisplatin-based chemotherapy in cancer patients with normal kidney function.
Materials and Methods

Eligibility
The trial design was parallel, prospective, randomized, placebo controlled, and single blinded. It was performed after the approval of the local institutional ethic committee review board of the Eberhard-Karls University of Tübingen. Written informed consent was given by each patient before study inclusion. The study followed the Declaration of Helsinki and good clinical practice guidelines.

Patients who had various types of cancer and were allocated to cisplatin-based combination chemotherapy and had normal renal function (measured by creatinine clearance > 60 ml/min) were eligible for the trial. Other eligibility criteria included age ≥ 18 yr, predicted life expectancy ≥ 2 mo, Karnofsky status ≥ 60%, and adequate baseline organ functions.

Patients were excluded when they had received previous treatment with aminophylline derivatives immediately before study entry; had an uncontrolled coronary heart disease, angina pectoris, arrhythmia, hyperthyroidism, acute infection, bilateral loss of hearing ≥ 110 dB according to World Health Organization criteria, sensory peripheral neuropathy, known adverse events in association with theophylline; or were pregnant and lactating or noncompliant.

Study Medication
The intravenous study medication (Bronchoparat, Klinge, Germany) was prepared in the Chemistry department of the University of Tübingen under sterile conditions and distributed in a blinded manner. Study medication was infused using an initial dosage of 4 mg/kg body wt intravenously within 30 min before cisplatin, then the infusion rate was reduced to 0.4 mg/kg body wt intravenously for a minimum duration of 6 h. After intravenous application, a peroral medication (350 mg three times daily; Theophylline retard ratiopharm, Ratiopharm, Germany) was started for 4 consecutive days until assessment on day 5 (see Figure 1 for details).

Chemotherapy Regimens and Precautions
Cisplatin was applied at a dosage of 50 mg/m² at day 1 combined with etoposide (500 mg/m²), ifosfamide (4 g/m²), and epirubicin (50 mg/m²; VIP-E) or with paclitaxel (80 mg/m²), 5-fluorouracil (2 g/m²), and folic acid (500 mg/m²; T-PLF). In addition, each patient received 3000 ml of isotonic electrolyte solution, including 60 mmol of magnesium and 6.9 mmol of calcium per 24 h and mannitol, 250 ml (20%), immediately before and after application of cisplatin in the control arm and in the verum arm.

Collection of Samples and Clearance Investigations
Before the start of treatment GFR (measured by inulin clearance), clinical parameters, and proteinuria were determined. GFR was assessed by renal clearance of inulin. Indwelling catheters were inserted into a vein of both forearms for infusion and contralateral blood sampling. Patients received a priming dose of 113 mg of inulin (InU test; Fresenius Pharma Austria, Linz, Austria) expressed per kilogram of body weight dissolved in sodium chloride solution (250 ml, 0.9%) infused within 30 min. Thereafter, inulin was continuously infused at a rate of 313 mg/kg body wt, dissolved in sodium chloride solution (500 ml, 0.9%) at a rate of 80 ml/h. After the equilibration period of 1 h, four 30-min baseline clearance periods were performed, during which urinary fluid losses were substituted orally by medicinal water (total time period 180 min). Urine was collected by spontaneous voiding, and blood samples were drawn at the midpoint of each urine collection period.

On day 5, a second inulin clearance, clinical parameters, and proteinuria were assessed. Concentrations of inulin in plasma and urine were determined by a colorimetric assay (15), plasma levels of theophylline and clinical parameters using standard methods in the laboratory of the University of Tübingen, and urinary protein excretion with a nephelometric assay.

Statistical Analyses
After inclusion, patients were randomly allocated to receive either placebo or study medication. A computer-based randomization procedure was used. A sample size of 40 patients was planned. Patients were stratified according to chemotherapeutic pretreatment. Statistical differences between groups were evaluated by t test for paired and unpaired samples. All values are expressed as the mean ± SEM. P values were determined by a two-sided calculation. Results were considered to be statistically significant at P < 0.05.

Results
Patients
Forty-one patients entered the study at the Medical Center II, Department of Oncology and Hematology, University of Tübingen, Germany. Five patients were ineligible (n = 2 fulminant
Effects on GFR

High urine volumes were observed in both groups during the clearance investigations on day −3 and day 5 (10.3 ± 0.71 ml/min for placebo and 10.7 ± 0.75 ml/min for verum on day −3; 9.4 ± 0.71 ml/min for placebo and 9.3 ± 0.65 ml/min for verum on day 5). After a single cycle of cisplatin-based combination chemotherapy, the median GFR values decreased by 21% (range, 11 to 31%) from 90.0 ml/min (±3.8) at baseline to 71.8 ml/min (±3.5) at day 5 in control patients (P < 0.01). In contrast, in the theophylline arm, the GFR was completely preserved with 91.5 ml/min (±3.7) before and 92.9 ml/min (±3.4) after application of chemotherapy (Figures 2 through 4). Twenty-six percent of patients (n = 5) who were treated with chemotherapy plus placebo had a GFR <60 ml/min at day 5 after treatment compared with 6% of patients in the verum arm (Table 1). Seven patients in the placebo arm had a >20% decrease of GFR compared with baseline, whereas this occurred in none of the patients who were treated with chemotherapy plus theophylline.

Effects on Serum Parameters

Neither differences among both treatment groups nor significant changes within the two treatment arms from pre- to postchemotherapy measurements in terms of serum magnesium or creatinine values were observed.

Effects on Urinary Protein Excretion

In the theophylline group, baseline protein excretion was slightly higher compared with the placebo arm (128 ± 35 versus 71 ± 10 mg/g creatinine). On day 5 after therapy, urinary protein excretion increased significantly in the verum group to 317 ± 69 mg/g creatinine and to a somewhat smaller extent in the placebo group (145 ± 30 mg/g creatinine). In total, urinary protein excretion was elevated approximately two- to threefold after chemotherapy in both groups at day 5 (Table 3). There were no signs of any pre- or postrenal causes for these changes, e.g., hemorrhagic cystitis.

Adverse Effects of Study Medication

All patients had received chemotherapy, study medication, or placebo on a phase I study ward. Online monitoring for BP and pulse rate were performed, and no toxicity related to study medication was observed (Table 4). All patients had taken the full oral dose of theophylline on 4 consecutive days after completion of treatment and discharge from the ward.

Theophylline Serum Concentrations

For all patients in the verum arm, blood samples revealed a theophylline serum level in the therapeutic range of 5 to 15 mg/L (9.41 ± 0.4 mg/L) after the end of the intravenous application.

Table 1. Serum parameters of renal toxicity

<table>
<thead>
<tr>
<th></th>
<th>Verum Arm (Mean ± SEM)</th>
<th>Placebo Arm (Mean ± SEM)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day −3</td>
<td>91.5 ± 3.7</td>
<td>90.0 ± 3.8</td>
<td>NS</td>
</tr>
<tr>
<td>day 5</td>
<td>92.9 ± 3.4</td>
<td>71.8 ± 3.5</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>% of patients with subnormal values</td>
<td>1 (6%)/1 (6%)</td>
<td>1 (5%)/5 (26%)</td>
<td></td>
</tr>
<tr>
<td>% of patients with &gt;20% decrease</td>
<td>—</td>
<td>7 (37%)</td>
<td></td>
</tr>
<tr>
<td>Serum magnesium (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day −3</td>
<td>0.80 ± 0.06</td>
<td>0.80 ± 0.10</td>
<td>NS</td>
</tr>
<tr>
<td>day 5</td>
<td>0.82 ± 0.07</td>
<td>0.79 ± 0.10</td>
<td>NS</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day −3</td>
<td>0.67 ± 0.20</td>
<td>0.76 ± 0.13</td>
<td>NS</td>
</tr>
<tr>
<td>day 5</td>
<td>0.69 ± 0.20</td>
<td>0.76 ± 0.19</td>
<td>NS</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
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<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Measured by inulin clearance (ml/min).
Discussion

The exact mechanisms of cisplatin-induced nephrotoxicity have not been fully elucidated. Like several nephrotoxic heavy metals (e.g., mercury), cisplatin may accumulate in the kidney, where it can interact with sulphydryl compounds, resulting in increased membrane fragility and depletion of intracellular glutathione. There is some evidence that cisplatin can induce apoptosis and necrosis of kidney cells in a dose-dependent manner (16–18). Renal damage is associated with several patterns of histologic changes, such as acute focal tubular necrosis and dilation of convoluted tubules and collecting ducts. Clinical manifestations are elevations in blood urea nitrogen, serum creatinine, proteinuria, and acute renal failure (ARF) (19). It is known that cisplatin induces a persistent reduction of GFR in a range of 20 to 40% from baseline in long-term follow-up studies (3), suggesting that these changes are partly irreversible. Today, intensive prophylactic hydration and forced diuresis is used for preservation of kidney function (20).

Adenosine has been proposed to exert important regulatory functions in the kidney, affecting renal blood flow, GFR, tubular water and electrolyte transport, tubuloglomerular feedback, and secretion of renin (7,21). In contrast to other vascular beds (e.g., brain, heart), vessels in the kidney respond to exogenous or endogenous adenosine with vasoconstriction of the afferent arterioles (5). This vasoconstriction is mediated by the adenosine A1 receptor and can be blocked by the nonselective adenosine receptor antagonist theophylline (7,10). Experiments in animals have shown that adenosine receptor antagonists such as

Table 2. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Verum Arm</th>
<th>Placebo Arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (patients)b</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>randomized</td>
<td></td>
<td></td>
</tr>
<tr>
<td>assessable</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>Genderb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>female</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Median age (yr [range])b</td>
<td>49 (25–63)</td>
<td>54 (29–70)</td>
</tr>
<tr>
<td>Primary tumor (n patients)b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gastric cancer</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>NHL</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Hodgkin’s</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>larynx cancer</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>cancer of unknown primary</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>testicular cancer</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Previous chemotherapy (n patients)b</td>
<td>13 (76%)</td>
<td>15 (79%)</td>
</tr>
<tr>
<td>Cisplatin-containing (n patients)b</td>
<td>6 (35%)</td>
<td>12 (63%)</td>
</tr>
<tr>
<td>Median cisplatin dose (range)b</td>
<td>75 (50–250) mg/m²</td>
<td>75 (50–800) mg/m²</td>
</tr>
<tr>
<td>Mean (SEM)b</td>
<td>35 (±17)</td>
<td>89 (±41)</td>
</tr>
</tbody>
</table>

aNHL, non-Hodgkin’s lymphoma.
bP > 0.05.

Figure 2. Changes of inulin clearance before and after completion of chemotherapy.

Figure 3. Intraindividual changes of GFR in placebo group (clearance 1 and 2).
as xanthinderivatives (e.g., theophylline) ameliorate or prevent the severity of ARF. These experimental models of ARF include renal ischemia, glycerol injection, drug-induced nephrotoxicity, and radiocontrast media as the underlying causative factors (7,22,23). In addition, a recent study demonstrated an upregulation of adenosine A₁ receptor in the rat kidney on day 3 induced by cisplatin infusion (24), indicating an increased sensitivity to adenosine in the case of repetitive applications of cisplatin.

To our knowledge, this is the first study to demonstrate a nephroprotective effect of theophylline in patients who receive cisplatin-containing chemotherapy. Despite hydration and forced diuresis, patients in the placebo arm had a significant decrease of GFR (median, −21%; range, 11 to 31%; *P* < 0.01) measured by the most sensitive available method (inulin clearance) after a single cycle of standard-dosage cisplatin (50 mg/m²) chemotherapy. Theophylline, a competitive adenosine receptor antagonist, administered before and during the maintenance phase, completely preserved kidney function in terms of GFR (median decrease, 1%; range, −4 to 5%). Side effects of theophylline were not observed, and the application was subjectively well tolerated. The results of this trial confirmed animal data that have demonstrated a nephroprotective effect of adenosine receptor antagonists after cisplatin application (14). No significant changes in serum creatinine, serum magnesium, or proteinuria were observed. Serum creatinine, as shown earlier (2), is an insensitive marker to detect acute glomerular impairment. A relevant effect of theophylline on proteinuria was not detected in our study; however, this can also be caused by tubular effects of cisplatin, which are not influenced by theophylline.

Other clinical investigations of theophylline have shown that this drug can prevent radiocontrast media–induced renal impairment (25). In addition, efficacy was demonstrated in patients with chronic renal insufficiency and application of radiocontrast media (26).

Several supportive measures have been proposed to prevent cisplatin-induced nephrotoxicity. Besides adequate hydration before and during cisplatin administration and afterward in combination with an osmotic diuretic such as mannitol (the current standard method), *e.g.*, prolongation of the infusion time of cisplatin (*e.g.*, 6 h instead of 2 h), dose fractionation over several days, the use of a chronomodulated schedule, and the use of nephroprotective agents such as organic thiosulfate compounds have been investigated in this setting (18,27–31). However, in general practice, a single-dose application of cisplatin as a 1-h infusion remains the common standard, and only dose fractionation over several days is being used in some cancer types (2,3). Experimental study drugs that may be useful in renal protection include BNP7787 (dimesna), selenium, amifostine, and silibinin (16,27–30,32–35).

Amifostine (WR-2721, ethyofos, 2-[3-aminopropyl]aminoethylphosphorothioic acid), an organic thiophosphate, is a prodrug that has cytoprotective properties as a result of free radical scavenging, hydrogen ion donation, or removal of DNA platinum adducts (36). In a randomized study of 242 patients who had advanced ovarian cancer and received intravenous cisplatin 100 mg/m² and cyclophosphamide 1000 mg/m² once every 3 wk, the calculated creatinine clearance decreased by >40% in two thirds of the control group compared with 12% of those in the group that received amifostine in a dosage of 910 mg/m² (37). In other studies, intravenous amifostine applied before cisplatin preserved GFR measured by creatinine clearance when it was co-administered with standard-dose cisplatin–containing or high-dose carboplatin–containing (>1.5 g/m³) regimens. Even after two cycles that contain intravenous cisplatin 50 mg/m² plus intravenous ifosfamide and etoposide or paclitaxel, GFR can decrease by >30%, but concomitant use of amifostine was able to counteract this decay (38,39). In a few studies, some preventive effects on renal tubulus function have also been observed, and even lower dosages of intravenous amifostine (*e.g.*, 740 mg/m²) may be effective (38,40). In contrast to amifostine, the treatment with aminophyllines results in a more cost-effective prevention strategy because theophylline seems to have the same magnitude of GFR preservation compared with amifostine, and it is associated with a more convenient toxicity profile.

In some studies, glutathione has been shown to reduce cisplatin-related toxicity without impairing its antineoplastic activity (41). However, a cisplatin dose-escalation study with concomitant administration of glutathione had to be terminated prematurely because of unacceptable ototoxicity (42), and glutathione has not yet received Food and Drug Administration approval for chemoprotection.

In the present trial, only the acute nephrotoxicity of cisplatin was examined. Therefore, further studies have to evaluate potential long-term effects of theophylline on kidney function. This is of great importance because cisplatin-based chemotherapy is used in a variety of patients with potentially curable cancer (*e.g.*, metastatic testicular cancer, ovarian carcinoma) and hematologic malignancies or in the adjuvant setting for ovarian carcinoma or as recently published for non–small-cell lung cancer (43). In addition, the present trial included only patients with normal creatinine clearance at baseline, and the potential nephroprotection of theophylline in patients with impaired kidney function or in patients who were treated with higher dosages of cisplatin (100 to 120 mg/m²) still remains to be determined.

Several drugs alter the pharmacokinetics of theophylline; how-
ever, there is no drug interaction described with cisplatin or other cytostatics used in this series (44) (Drugdex database search).

In conclusion, the current pilot trial demonstrated that theophylline may effectively prevent the acute decrease of GFR induced by cisplatin-based chemotherapy in combination with forced diuresis and hydration compared with forced diuresis and hydration alone.

Acknowledgments

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