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ORIGINAL ARTICLE

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Cytotoxicity of the organic ruthenium anticancer drug Nami-A is correlated with DNA binding in four different human tumor cell lines

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Abstract *Purpose*: The cytotoxicity, intracellular accumulation and DNA adduct formation of the ruthenium complex imidazolium *trans*-imidazoledimethylsulfoxide tetrachlororuthenate (ImH[*trans*-$RuCl_4$(DMSO)Im], Nami-A) were compared in vitro with those of cisplatin in four human tumor cell lines: Igrov-1, 2008, MCF-7, and T47D. *Methods*: Cytotoxicity was assessed in vitro using a growth inhibition assay. Accumulation was determined by flameless atomic absorption spectroscopy (AAS). GG and AG intrastrand adducts were measured using the ^{32}P-postlabeling assay. *Results*: Nami-A was on average 1053 times less cytotoxic than cisplatin. The cytotoxicity of cisplatin was linearly related to both intracellular platinum accumulation and DNA binding, while the cytotoxicity of Nami-A was significantly related only to DNA binding and not to intracellular ruthenium accumulation. The levels of accumulation of Nami-A measured as ruthenium and of cisplatin measured as platinum were correlated linearly with the incubation concentration over a concentration range of 0 to 600 μM of both drugs. Ruthenium intracellular accumulation and DNA binding were on average 4.8 and 42 times less, respectively, than those of cisplatin. In addition, the numbers of GG and AG intrastrand adducts induced by Nami-A were 418 and 51 times fewer, respectively. Nami-A and cisplatin had the same binding capacity to calf thymus DNA. Nami-A was 25–40% less bound to cellular proteins than cisplatin. *Conclusions*: There was no saturation of the uptake and DNA binding capacity of either Nami-A or cisplatin. Furthermore, the low binding of Nami-A to cellular DNA cannot simply be explained by a lower capacity to bind to DNA, because the absolute level of binding in vitro to calf thymus DNA was the same for Nami-A and cisplatin. Finally, the lower cytotoxicity of Nami-A on a molar basis than that of cisplatin can at least partly be explained by its reduced reactivity to DNA in intact cells.

Keywords Nami-A · Cisplatin · Cytotoxicity · Accumulation · DNA binding

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Introduction

Cisplatin has become the key anticancer drug in the therapy of a wide spectrum of cancers. Despite its activity against many tumors, cisplatin is ineffective in others, and it can also induce major toxicity. In addition, many tumors that are initially sensitive to cisplatin become resistant after a limited number of courses [35]. These limitations have stimulated research on organic analogs of platinum and other metals with the aim of improving therapeutic efficacy [13, 14]. The ruthenium (Ru) complexes have been identified as favorable anticancer compounds [6, 18]. One of the most intensively studied analogs is Na[*trans*-$RuCl_4$(DMSO)Im] (Nami), a compound active against Lewis lung carcinoma, B16 melanoma and MCa mammary carcinoma, and a compound that has shown better efficacy in xenograft studies than cisplatin [24, 25]. This is due to a possible antimetastatic effect, not shown by cisplatin or cisplatin-like compounds [26, 27]. Nami-A ([ImH][*trans*-$RuCl_4$(DMSO)Im]) is a compound with better chemical characteristics and is derived from Nami after replacement of Na^+ by ImH^+. Nami-A is not hygroscopic and is therefore very stable in the solid state, while showing

good water solubility. In some studies Nami-A has been found to be more active against solid tumors than Nami [19]. Nami-A, unlike cisplatin, appears to be more active and selective against tumor metastases, with no appreciable organ toxicity against liver, kidney or lungs in animal models, nor direct relevant in vitro cytotoxicity in tumor cells [10, 22, 28, 29]. Nami-A has entered phase I clinical trials at the Netherlands Cancer Institute in Amsterdam.

It is commonly believed that the main target for ruthenium(III) drugs and other antitumor metal complexes is DNA, as shown for platinum(II) drugs. The antitumor action of ruthenium(III) complexes would be the consequence of direct DNA binding and damage [9]. Novakova et al. [21] have reported that the complex *mer*-[Ru(III)(terpy)Cl_3], which has significant cytotoxic properties, is able to bind DNA firmly and to modify its structural conformation. Extensive DNA damage correlates with high cytotoxicity [21]. In contrast to the view that DNA is the main target for ruthenium drugs, other authors have claimed that DNA-independent mechanisms, such as inhibition of metalloproteinases, interference with adhesion processes, and scavenging of nitric oxide are responsible for the antitumor and antimetastatic activity of these compounds [28, 29, 30].

The aim of the present investigation was to compare the pharmacological effects of Nami-A and cisplatin in vitro in two human ovarian and two human mammary tumor cell lines. The study focused on the cytotoxicity of Nami-A in vitro, and on cellular uptake and DNA binding of Nami-A during treatment. To elucidate the type of DNA damage that Nami-A induces and to determine the nature of the interaction, we investigated in vitro the binding of Nami-A and cisplatin to calf thymus DNA. Finally, we investigated in vitro the binding of Nami-A and cisplatin to cellular proteins and the influence of glutathione (GSH) depletion on DNA binding in tumor cells.

Materials and methods

Chemicals

Cisplatin was obtained from TEVA Pharma (Mijdrecht, The Netherlands). Nami-A was a generous gift from POLYtech (Trieste, Italy) and was formulated by the Slotervaart Hospital, Amsterdam, to a clinically usable form of the compound. It was obtained as a freeze-dried cake, consisting of 50 mg Nami-A, 125 mg mannitol and 4.8 mg citric acid anhydrate per vial. Nami-A was diluted with phosphate-buffered saline (PBS) to the desired concentration and used directly in the in vitro experiments. Calf thymus DNA was purchased from Sigma (St. Louis, Mo.).

Growth inhibition assay

The cytotoxicity of Nami-A and cisplatin were determined in a growth inhibition assay, as described previously [34]. Briefly, exponentially growing cells were harvested by trypsinization and plated in 96-well microplates and allowed to attach for 48 h at 37°C under an atmosphere containing 5% CO_2. After this attachment period, the cells were continuously exposed for 4 days in 96-well plates to Nami-A or cisplatin in a range of concentrations with a dilution factor of three between each well. At the end of the incubation period, adherent cell cultures were fixed in situ by addition of 100 µl cold 50% (w/v) trichloroacetic acid (TCA) and were kept for 60 min at 4°C. The supernatant was then discarded, and the plates were washed three times with distilled water. Sulforhodamine (SRB) solution (0.4% w/v in 1% acetic acid) was added and the cells were allowed to stain for 30 min at room temperature. Unbound SRB was removed by washing three times with 1% acetic acid. Then the plates were air-dried. Bound stain was dissolved with unbuffered 10 m *M* Tris base (Tris-hydroxymethyl-aminomethane) and the optical density was read on a spectrophotometer (Multiscan MCC/340; Labsystems, Farnborough, UK). Cytotoxicity was evaluated in terms of cell growth inhibition in treated cultures versus that in untreated controls. IC_{50}, the concentration of compound at which cell proliferation was 50% of that observed in control cultures, was determined by linear regression analysis.

Treatment of tumor cell lines with Nami-A and cisplatin with or without buthionine sulfoximine

The four tumor cell lines used, Igrov-1, 2008, MCF-7 and T47D, were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). The cells were kept in logarithmic growth at 37°C in a humidified atmosphere of air containing 5% CO_2. Cells were exposed to 0, 75, 150, 300 and 600 µ*M* Nami-A or cisplatin in RPMI without FCS for 4 h at 37°C in a humidified atmosphere of air containing 5% CO_2. Igrov-1 and MCF-7 cells were depleted of GSH by a 24-h exposure to 50 or 500 µ*M* buthionine sulfoximine (BSO), respectively, which corresponded to 10% inhibitory concentrations (IC_{10}) as determined in a 3-day continuous exposure cytotoxicity assay. Total GSH levels were determined in untreated and BSO-exposed cells with a GSH assay (Cayman, Ann Arbor, Mich.). GSH-depleted and control cells were exposed to 600 µ*M* Nami-A or cisplatin for 4 h. Subsequently, cells were washed twice with ice-cold PBS and harvested by scraping under ice-cold conditions. After lysing the cells in 1 ml distilled water, the protein content was measured using the Bradford assay [4].

DNA isolation

DNA from the tumor cell lines was isolated as described previously [17, 32]. Briefly, PBS-washed cells were lysed in nuclear buffer containing 10 m *M* Tris-HCl, 2.32% NaCl and 2 m *M* EDTA, pH 7.3. Subsequently, 1% sodium dodecylsulfate and 0.1 mg/ml proteinase K were added, followed by incubation overnight at 42°C. DNA was purified by a high salt extraction as described by Miller et al. [20]. The purified DNA was dissolved in 400 µl 10 m *M* Tris-HCl, pH 7.8. Subsequently, the DNA concentration was determined by measuring the absorbance at 260 nm and the purity was checked by determining the absorbance ratio at 260 and 280 nm. Ratios between 1.8 and 2.0 were routinely obtained.

Measurement of ruthenium and platinum content

An aliquot of lysed cells or DNA solution was dried under vacuum, after which the pellet was digested in 65% nitric acid at 70°C for 2 h in a closed vial. The total amounts of intracellular and DNA-bound ruthenium and platinum were determined by flameless atomic absorption spectroscopy (FAAS) on a Zeeman Varian 4775 instrument, as described previously [7]. Before daily analysis, a five-point calibration curve was prepared for both drugs. Heavy metal concentrations were calculated as nanomoles per milligram protein or per milligram DNA. All measurements were done in duplicate and included three quality controls according to standard operating procedures.

Treatment of calf thymus DNA with Nami-A and cisplatin

Calf thymus DNA was incubated with 0, 0.10, 0.5, 2.0, 10.0, 50 and 200 μ*M* Nami-A or cisplatin in PBS for 2 h at 37°C. Subsequently, unbound drug was removed by precipitation with 100% ice-cold ethanol, followed by two washings with ice-cold 70% ethanol. The air-dried pellet was dissolved in 10 m *M* Tris-HCl, pH 8.0. The final DNA concentration was determined by measuring the absorbance at 260 nm using a dual beam spectrophotometer (Lambda Bio 20A; Perkin Elmer, Norwalk, Conn.), and the purity was checked by determining the absorbance ratio at 260 and 280 nm. Ratios between 1.8 and 2.0 were routinely obtained.

Measurement of ruthenium- and platinum-intrastrand DNA adducts

Ruthenium and platinum intrastrand 2′-deoxyguanylyl(3′ → 5′)-2′-deoxyguanosine (GpG) and 2′-deoxyadenylyl(3′ → 5′)-2′-deoxyguanosine (ApG) adducts were determined using the improved ^{32}P-postlabeling assay as described by us [23]. Briefly, the DNA was digested, after which adducts were separated on the basis of their positive charge by strong cation-exchange chromatography (SCX). Subsequently, the adducts were deplatinated and the resulting dinucleotides labeled with [γ-^{32}P]ATP. Finally, they were separated by high-performance liquid chromatography (HPLC) and detected by on-line scintillation counting. Adduct levels were calculated as femtomoles per microgram DNA.

Determination of the protein-bound fraction after Nami-A or cisplatin accumulation

The amounts of protein-bound Nami-A and cisplatin were determined by precipitation of the proteins by TCA [16]. Cells were exposed to 600 μ*M* Nami-A or cisplatin in RPMI for 4 h at 37°C in a humidified atmosphere of air containing 5% CO_2. Subsequently, cells were washed twice with ice-cold PBS and harvested by scraping under ice-cold conditions. After lysing the cells in 0.5 ml distilled water, the protein content was measured using the Bradford assay. An aliquot of the lysed cells was mixed 1:1 with ice-cold 20% TCA and incubated for 10 min on ice. The precipitated proteins were then removed by centrifugation at 10,000 *g* for 5 min at 4°C. After removal of the supernatant, the pellet was dried. Platinum and ruthenium contents were determined as for the accumulation experiments described above.

Statistical analysis

Statistical analysis was performed using Student's *t*-test. *P* values < 0.05 were considered significant.

Results

Determination of the cytotoxicity of Nami-A and cisplatin

The four cell lines were continuously exposed to Nami-A or cisplatin for 4 days in a growth inhibition assay. The cytotoxicity of Nami-A and cisplatin was determined as the IC_{50}. IC_{50} values for Nami-A were 2000, 510, 800 and 900 μ*M* in Igrov-1, 2008, MCF-7 and T47D cells, respectively. A much higher toxicity was found after incubation with cisplatin, which resulted in IC_{50} values of 1.00, 0.37, 3.50 and 4.00 μ*M* in Igrov-1, 2008, MCF-7 and T47D cells, respectively. These results indicate a large difference, in the order of 225–2000 depending on the cell line tested, in cytotoxicity between Nami-A and cisplatin under these incubation conditions.

Accumulation of Nami-A and cisplatin in tumor cell lines

We exposed two ovarian and two breast tumor cell lines to 0–600 μ*M* Nami-A or cisplatin for 4 h. Igrov-1, 2008, MCF-7 and T47D cells accumulated 4.1, 10.3, 2.3 and 2.5 times less Nami-A than cisplatin, respectively (Fig. 1). Furthermore, a linear relationship between the accumulation and the incubation concentration of Nami-A and cisplatin was found. These results indicate that the transport of both Nami-A and cisplatin could not be saturated at concentrations in the range used, which seems to indicate passive transport. Control experiments were performed with cells exposed to 600 μ*M* Nami-A or cisplatin at 0°C for 4 h. This resulted in a strong reduction in the accumulation of Nami-A and cisplatin in the four cell lines to $14.9 \pm 1.5\%$ and $6.3 \pm 2.3\%$ as compared to the accumulation at 37°C, respectively (Fig. 1). Almost the entire accumulation of Nami-A and cisplatin was abrogated at 0°C, which combined with the unsaturable accumulation points to a form of passive or facilitated carrier-mediated transport.

Determination of the total amount of Nami-A and cisplatin bound to DNA in tumor cell lines

Cellular DNA was isolated and the total amount of adducts on the DNA was determined after exposure of the four tumor cell lines to Nami-A or cisplatin at 37°C under the same conditions a described in the previous section. The total number of DNA adducts correlated linearly with the incubation concentration of Nami-A and cisplatin (Fig. 2). The DNA adduct levels were 85, 44, 23, and 15 times less after incubation with Nami-A than after incubation with cisplatin in Igrov-1, 2008, MCF-7 and T47D cells, respectively.

After incubation of Igrov-1 and MCF-7 cells for 24 h with BSO at the IC_{10} concentrations, total GSH levels were depleted to $2.5 \pm 0.5\%$ and $15.3 \pm 3.2\%$ of baseline values, respectively. Subsequently, the cells were exposed for 4 h to 600 μ*M* Nami-A or cisplatin. GSH depletion had no significant effect ($n = 3$, data not shown) on Nami-A or cisplatin binding to DNA in either cell line.

Correlation between cytotoxicity and cellular DNA binding

In order to determine whether the cytotoxicity of Nami-A and cisplatin was correlated with the accumulation and/or DNA binding in the four tumor cell lines, the

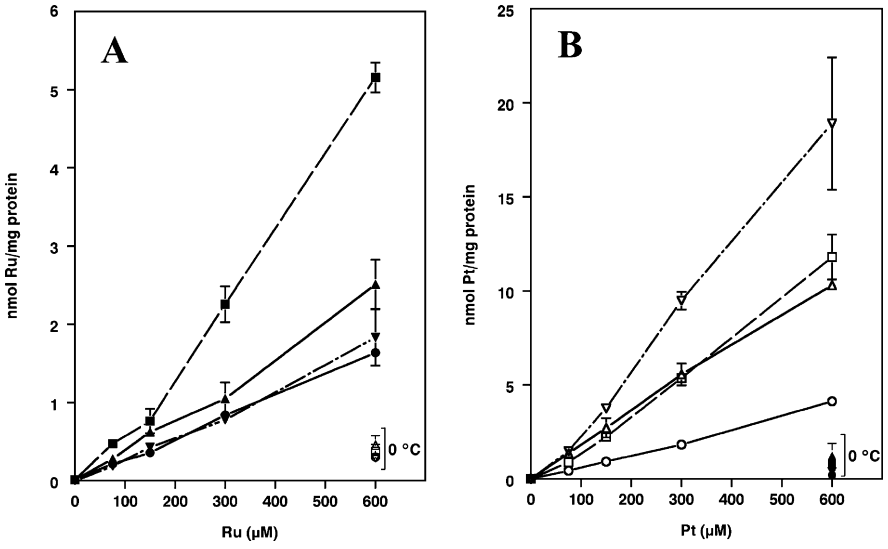

Fig. 1A, B Intracellular accumulation after 4 h continuous exposure of Igrov-1 (△ and ▲), 2008 (▽ and ▼), MCF-7 (□ and ■) and T47D (○ and ●) cells to 0–600 μ*M* Nami-A (**A**) or cisplatin (**B**) at 37°C (*closed symbols*) or 0°C (*open symbols*). The data are the results of three independent experiments presented as the means ± SD. Ruthenium (Ru) and platinum (Pt) were measured

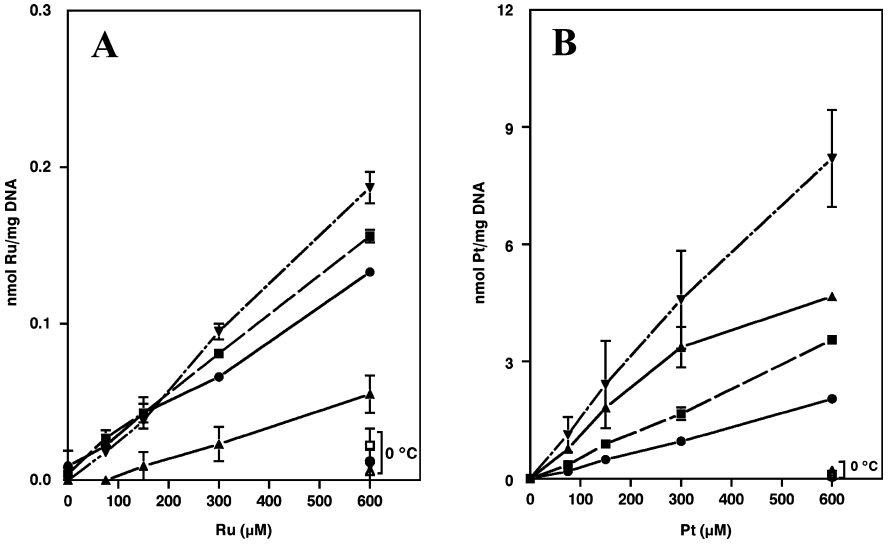

Fig. 2A, B DNA binding after 4 h continuous exposure of Igrov-1 (△ and ▲), 2008 (▽ and ▼), MCF-7 (□ and ■) and T47D (○ and ●) cells to 0–600 μ*M* Nami-A (**A**) or cisplatin (**B**) at 37°C (*closed symbols*) or 0°C (*open symbols*). The data are the results of three independent experiments presented as the means ± SD

previously obtained data were subjected to linear regression analysis (Fig. 3). We found a significant correlation between cytotoxicity and cellular accumulation of cisplatin ($R^2 = 0.76$, $P = 0.007$), as well as DNA binding ($R^2 = 0.89$, $P < 0.001$). However, the cytotoxicity of Nami-A correlated only with DNA binding ($R^2 = 0.99$, $P < 0.001$) and not with cellular accumulation ($R^2 = 0.05$, $P = 0.87$).

Binding of Nami-A and cisplatin to calf thymus DNA

We determined the binding of both Nami-A and cisplatin to naked calf thymus DNA after incubation with 0.1–200 μ*M* of both compounds in PBS (Fig. 4). Under these conditions, there was no significant difference between DNA binding of Nami-A and cisplatin. Furthermore, the DNA binding was linearly related ($R^2 = 0.99$, $P < 0.001$) to the incubation concentration of both Nami-A and cisplatin over the tested concentration range. These results indicate that the low binding of Nami-A to cellular DNA in all four tumor cell lines, as compared to that of cisplatin, is probably not caused by a lower DNA binding capacity of Nami-A.

Intrastrand adduct formation of Nami-A and cisplatin on calf thymus DNA

We determined the intrastrand adduct formation after incubation with 0.2–20 μ*M* Nami-A and cisplatin on naked calf thymus DNA under the same conditions as used for the binding studies discussed above. Intrastrand Ru-GG adduct levels were 418 and 54 times lower after incubation with 0.2 and 20 μ*M* Nami-A, respectively, than the levels of intrastrand Pt-GG adduct after exposure to 0.2 and 20 μ*M* cisplatin (Fig. 5A). The intrastrand Ru-AG adduct levels were 51 and 13 times

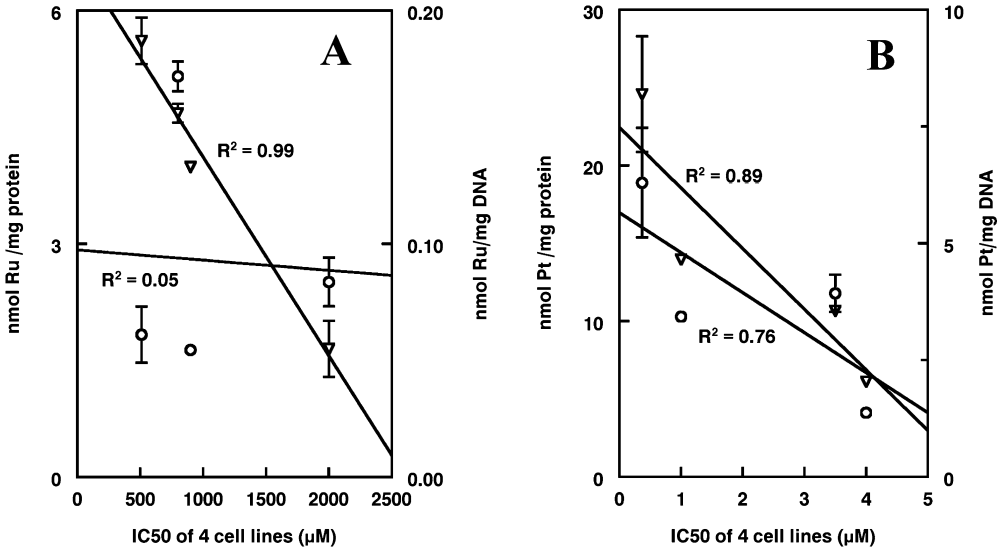

Fig. 3A, B Relationship between the cytotoxicity of Nami-A (**A**) and cisplatin (**B**) and the amount of the two compounds bound to the DNA (▽) or present in the cell lysate (○) of four tumor cell lines. The data are the results of three independent experiments presented as the means ± SD

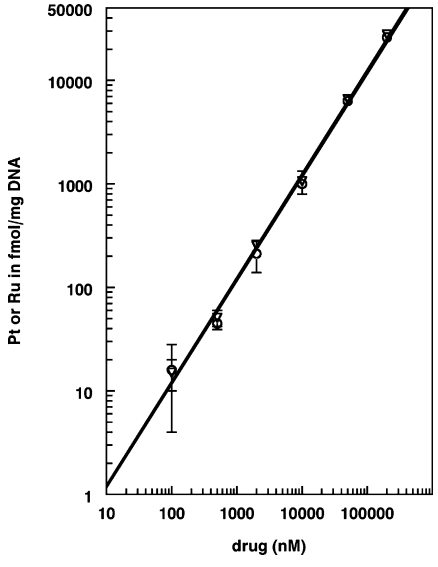

Fig. 4 Linear relationship between the Nami-A (○) and cisplatin (▽) incubation concentration and the total amount of compound bound to calf thymus DNA. The data are the results of three independent experiments presented as the means ± SD

lower after incubation with 0.2 and 20 μ*M* Nami-A, respectively, than the levels of intrastrand Pt-AG adduct levels after incubation with 0.2 and 20 μ*M* cisplatin (Fig. 5B). Nami-A seems to have a much lower intrastrand adduct formation capacity than cisplatin, and Nami-A forms about four times as many AG adducts in relation to GG adducts than cisplatin.

Protein binding of Nami-A and cisplatin in tumor cell lines

We determined the protein binding in Igrov-1, 2008, MCF-7 and T47D tumor cell lines after 4 h continuous exposure to 600 μ*M* Nami-A or cisplatin at 37°C. There was no significant difference between total accumulated and protein-bound platinum after incubation with cisplatin (Fig. 6). Conversely, a significant proportion (25–40%, $P < 0.038$ for all four cell lines) of ruthenium was non-protein-bound after incubation with Nami-A. Possibly, Nami-A has a higher affinity for other peptides excluding glutathione, which are not precipitated by TCA.

Discussion

Previously published studies have demonstrated that the uptake of the antitumor drug cisplatin is linear in Ehrlich ascites tumor cells, murine L1210 and 2008 cells up to 3.33 m *M*, the limit of its solubility in saline [2]. Many studies directed towards the mechanism of action of the antitumor drug cisplatin have contributed to the widely accepted view that its biological activity correlates with its ability to interact with DNA [15], but there is still significant debate as to which adducts are responsible for the different biological effects [33]. Many studies have revealed the capacity of ruthenium complexes to bind to DNA of isolated plasmids, or eukaryotic cells [5]. Other investigators seem to suggest that these complexes cannot easily penetrate cell membranes, preferring extracellular components as binding sites [8, 12].

In the present study, we compared total cell accumulation and intracellular DNA binding with the cytotoxicity of equimolar amounts of cisplatin and Nami-A in four human tumor cell lines. Furthermore, we investigated the binding capacity of both cisplatin and Nami-A to calf thymus DNA, after which we determined the relative amounts of intrastrand Ru- and Pt-adducts present on the DNA. Finally, we compared the protein binding of cisplatin and Nami-A in the four tumor cell lines.

In order to gain an insight into the accumulation of Nami-A in tumor cells, we incubated four tumor cell

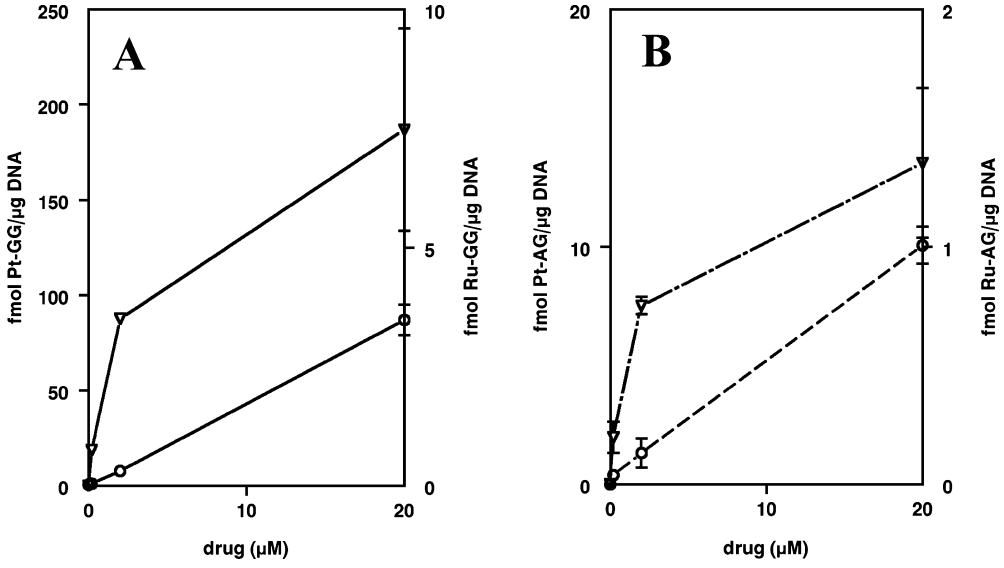

Fig. 5A, B Relationship between the number of GG-adducts (**A**) and AG-adducts (**B**) formed after incubation of calf thymus DNA with 0.2 to 20 µ*M* Nami-A (○) or cisplatin (▽). The data are the results of three independent experiments presented as the means ± SD

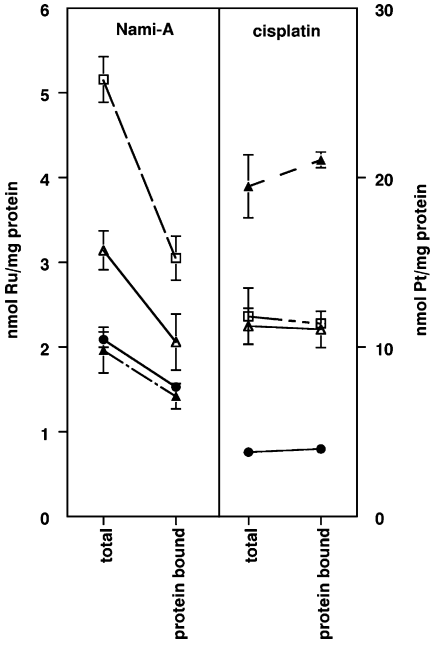

Fig. 6 Comparison of the protein binding of Nami-A and cisplatin relative to the total amount of accumulated compound in Igrov-1 (△), 2008 (▲), MCF-7 (□) and T47D (■) cells. The data are the results of three independent experiments presented as the means ± SD

lines in vitro at equimolar concentrations of Nami-A and cisplatin. We demonstrated that Nami-A accumulation, as has previously been reported for cisplatin [11], was linearly related to the drug concentration up to the highest concentration tested of 600 µ*M*. This indicates that there is no saturation of the uptake of either drug at concentrations in the range used. Depending on the tumor cell line tested, Nami-A accumulation was 2.5 to 10 times lower than that of cisplatin. In accordance with what has been found for cisplatin [11], Nami-A seems to enter cells via a passive or facilitated passive transport mechanism, but apparently with greater difficulty than cisplatin. In contrast to studies with TS/A adenocarcinoma cells, where a plateau of intracellular concentration occurs after 2 h of exposure to 100 µ*M* Nami-A [3], we found no evidence for saturation of accumulation using Nami-A at concentrations up to 600 µ*M* in our four tumor cell lines.

Cellular DNA binding of Nami-A and cisplatin was tested in vitro in the four tumor cell lines under the same conditions as those used for the accumulation experiments. A large discrepancy was found between Nami-A and cisplatin in their capacity to bind to cellular DNA. Although Nami-A accumulated 2.5 to 10 times less than cisplatin, the binding of Nami-A to cellular DNA was 15 to 85 times lower than that of cisplatin.

Accumulation as well as DNA binding of cisplatin was well correlated with the cytotoxicity of this drug in the four tested tumor cell lines. This is in contrast to Nami-A, the cytotoxicity of which in the four tumor cell lines was correlated only with the level of DNA binding and not, as for cisplatin, with drug accumulation in the cells. Although these data suggest that the cytotoxicity of Nami-A is exerted through binding to DNA, we have to keep in mind that the cytotoxicity of Nami-A is much lower than that of cisplatin. The concentrations of Nami-A needed to reach cytotoxicity in vitro are much higher than the Nami-A concentrations of about 100 µ*M* that inhibit the formation of metastases [31], indicating that the antimetastatic activity of Nami-A is probably not due to a reduction in cell viability.

In order to find an explanation for the relatively low binding of Nami-A to cellular DNA in the four tumor cell lines, we incubated calf thymus DNA with 0 to 200 µ*M* Nami-A or cisplatin in vitro. We found no significant difference between the DNA binding capacities of Nami-A and cisplatin, and both drugs bound DNA in a linear fashion over the concentration range tested. These data are in accordance with a study performed on pBR322 plasmid, in which Nami-A was as

effective as cisplatin in causing termination sites for replication [3]. These data suggest that Nami-A has a higher affinity than cisplatin for cell components other than cellular DNA.

Since previous studies have shown that the main target for cisplatin is DNA [15] and the main type (>80%) of adducts formed are Pt-GG and Pt-AG intrastrand adducts, we focused our attention on the characterization of DNA intrastrand adducts induced in vitro on calf thymus DNA by Nami-A. We showed that Nami-A was able to form both Ru-GG and Ru-AG intrastrand adducts. However, Nami-A formed far fewer intrastrand adducts than cisplatin. Another difference between Nami-A and cisplatin is the GG:AG adduct ratio, which was about four times higher for Nami-A. Previous studies have shown that Nami-A forms very few DNA interstrand crosslinks, whereas the number of DNA-protein crosslinks has been found to be comparable after cisplatin exposure [1]. Therefore it seems that Nami-A interacts with DNA in a different manner to cisplatin, leading to yet-unknown types of adducts.

Our experiments pointed towards the relatively high affinity of Nami-A, as compared to cisplatin, for cell constituents other than cellular DNA, and therefore we tested the protein binding capacity of both drugs in vitro in the four tumor cell lines. Depending on the cell line tested, Nami-A was 25% to 40% less bound to cellular protein than cisplatin. A probable explanation for this substantial difference in protein binding between Nami-A and cisplatin is a higher affinity of Nami-A for detoxification molecules such as GSH or metallothioneins (MTs), which were not determined in the protein precipitation assay. Therefore, we tested the effect of an almost complete depletion of total GSH levels by BSO in Igrov-1 and MCF-7 cells, and found no significant difference in binding of Nami-A and cisplatin to DNA after exposure of cells to 600 μ*M* of either drug.

In conclusion, our results indicate that Nami-A is able to enter cells in substantial amounts via a probably unsaturable transport mechanism, but this cannot explain the lower cytotoxicity of Nami-A than of cisplatin. However, the low cytotoxicity of Nami-A may be explained by its low capacity to bind to cellular DNA, as compared to cisplatin, possibly due to intracellular inactivation. We found that the cytotoxicity of Nami-A, as of cisplatin, is correlated with DNA binding. Our results demonstrate that the Ru intrastrand adducts are most likely not involved in this process. The type of Ru adducts that induce the cytotoxicity of Nami-A remains to be elucidated. Further investigations are also needed to elucidate the lower protein binding capacity of Nami-A in tumor cells.

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