INFLUENCE OF HYDROLYSIS PRODUCTS OF AQUA(1,1-BIS(AMINOMETHYL)CYCLOHEXANE)SULFATOPLATINUM(II) ON TOXICITY IN RATS

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SUMMARY

Aqueous solutions of the cisplatin analog aqua(1,1-bis(aminomethyl)cyclohexane)sulfato Platinum(II) (TNO-6, spiroplatin) principally contain hydrolyzed and oligomerized molecules. Sodium sulfate reduces the hydrolysis of the sulfato ligand. We investigated the influence of the equilibrium state on nephrotoxicity of spiroplatin in rats receiving 3 doses of 3 mg/kg with an interval of 8 days. Rats (n = 6) treated with spiroplatin solubilized in isoosmotic sodium sulfate (impaired hydrolysis) showed less toxicity as measured by proteinuria, platinum excretion and body weight, than the group treated with spiroplatin solubilized in 5% glucose. This result indicates that the presence of hydrolysis products plays a role in the toxicity of spiroplatin.

INTRODUCTION

The second generation antitumor platinum complex aqua(1,1-bis(aminomethyl)cyclohexane)sulfato Platinum(II) (TNO-6, spiroplatin) was reported to be less nephrotoxic than cisplatin in preclinical studies [2,5,6]. However during phase I clinical trials, proteinuria appeared [14] and several cases of acute renal failure were observed in phase II trials [3,15].

The structure-activity relationship of platinum complexes is not fully understood. In this regard, an interesting fact is that spiroplatin in aqueous solutions is only present as such to a small amount, because it hydrolyses to several aquated species after dissolution (Table 1) (Elferink et al., unpublished). We developed liquid chromatographic analysis, with which these aquation products were individually monitored. Investigations of aqueous
TABLE 1
PLATINUM COMPLEXES PRESENT IN A SOLUTION OF SPIROPLATIN

![Chemical structure](image)

<table>
<thead>
<tr>
<th>X₁</th>
<th>X₂</th>
<th>Complex</th>
<th>Peak No.ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O</td>
<td>SO₄²⁻</td>
<td>Monoaqua-monosulfato (spiroplatin)</td>
<td>2</td>
</tr>
<tr>
<td>H₂O</td>
<td>H₂O</td>
<td>Diaqua</td>
<td>1</td>
</tr>
<tr>
<td>H₂O</td>
<td>Cl</td>
<td>Monoaqua-monochloro</td>
<td>3</td>
</tr>
<tr>
<td>Cl</td>
<td>Cl</td>
<td>Dichloro</td>
<td>4</td>
</tr>
<tr>
<td>OH-PrR-OHᵇ</td>
<td></td>
<td>Dimer</td>
<td>5</td>
</tr>
</tbody>
</table>

ᵃ Peak numbers refer to Fig. 1.
b R = 1, 1-bis(aminomethyl)cyclohexane.

solutions of spiroplatin, including infusion fluids, showed that the hydro-
lytic equilibria were affected by platinum concentration, pH and sulfate
concentration (unpublished data).

Aquated and oligomerized products of cisplatin are known to have more
toxic and less antitumor activity than the parent compound [12]. Addition
of sodium chloride to solutions of cisplatin reduces hydrolysis of the drug.
Litterst [9] showed that this resulted in a beneficial effect on cisplatin toxici-
ty in rats.

Sodium sulfate added to solutions of spiroplatin did not completely
suppress the aquation process. Nevertheless, the differences observed be-
tween the spiroplatin solutions with and without sodium sulfate provoked
to study the difference in nephrotoxicity of both solutions in the rat.

MATERIALS AND METHODS

Male Wistar rats (180—230 g) were individually kept in stainless steel
metabolism cages with free access to standard food (RMH-B, Hope Farms,
Woerden, The Netherlands) and tap water. They were divided into 4 groups
of 6 animals and treated as follows. Group A, 3 mg/kg of spiroplatin as a
solution of 0.3 mg/ml glucose 5%; group B, glucose 5%; group C, 3 mg/kg
spiroplatin as a solution of 3 mg/ml sodium sulfate 0.12 M; group D, sodium
sulfate 0.12 M. Animals were anesthetized with ether, weighed and solutions
were injected in the caudal vein in 0.5 min. This procedure was repeated
twice with intervals of 8 days. Six days after the last injection the animals
were weighed and kidneys were removed under anesthesia, just before they
were killed by bleeding from the heart.

Parts of kidney cortex and medulla were fixed in Tellyesniczky fixative
for 3 h, washed in ethanol 70% [8] and further processed for plastic embed-
ding in glycol methacrylate [13]. One-micron sections of the plastic embed-
ded material were stained with hematoxylin and eosin, periodic acid-Schiff
without diastase pretreatment, methenamine reticulin stain and elastica van
Gieson stain.

Injection fluids were prepared from spiroplatin ampules, containing 10 mg
in 1 ml water (supplied by Bristol Myers Co., Brussels), 24 h in advance of
each administration to be sure of completely equilibrated solutions. Glucose
5% infusion fluid was from Lansberg (Uden, The Netherlands) and sodium
sulfate was of analytical grade (Merck, Amsterdam, The Netherlands). The
solutions were chromatographed just after injection. Chromatography was
performed on a Spherisorb S5 ODS2 column (11 × 0.46 cm) with a mobile
phase of 15% methanol v/v in 0.05 M sodium sulfate (pH 3) and a flow-rate
of 1 ml/min. Differential pulse amperometric (DPA) detection was applied
at −540 mV (pulse −100 mV) as described previously (unpublished data).

Urine of all animals was collected daily. Volume and protein content were
measured in all samples. Platinum was determined by atomic absorption
spectrometry only in urine collected during the intervals of 0–5 h, 5–24 h
and 24–48 h after each administration. Urinary protein was determined by
the Bio-Rad protein assay (Bio-Rad Laboratories B.V., Utrecht, The Nether-
lands). A protein spectrum was made from urine samples of one control (B)
and two treated (A and C) rats 5 days after the first injection. Creatinine
clearance was calculated from the concentrations determined in the final
24-h urine and the blood taken from the caudal vein and/or kidney artery
just before the animals were killed. Statistical evaluation was based on
Student t-tests.

RESULTS AND DISCUSSION

Hydrolysis of spiroplatin is limited by keeping the concentration high and
by the addition of sodium sulfate (unpublished data). For the animal experi-
ment tolerable extremes in drug constitution were prepared: spiroplatin
0.3 mg/ml glucose 5% (group A) and spiroplatin 3 mg/ml isosmotic 0.12 M
sodium sulfate (group C). These solutions were the utmost extremes to be
tolerated by the rat and to be injected accurately in a dose of 3 mg/kg
(LD₅₀ = 4 mg/kg). The composition of both solutions is shown in the chro-
natograms of Fig. 1. The chloro complexes originate from a precursor of
spiroplatin during synthesis (Meinema et al., unpublished data). The 3 mg/
ml solution contained less diaqua and dimer species and 7 times more mono-
aqua-monso sulfato complex than the 0.3 mg/ml solution. The pH of both
solutions was 3.9.

Change in body-weight is shown in Fig. 2. Group C seemed to exhibit less
general toxicity than group A, but the difference was not statistically signi-
ficant.

Proteinuria was measured as a parameter of nephrotoxicity, because in
contrast to observations in man [14] and in dogs [7], serum BUN and
Fig. 1. Chromatograms of spiroplatin 0.3 mg/ml in glucose 5% (a) and spiroplatin 3 mg/ml in sodium sulfate 0.12 M (b) at two sensitivities. Column, Spherisorb S5 ODS2; mobile phase, 15% methanol in 0.05 M Na$_2$SO$_4$ (pH 3); detection, DPA –540 mV vs. Ag/AgCl. Peak identities see Table 1, peak 6 = unknown (not detected in a).

Creatinine concentrations did not substantially rise after spiroplatin in rats [6]. Figure 3 shows that the first injection induced a significant rise of proteinuria in both groups of rats treated with spiroplatin solutions, with a maximum on day 5. The excreted protein mainly consisted of albumin. After the second and third injection proteinuria was less pronounced in group C than in group A. Only on day 12, protein excretion in group A differed significantly ($P < 0.05$) from that in group C. During the second half of the study a white precipitate appeared in the urine of most treated rats. Its origin is as yet unknown.

The cumulative excretion of platinum in urine after the first and third injection of spiroplatin is shown in Fig. 4. The cumulative excretions after the first injection (37.2% and 34.9%) were in agreement with those found in dogs (28.9%, $n = 3$) and in man (34.8%, $n = 18$) (own unpublished observations). Platinum excretion after the third administration was lower than after the first injection in both groups ($P < 0.01$), being significantly lower in group A than in group C ($P < 0.01$). It might be caused by renal damage not restored during the 8-day interval. This reduction was more pronounced in group A than in group C.
Serum creatinine concentrations, determined on the last day of the experiment, did not reveal renal damage: 48.2 ± 3.5, 46.5 ± 4.8, 56.5 ± 9.4 and 47.0 ± 6.9 μmol/ml in group A, B, C and D, respectively. However, creatinine clearances of the treated groups, being 0.94 ± 0.17 and 0.85 ± 0.37 ml/min for group A and C, respectively, were lower ($P < 0.01$) than those of the controls, being 1.42 ± 0.22 and 1.48 ± 0.26 ml/min for group B and D, respectively.

Microscopical evaluation of the kidneys showed major alterations in the tubuli contorti I and II in both groups A and C. In the tubuli contorti I the brush border was impaired. In both I and II there was a marked nuclear polymorphism and no mitotic activity. Multifocal fragmentation of tubular epithelial cells was observed. In all four groups there were marked hydropic changes, probably due to ischemia. The tubular basal membrane was intact. The glomeruli showed no abnormalities at light microscopic level. The interstitium was edematous and contained only focally a few inflammatory cells (lymphocytes, histiocytes and granulocytes) in group A and C. The vessels were normal in all groups. The observed kidney lesions in the treated groups are in accordance with Ref. 6, although De Jong et. al. [2] did not find histological alterations, but they administered lower doses of spiroplatin (up to 2 mg/kg). With light microscopy no histopathological differences
between both treated groups could be observed at necropsy 6 days after the last dose.

Differences in urinary excretion of platinum are especially measurable during the first 5 h after administration of the drug, because non-protein bound drug is only available during that period of time [4]. It is known for cisplatin to be excreted by glomerular and tubular processes [1,11]. This makes it plausible that the decreased platinum excretion in urine over the first 5 h after the third injection is due to tubular lesions still existing from previous administrations, being less in group C than in group A.

Kidney damage in the treated groups was also observed by a significantly decreased creatinine clearance compared to the control groups. However, the serum creatinine concentrations were only slightly increased, which is in agreement with previous observations [6]. It might be caused by the low muscle mass of the treated animals compared to the controls [10], although other unknown processes may also be involved. In many toxicological studies [e.g. 2,5–7,9] serum creatinine is used as a parameter of nephrotoxicity. Our result shows that the absence of serum creatinine increase is not a firm base to exclude renal damage.
Severe proteinuria was one of the toxic symptoms of spiroplatin during phase I trial. It could be limited by extending the duration of infusion from 10 min to 4 h [14]. Nephrotoxicity might be caused by hydrolyzed and oligomerized products of spiroplatin present in the administration vehicle, as well as those formed by hydrolysis in the body. Within the physiologically permitted conditions nephrotoxicity could be decreased in the rat model by suppressing spiroplatin hydrolysis in the injection fluid, but not to such an extent that a new clinical trial will be justified. This study also indicates that future anticancer platinum complexes should be examined for the extent of hydrolysis in an early stage of development.
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REFERENCES


