Investigation on pharmacokinetics, tissue distribution and excretion of a novel platinum anticancer agent in rats by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

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Abstract

1. DN604 is a new platinum agent with encouraging anticancer activity. The present study was to explore the pharmacokinetic profiles, distribution and excretion of platinum in Sprague–Dawley rats after intravenous administration of DN604. A sensitive and selective inductively coupled plasma mass spectrometry (ICP-MS) method was established for determination of platinum in biological specimens. The pharmacokinetic parameters were calculated by a non-compartmental method.

2. The area under concentration–time curve AUC0-t and AUC0-∞ for platinum originating from DN604 at 10 mg/kg were 25.15 ± 1.29 and 28.72 ± 1.04 g/h/ml, respectively. The mean residence time MRT was 36.59 ± 6.65 h. The volume of distribution Vz was 11.42 ± 2.49 l/kg and clearance CL was 0.18 ± 0.01 l/h/kg. In addition, the elimination half-life T1/2z was 44.83 ± 9.75 h. After intravenous administration of DN604, platinum was extensively distributed in most of tested tissues except brain. The majority of platinum excreted via urine, and its accumulative excretion ratio during the period of 120 h was 63.5% ± 7.7% for urine, but only 6.94% ± 0.11% for feces.

3. The satisfactory half-life, wide distribution and high excretion made this novel platinum agent worthy of further research and development.

Keywords

Distribution, DN604, excretion, ICP-MS, pharmacokinetics

Introduction

During the treatment of lung, testicular, head and neck, cervical, ovarian and bladder cancer, platinum compounds have been widely used together with other anticancer drugs (Sprowl et al., 2013). Cisplatin, as the first generation platinum compound, is still a first-line drug to combat cancer and substantial relevant researches have been done since its anticancer activity was discovered (Rosenberg et al., 1965). Simultaneously, the adverse effects including gastrointestinal, renal and neurological toxicity (McKeage et al., 1995) as well as the evident acquired resistance to cisplatin (Sprowl et al., 2013) have limited its clinical application (Gore et al., 1989).

For the past decades, many efforts have been made to develop new platinum derivatives and to further overcome the aforesaid adverse effects. Carboplatin, which was clinically available in 1984, displayed a similar spectrum of anticancer activity with cisplatin but much less nephrotoxicity (Zhao et al., 2012), resulting in an improved quality of patient’s life during Pt-based chemotherapy (Rixe et al., 1996). Nevertheless, its cross-resistance and the inferiority to cisplatin in the treatment of testicular and neck-head cancer are still noticeable problems (Sprowl et al., 2013; Wang et al., 2007). Oxaliplatin, a third generation platinum drug with no cross-resistance to cisplatin, approved as the first platinum-based anticancer drug for the treatment of colorectal cancer, a disease that is unresponsive to other platinum chemotherapeutics (Cvitkovic & Bekradda, 1999; Luo et al., 1999; Tashiro et al., 1989). Unfortunately, it is only available in a few countries such as France, due to the unpredictable neurotoxicity (Desoize et al., 2002; Jadhav et al., 2010). With the above given information, it is essential and urgent to develop new platinum compounds with broad spectrum of excellent antitumor activity and less adverse reaction.

While synthesizing and screening platinum-containing antitumor compounds, we acquired DN604 (cis-diammine-1, 1-(3-cyclobutanone) dicarboxylate platinum II) (chemical
structure shown in Figure 1), which in subsequent experiments revealed this compound has encouraging anticancer activity and high solubility. Platinum agents are usually composed of platinum, leaving groups and carrier groups. It has been reported that modification of platinum-based agents with less liable leaving groups can reduce the toxicity of the drug (Canetta et al., 1985; Harrap et al., 1985; Wang et al., 2007). Also 1, 1-(3-cyclobutanone) dicarboxylate, as a leaving group in DN604, has higher pKa than Cl\(^-\), suggesting 1, 1-(3-cyclobutanone) dicarboxylate is a less liable leaving group compared with cisplatin. Thus, it is reasonable for us to speculate that the toxicity of DN604 may be less than that of cisplatin. DN604 showed a higher sensitivity in human colorectal cancer cell line HCT116 and human breast cancer cell line MCF-7 compared with cisplatin (Table 1). According to the in vitro and in vivo biological evaluations, DN604 has the potential to be further developed as a novel platinum anticancer drug.

Many promising compounds failed to become new drugs because of their poor pharmacokinetic characteristics such as low bioavailability and undesirable half-life. Thus, exploring the preclinical pharmacokinetic profile of a novel drug candidate is necessary and important. This provides a good knowledge of the disposition of drug, including its absorption, distribution, metabolism and excretion, to better select drug candidates for clinical studies. Nowadays, several methods are available for quantitative analysis of platinum agents, such as high performance liquid chromatography (HPLC), flameless atomic absorption spectroscopy (FAAS) and inductively coupled plasma mass spectrometry (ICP-MS) (Zhao et al., 2012). However, the application of the former two methods is limited due to non-specificity or inadequate sensitivity for pharmacokinetic studies (Liu et al., 2009), whereas, ICP-MS is more welcomed because of its excellent selectivity and sensitivity. Actually, ICP-MS has become the first choice for detection of metal-based compounds in pharmacokinetic studies.

In this study, we fully evaluated the pharmacokinetic profile of DN604 including pharmacokinetics, tissue distribution and excretion in rats by platinum analysis using ICP-MS.

### Materials and methods

#### Chemicals and reagents

DN604, whose chemical structure has been characterized with element analysis, ultraviolet spectrometry, infrared spectrometry, nuclear magnetic resonance and single crystal X-ray diffractometer, was provided by Southeast University (Nanjing, China). Platinum (Pt) and rhenium (Re) standard solutions were purchased from ANPEL Co., Ltd. (Shanghai, China). N-Butyl alcohol of analytical grade purity, ammonia water (25%, 13 mol/l) of analytical grade purity and hydrogen peroxide solution (30%) of analytical grade purity were obtained from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Triton X-100 of chemical purity and nitric acid of MOS grade were purchased from Sinopharm Chemical Reagent Company (Shanghai, China).

#### Animals

The Sprague–Dawley (SD) rats (200±20 g) were purchased from the Shanghai Sino-British Sippr/BK LAB Animal Co. Ltd (Shanghai, China). Beagle dogs (10.0±0.5 kg) were provided by Southeast University Laboratory Animal Center (Nanjing, China). All animals were maintained in a normally controlled breeding room (temperature: 22±2°C, humidity: 50±5%, 12 h dark/light cycle) with standardized diet and acclimatized for 7 days, prior to the experiments. The rats were fasted 12 h before the administration of DN604 but with unrestricted access to water. All animal experiments were approved by the China Pharmaceutical University Animal Ethics Committee.

#### Pharmacokinetic studies in rats

Eighteen rats were randomly divided into three groups, each six rats (three male, three female), and intravenously administrated with the DN604 at the dose of 5, 10 and 20 mg/kg (equivalent to platinum at 2.532, 5.064 and 10.128 mg/kg). About 100 µl blood samples were collected at 0, 0.033, 0.083, 0.16, 0.5, 1, 2, 3, 5, 8, 12, 24, 36, 48, 72 and 96 h after the treatment of DN604. All blood samples were centrifuged at 12 000 g for 3 min, and the resulting 50 µl plasma was transferred into a clean tube immediately, and maintained at −80°C till quantitative determination of platinum by ICP-MS.

#### Tissue distribution and excretion studies in rats

Other 18 rats (nine male, nine female) received drug solution at 10 mg/kg body weight. The tested animals were sacrificed by bleeding the femoral artery at 0.5 h, 2 h, and 12 h after administration (six animals per time point, three male and three female), and the heart, liver, spleen, lung, kidney, stomach, intestine, brain, muscle, adipose, testicles or ovaries were excised immediately. The blood samples were collected at the same time. All the tissues were washed in...
normal saline (4°C), blotted on filter paper, accurately weighed, and stored at −80°C till quantitative determination of platinum by ICP-MS.

Another six rats (three male, three female) were placed in metabolic cages and the urine and feces were collected for 24 h as blank samples. Animals received intravenous administration of drug solution at 10 mg/kg body weight, and the urine and feces were collected at 1–2 h, 2–4 h, 4–8 h, 8–12 h, 12–24 h, 24–36 h, 36–48 h, 48–72 h, 72–96 h and 96–120 h. The volumes of urine samples were measured and feces samples were weighed. The urine and the feces samples were stored at −80°C till quantitative determination of platinum by ICP-MS.

### Preparation of blood, tissue, urine and feces samples

An aliquot of 25 μl rat plasma or urine sample was diluted to a final volume of 2.5 ml using aqueous solution containing 0.01% Triton X-100, 2% nitric acid and 2% N-butyl alcohol. Then the aliquot was injected directly for analysis by ICP-MS after sufficient mixing.

Tissue or feces samples were digested by 65% nitric acid (0.4 ml) and 30% H2O2 (0.8 ml), followed by heating at 80°C for 1 h to get a sufficient digestion. In order to neutralize the excess nitric acid, 25% ammonia water (0.4 ml) was added when cooling. Then the mixture was diluted by ultrapure water to 2 ml. After filtration, the digested solution for every tissue was harvested, and this solution was diluted with diluents which are described above and was injected directly for analysis by ICP-MS.

### ICP-MS condition

All determinations were performed on an ICP-MS (X series 2, Thermo Scientific, Waltham, MA), which was equipped with the Plasma Screen Plus sensitivity enhancement option fitted, Xt interface cones and Peltier cooling of the spray chamber (3°C). The ICP-MS was operated in standard instrument operation mode. Plasma Lab software was used to instrument control, data acquisition and analysis. The instrumental and operating condition were optimized with the commented tune solution. The major isotope of platinum and rhenium (IS) was monitored at m/z of 195 and 185, respectively.

### Results

#### Qualification of analytical methods

The calibration curve of platinum in rat biological samples was linear within the range from 20 to 20000 ng/ml (20, 50, 100, 500, 1000, 2000, 5000, 10 000 and 20 000 ng/ml) with the lower limit of quantification (LLOQ) of 20 ng/ml. R² for all standard curves were >0.9995. The relative standard deviation of quality control samples (nine replicate samples) was 9.1%, 2.1% and 3.9% at 50, 1000 and 16 000 ng/ml, respectively. The accuracies determined for intra and inter-day (three days) were all within 100±10% of the nominal values. Moreover, no significant matrix effect was observed after dilution. Thus, this optimized ICP-MS method was sensitive, selective and rapid for the measurement of platinum originating from DN604 in rat plasma, tissue, urine and feces samples.

#### Pharmacokinetic parameters

A summary of the pharmacokinetic parameters for platinum originating from DN604 in rats after intravenous administration at the dose of 5, 10 and 20 mg/kg is given in Table 2, and the plasma concentration–time profile of platinum is presented in Figure 2. Total platinum in rat plasma eliminated bi-phasically with a rapid initial phase and a prolonged elimination phase. Take the group treated with DN604 at 10 mg/kg body weight for example, a peak plasma concentration for total platinum of 20.4 ± 1.61 μg/ml was reached immediately and declined sharply to 0.88 ± 0.20 μg/ml within 2 h. At the twenty-fourth hour after administration, the total platinum concentration in rat plasma dropped to 0.20 ± 0.02 μg/ml. The plasma pharmacokinetic parameters calculated according to a non-compartmental method were as follows: AUC(0→t) = 25.15 ± 1.29 μg/ml/h, CL = 0.18 ± 0.01 l/h/kg, MRT = 36.59 ± 6.65 h, Vz = 11.42 ± 2.49 l/kg and T1/2z = 44.83 ± 9.75 h. It is noteworthy that both AUC(0→t) and AUC(0→∞) were dose proportional, and the R² could be 1 and 0.993 respectively.

#### Tissue distribution in rats

The concentrations of platinum from DN604 after intravenous administration at 10 mg/kg in tissues and plasma at 0.5 h, 2 h and 12 h are presented schematically in Figure 3. The highest concentrations of Pt were detected in the kidney, followed by the liver. Half an hour after DN604 treatment, the mean platinum concentration in the kidney (16.32 ± 2.57 μg/g tissue) was more than three times higher than that in the liver (5.18 ± 0.96 μg/g tissue). The tissue distribution profile showed that the lowest platinum concentration was found in

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Table 2. Mean pharmacokinetic parameters of platinum originating from DN604 in rats after intravenous administration at the dose of 5, 10 and 20 mg/kg (n = 6).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dose (mg/kg)</th>
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<tr>
<td></td>
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<tr>
<td>T1/2z (h)</td>
<td>39.40 ± 8.67</td>
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<tr>
<td>Cmax (μg/ml)</td>
<td>9.56 ± 1.39</td>
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<tr>
<td>AUC(0→t) (μg/h·ml)</td>
<td>10.75 ± 1.10</td>
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<tr>
<td>AUC(0→∞) (μg/h·ml)</td>
<td>11.91 ± 1.09</td>
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<tr>
<td>CLz (l/h·kg)</td>
<td>0.21 ± 0.02</td>
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<tr>
<td>Vz (l/kg)</td>
<td>12.01 ± 2.75</td>
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<tr>
<td>MRT (h)</td>
<td>31.09 ± 6.75</td>
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Figure 2. Mean plasma concentration-time profiles of total platinum originating from DN604 in rats after intravenous administration at the dose of 5, 10 and 20 mg/kg (n = 6).
the brain (0.09 ± 0.03 μg/g tissue at 30 min), likely due to the blood-brain-barrier (BBB), which was consistent with the earlier studies reporting little uptake of cisplatin or carboplatin into brain (Johnsson et al., 1995). It is noteworthy that Pt concentration in uterus ranked the third place among the tested tissues (2.78 ± 0.18 μg/g tissue). Apart from brain, platinum was extensively distributed in tested tissues. It seems that the removal of platinum in tissues was biphasic, with most of platinum removed within 2 h and the remaining platinum declined slowly with time.

Excretion into urine and feces

The accumulative excretion of DN604 by urine and feces from 0 h to 120 h, after intravenous administration at 10 mg/kg, is showed in Figure 4. It is clear that the major route of excretion of DN604 was via urine, 63.5% ± 7.7% of platinum being excreted within 120 h. Urinary excretion reached the maximum nearly 12 h after the treatment of DN604 (58.5% ± 8.4% of dose/12 hour period). The contribution of feces made for the elimination of DN604 was insignificant, which only dealt with 6.94% ± 0.11% amount of drug tested. The results were in agreement with the previous studies about the main excretion path of other common platinum-based drugs, where renal excretion is responsible for the majority of platinum elimination. As the half-life was 45 h, more than 120 h is needed to achieve >90% excretion.

Discussion

In order to develop a platinum agent with satisfactory anticancer activity, thousands of platinum-based compounds have been synthesized and evaluated in the past forty years. The IC\textsubscript{50} values of the novel platinum agent DN604 for HCT116 and MCF-7 cell line were 17.5 and 32.5 μM, respectively (Table 1). In contrast, IC\textsubscript{50} values of cisplatin were 17.8 and 5.52 μM for the same two cell lines, respectively, while those of carboplatin were 175 and 134 μM (Fang et al., 2013; Sun et al., 2012). The differences between their IC\textsubscript{50} values indicated that DN604 showed the similar antitumor activity with cisplatin but 5–10 times stronger activity than carboplatin after the selected tumor cells were exposed to each drug for 72 h. In the light of developing a new promising platinum drug, pharmacokinetics studies, tissue distribution and excretion of platinum originating from DN604 have been performed to help us better understand its \textit{in vivo} disposition.

In this study, we found that the plasma elimination half-life of total platinum originating from DN604 was 44.83 ± 9.75 h in rats. In contrast, the plasma half-life of carboplatin in rats was 24.25 ± 12.87 h according to our previous investigation (data not shown), which was almost half of that of DN604. It is known that the main goal of chemical modification towards platinum-based agents was to increase the plasma half-life, aiming at continuously delivering drugs to the
extracellular fluid of tumors for a long period of time (Desoize et al., 2002). The combination of superior anticancer activity and long action time may enhance the efficiency of DN604.

It is widely known that the area under the curve (AUC) is critical to the chemotherapy effect of platinum-based drugs. Results from the treatment of patients with germ cell tumors of the testis indicated that proper AUC value might be the sign whether the disease would relapse. No relapses were observed in patients whose AUCs were greater than 4 mg/ml min. In contrast, relapses occurred in five out of eight patients whose AUCs were under 4 mg/ml min (Horwich et al., 1991). Consequently, the comparison between the dose-normalized AUC of platinum-based compounds plays an important role in evaluating their efficiency, which is expressed as AUC0–∞/dose. From the results, the AUC0–∞/dose of DN604 (5.64 ± 0.92 kg/h/l) was almost the same with that of cisplatin (5.61 ± 0.99 kg/h/l), and much higher than that of carboplatin (1.12 ± 0.09 kg/h/l) (Wang et al., 2007). Importantly, the rat plasma protein binding of DN604 is only about 20% (data not shown), which is much less than that of cisplatin (90%) (O’Dwyer et al., 2000). This implies that more free drug may be achieved after the administration of DN604, which is regarded as the pharmacologically active species, and thus a better anticancer efficiency may be achieved compared with cisplatin.

The tissue distribution study demonstrated that the major- ity of platinum was in kidney, followed by the liver (Figure 3). This is similar to the earlier studies on cisplatin and carboplatin (Kizu et al., 1993; Siddik, 2003). Apart from these two tissues, DN604 had no other specific tissue targets, which may be another goal of platinum compound development to become a broad spectrum anticancer drug. In particular, it is surprising and interesting that Pt concentration in uterus ranked the third place among the tested tissues (2.78 ± 0.18 µg/g tissue), revealing that DN604 might become a promising drug for the treatment of cervical cancer. Moreover, the central nervous system is not likely to be impaired by DN604 due to the low platinum concentrations in the brain. The concentrations of platinum in tested tissues peaked at 30 min, signifying the platinum in plasma redirected to tissues within 30 min. These results were in agreement with the profile of platinum in plasma, which dropped dramatically in the initial stage. About 12 h after the intravenous administration of DN604, platinum concentration in kidney (1.64 µg/g tissue/dose) was roughly 60% of that in the treatment of cisplatin (2.6 µg/g tissue/dose) (Wang et al., 2007).

J. D. Blachley concluded that the accumulation of cisplatin in kidney and its retention for days after single dosage was mainly responsible for its severe nephrotoxicity (Blachley et al., 1981). Thus, it is reasonable to expect that DN604 may be not prone to cause such severe nephrotoxicity due to the lower accumulation of platinum in the kidney.

It is widely acceptable that the main elimination path of platinum-based antitumor drugs is via urine, with very little platinum recovered in feces. The renal excretion of DN604 (63.5% ± 7.7%) was high, which was between that of carboplatin (80~90%) and that of cisplatin (43~48%) (Siddik et al., 1987). There are two possible reasons accounting for their differences in renal excretion. Firstly their different reactivity leads to different protein binding characteristics. The protein binding of both DN604 and carboplatin is less than that of cisplatin and a high percentage of irreversible protein binding will result in a low urinary excretion of cisplatin (Daley-Yates et al., 1985). The main clearance mechanism of cisplatin is irreversible binding in plasma and tissues. In contrast, carboplatin is eliminated by glomerular filtration (McKeage, 1995a). Therefore, the urinary excretion of carboplatin and DN604 are higher than that of cisplatin. Secondly the participation of active transporters may result in varying renal elimination of platinum-based compounds. It has been demonstrated that the lower renal excretion of cisplatin might be caused by a tubular re-absorptive process, which was mediated by organic action transporter OCT2, a drug trans- porter strongly expressed and localized in the basolateral membrane of proximal tubule epithelial cells (Motohashi et al., 2002). It is reported that OCT2 facilitates the uptake of cisplatin from the blood into tubular epithelial cells, increasing the platinum accumulation in kidney and leading to platinum-associated nephrotoxicity (Filipski et al., 2008; Motohashi et al., 2013). In contrast, carboplatin is not a substrate of OCT2 and its elimination is mainly through glomerular filtration (Burger et al., 2010). Without tubular reabsorptive process, renal elimination of carboplatin is extraordinarily higher than that of cisplatin and carboplatin does not induce nephrotoxicity (Horwich et al., 1991). Considering the high renal excretion of DN604, we speculated that DN604 was possibly not a substrate of OCT2, which also requires further investigation. The results indicated that the renal handling and reactivity with macromolecules of platinum-based compounds are the determinants of their major pharmacokinetic differences (Siddik et al., 1987).

Conclusion

In summary, we fully explored the pharmacokinetic profiles of novel platinum compound DN604 in rats for the first time by using inductively coupled plasma mass spectrometry (ICP-MS). The pharmacokinetics, tissue distribution and excretion characteristics can help us explain the lower toxicity of DN604. Because of its less reactivity compared with cisplatin, DN604 exhibited lower protein binding, less tissue accumulation and higher urinary excretion. Since the concentration of platinum from DN604 dropped quickly and there were no obvious platinum accumulations in tested tissues and plasma, these characteristics reveal, this complex is expected to reduce adverse effects during cancer chemotherapy. Moreover, a higher dose of DN604 might be achieved, in order to improve the chemotherapy for cancer. Consequently, DN604 is a promising candidate for clinical evaluation.

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Declaration of interest

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References


Notice of Correction:

Following the initial early online publication of this article (14th February 2014), the authors noticed an error resulting from a miscalculation. The error was present in three locations and has been corrected in this final online and print version of the article.

In section 2 of the abstract, and in paragraph 1 of the ‘Results’ section, subsection ‘Excretion into urine and feces’, the correct accumulative excretion of platinum by feces was 6.94% ± 1.09%, and not 0.69% ± 1.09% as previously stated. Figure 4B was also affected. This error did not affect the conclusions as renal excretion is still responsible for the majority of platinum elimination.

The authors apologise for the mistake.