Cancer Chemother Pharmacol (2001) 47: 498–504
DOI 10.1007/s002800000264

ORIGINAL ARTICLE

**Anna Riccardi · Daniela Meco · Cristiano Ferlini
Tiziana Servidei · Giovanni Carelli · Giuseppe Segni
Carla Manzotti · Riccardo Riccardi**

In vitro and in vivo antitumor activity of the novel trinuclear platinum complex BBR 3464 in neuroblastoma

Received: 27 September 2000 / Accepted: 8 December 2000 / Published online: 21 February 2001

Abstract *Purpose*: BBR 3464 is a promising new trinuclear platinum complex that has been shown to circumvent the resistance to cisplatin in a panel of tumor cell lines and xenografts with acquired or intrinsic resistance to cisplatin. The in vitro and in vivo antitumor activity of BBR 3464 was evaluated and compared with that of cisplatin in neuroblastoma. *Methods*: In in vitro studies, the short- and long-term cytotoxicities, cell cycle perturbations, the ability to induce apoptosis, the intracellular platinum accumulation and DNA platination were evaluated in three neuroblastoma cell lines exposed to appropriate drug concentrations for 1 h. In in vivo studies, BBR 3464 was administered i.v. at doses of 0.30 and 0.35 mg/kg three times at intervals of 4 days (q4d×3), and cisplatin was administered i.v. according to two different schedules (at 2 and 4 mg/kg three times at intervals of 4 days and at 6 and 12 mg/kg as single doses). *Results*: In a short-term growth inhibition assay, BBR 3464 was shown to be up to 100-fold more potent than cisplatin and it was even more potent in a clonogenic assay. The difference in the antitumor effect of BBR 3464 on the different cell lines was evident in both assays, while cisplatin exerted a comparable antitumor activity in all lines tested. Cell cycle analysis demonstrated a longer-lasting block in G_2/M phase induced by BBR 3464 without the early S phase accumulation induced by cisplatin. The higher potency of BBR 3464 appeared to be unrelated to the induction of apoptosis, that was lower or at most comparable to cisplatin. Cellular platinum accumulation and platinum-DNA adduct formation following BBR 3464 exposure was higher than following cisplatin exposure. These differences may have resulted from a different mechanism of action and may explain the lack of cross-resistance with cisplatin. In xenografts of neuroblastoma, BBR 3464 was confirmed to be very potent as compared to cisplatin (MTD 0.35 mg/kg and 4 mg/kg for BBR 3464 and cisplatin, respectively). The efficacy of BBR 3464 was superior to that of cisplatin when both drugs were administered on a fractionated schedule (q4d×3), while BBR 3464 appeared equally active to 12 mg/kg cisplatin administered as a single dose. *Conclusions*: Our findings indicate that BBR 3464 has a definite antitumor effect in neuroblastoma lines and may be a candidate for early clinical trials in children with neuroblastoma.

Keywords Trinuclear platinum complex · BBR 3464 · Cisplatin · Neuroblastoma

A. Riccardi · D. Meco · T. Servidei
G. Segni · R. Riccardi (✉)
Department of Paediatrics,
Catholic University of Rome,
Largo A. Gemelli 8, 00168,
Rome, Italy
E-mail: riccardi@rm.unicatt.it
Tel.: +39-6-3058203
Fax: +39-6-3052751

C. Ferlini
Department of Obstetrics and Gynaecology,
Catholic University of Rome, Largo A. Gemelli 8,
00168, Rome, Italy

G. Carelli
Department of Occupational Medicine,
Catholic University of Rome, Largo A. Gemelli 8,
00168, Rome, Italy

C. Manzotti
Novuspharma SpA, Viale G.B. Stucchi, 110,
20052 Monza, Milan, Italy

Introduction

Neuroblastoma is one of the most common pediatric solid tumors, but despite intensive chemotherapy there has been no substantial improvement in survival rates of children with advanced disease [5, 13]. Cisplatin either as a single agent or in combination is one of the most active compounds in many forms of cancer, including neuroblastoma. Although initial responses to chemotherapy in disseminated or unresectable neuroblastoma are common, these are invariably followed by the development of drug resistance and tumor progression. The severe toxicity of cisplatin, mainly nephrotoxicity and periph-

eral neuropathy, prompted the development of second-generation platinum compounds with the purpose of circumventing the resistance to cisplatin and of achieving the lowest possible toxic profile. Some side effects are less severe with carboplatin than with cisplatin, but the spectrum of clinical activity is similar and carboplatin is unable to circumvent cisplatin resistance.

The attempt to develop new platinum compounds with comparable antitumor activity but no cross-resistance with cisplatin and carboplatin has led to the development of a large number of mononuclear and polynuclear platinum compounds [7, 10]. Recently, from a systematic study of bifunctional DNA-binding agents based on charged, polynuclear platinum complexes, BBR 3464 (*trans*-{bis[*trans*-diamminechloroplatinum (μ1,6-hexanediamine)]}diammineplatinum tetranitrate salt) has been identified as the most promising agent based on its outstanding efficacy in a panel of human tumor cell lines and xenografts of different histotype, with intrinsic or acquired resistance to cisplatin [8, 12]. The purpose of our study was to define the cellular pharmacological profile of BBR 3464 in three human neuroblastoma cell lines: SK-N-DZ, LAN-1 and BE(2)M17. These cell lines were selected as representative of progressive stages of maturation [4]. We also evaluated the antitumor activity of BBR 3464 against SK-N-DZ and BE(2)M17 human tumor xenografts in nude mice. All studies were conducted comparing BBR 3464 with cisplatin in parallel.

Materials and methods

Cell lines, growth conditions and drugs

The neuroblastoma cell line SK-N-DZ was kindly provided by Dr. M.A. Israel (University of California, San Francisco), and LAN-1 and BE(2)M17 were a gift from Dr. J.L. Biedler (Memorial Sloan Kettering, New York). Cell lines were grown as monolayer cultures in RPMI-1640 medium (Bio Whittaker, Verviers, Belgium) with l0% fetal calf serum. At least three different experiments were performed in duplicate for each cell line and for each platinum complex.

BBR 3464 prepared as the NO_3^- salt was supplied by Novuspharma, Monza, Milan, Italy. Cisplatin was obtained from Bristol-Myers Squibb.

Growth inhibition assay

Cell lines were exposed to BBR 3464 and cisplatin for 1 h. They were then washed and fresh medium was added. Cell counting by trypan blue dye exclusion was then performed after an additional 72 h of culture. The results are expressed as the concentration able to induce half-maximal inhibition (IC_{50}).

Clonogenic survival assay

Approximately $0.8–1 \times 10^3$ cells were seeded in triplicate into 25-cm^2 flasks and exposed, after 24 h attachment, to the appropriate drug concentration for 1 h. The flasks were then washed gently with PBS, the medium was replaced and the flasks incubated at 37°C. After 10 to 12 days, the cells were stained with 1% methylene blue in 70% ethanol/water and colonies evaluated by visual counting. The number of colonies in treated cultures are expressed as a percentage of control cultures. The mean ±SD of colony counts from replicate cultures was calculated, and IC_{50} values determined from survival curves.

Cell cycle analysis

Progression of cells through the cell cycle was examined by flow cytometry. Exponentially growing cells were treated with serial concentrations of BBR 3464 and cisplatin or with the medium alone for 1 h. Cell cycle analysis was carried out after additional 24, 48 and 72 h of culture. The cells were harvested and the nuclei isolated and stained using a solution containing 0.1% (w/v) sodium citrate, 0.1% (v/v) NP40, 4 m*M* EDTA and 50 mg/ml propidium iodide as a DNA dye [6]. In order to calculate the amount of DNA fragmentation, all the events falling in the $subG_1$ region were computed and indicated as percent of DNA fragmentation. These events were excluded from the cell cycle analysis. DNA was analyzed flow cytometrically by acquiring a minimum of 10,000 nuclei using a FacScan flow cytometer (Becton Dickinson Immunocytometry Systems, San José, Calif.). DNA fluorescence was recorded in linear and log modes and pulse signal processing was used to set a doublet discrimination gate. Cell cycle analysis was performed using the Multicycle software package (Phoenix, San Diego, Calif.).

Morphological analysis of apoptosis

The ability of BBR 3464 and cisplatin to induce apoptosis was measured by the TUNEL assay using a commercial kit (Apoptos-I.S., Ylem, Avezzano, Italy), according to the manufacturer's instructions. The cells were exposed to equitoxic concentrations (IC_{30}, IC_{50}, IC_{80}, and multiples) of BBR 3464 and cisplatin for 1 h and analyzed after an additional 72 h of culture. A minimum of 500 cells were examined. Counting and image analysis were performed using a IAS 2000 System (Delta Sistemi, Rome, Italy). The morphological evaluation was compared with the DNA fragmentation data obtained by cell cycle analysis.

Platinum accumulation

Total intracellular platinum was measured after 1 h of incubation with BBR 3464 and cisplatin (10–100 μ*M*, five graded concentrations). The cells were washed twice with ice-cold PBS before harvesting and collection. An aliquot was used for cell counting, and the platinum assay was performed on the remaining cells. The cells were dried and digested with 100 μl 70% nitric acid and 30 μl 30% H_2O_2 in a microwave oven. Distilled water (800 μl) was then added to the samples and a 30-μl aliquot was injected into a Perkin-Elmer model 4000 atomic absorption spectrometer.

DNA platination

Approximately 1×10^7 cells were harvested after a 1-h treatment with BBR 3464 and cisplatin (10–100 μ*M*), and washed twice with PBS. Cell pellets were incubated overnight at 50°C with 0.5 ml lysis buffer (10 m*M* Tris-HCl, pH 8, 1 m*M* EDTA, 0.5% SDS, 20 μg/ml proteinase K; Sigma). The lysates were then treated with 20 μg/ml RNase A (Sigma) for 1 h at 37°C. DNA was extracted with phenol-chloroform and its content was determined by spectrophotometry. The platinum assay was performed by atomic absorption spectrometry as above. The DNA-bound platinum levels are expressed as picograms platinum per microgram DNA.

In vivo evaluation in human tumor xenograft models

CD1 nu/nu male nude mice (Charles River, Calco, Italy) at 5 weeks of age were used throughout the study. All experimental animal investigations complied with the guidelines of the Istituto Superiore di Sanita' (Rome, Italy) on experimental neoplasia in animals. Mice

were injected subcutaneously with 30×10^6 cells of both SK-N-DZ and BE(2)M17 cell lines. We tried to obtain LAN-1 xenografts in nude mice twice, but tumors grew only in a limited percentage of mice. Drug administration was started 2 weeks after transplantation when tumors weighed approximately 100–200 mg. BBR 3464 was administered three times i.v. at doses of 0.30 and 0.35 mg/kg every 4th day (q4d×3), which had previously been determined as the maximum tolerated dose (MTD), with no toxic deaths. This dose resulted in a mean weight loss of 10–15% of the initial weight. Cisplatin was administered i.v. according to two different schedules: 2 and 4 mg/kg q4d×3 and 6 and 12 mg/kg as single doses. The fractionated schedule enabled cisplatin given as repeated doses to be compared with BBR3464 and the cisplatin single doses, so that the two drugs administered at their optimal schedule were compared. In all the experiments control mice received the corresponding vehicles.

For evaluation of antitumor activity, each control or drug-treated group included five mice bearing bilateral subcutaneous tumors. Tumor growth was monitored and tumor weight (TW) was determined by measuring the tumor diameters with a vernier caliper and using the formula: TW = tumor volume = $d^2 \times D/2$, where d and D are the shortest and longest diameter, respectively. Drug efficacy was assessed as the percentage tumor weight inhibition (TWI) in treated versus control mice and as $\log_{10}$ cell kill (LCK) achieved by the drug treatment, according to the formula $T - C/DT \times 3.32$, where T is the mean time (days) required for treated tumors and C for the control tumors to reach an established weight (500 mg), and DT is the mean doubling time of control tumors. The drug was considered effective when TWI was ≥80% and/or LCK was ≥1 [11]. The statistical significance of differences in TWI between cisplatin- and BBR 3464-treated mice was evaluated using Student's *t*-test.

Results

Cytotoxicity

Short- and long-term cytotoxicities were evaluated by a 3-day growth inhibition assay and a clonogenic survival assay, respectively. In both assays BBR 3464 was more potent than cisplatin. IC_{50} values calculated from the dose-survival curves obtained from the short-term cytotoxicity assay are shown in Table 1. The LAN-1 cell line showed the highest sensitivity to BBR 3464. The IC_{50} values obtained from the clonogenic assay are shown in Table 2. The IC_{50} values for cisplatin from the two assays were very similar, while BBR 3464 was dramatically more cytotoxic in the long-term assay. The LAN-1 cell line was by far the most sensitive line, followed in order by BE(2)Ml7 and SK-N-DZ.

Cell cycle analysis and apoptosis

Progression of cells through the cell cycle was evaluated 24, 48 and 72 h after a 1-h drug exposure (Figs. 1 and 2). In all the cell lines both drugs induced a dose-dependent increase in cells blocked in the G_2/M phase. However, the two drugs exhibited different behavior. In the case of cisplatin, at the highest doses, the G_2/M block observed at 48 h (data not shown) was preceded by an accumulation in the S phase at 24 h (Fig. 1C). On the other hand, equipotent doses of BBR3464 (as detected by the 3-day growth inhibition assay) induced a dose-dependent G_2/M cell cycle arrest at 24 h (Fig. 1B) that lasted for up to 72 h (Fig. 2B), with no early S phase accumulation. Following this finding, we tested the hypothesis that BBR3464 might induce an S phase block with an earlier kinetics with respect to cisplatin. Therefore, cell cycle analysis was performed after 12 h of culture, and again no S phase arrest was found in BBR 3464-treated cells (data not shown).

Along with the dose-dependent accumulation in the G_2/M phase, we also noted an increase in DNA fragmentation over time. This behavior is a hallmark of the presence of apoptosis. Therefore, we performed a morphological analysis of apoptosis by the TUNEL assay after a 1-h exposure to equitoxic doses (IC_{30}, IC_{50}, IC_{80}) of the drugs and an additional 72 h of culture. As shown in Table 3, the ability of BBR 3464 to induce apoptosis at the doses tested in all three cell lines was lower or at most comparable to that of cisplatin.

Intracellular platinum accumulation

Up to the highest concentration tested (100 μ*M*), a nonsaturable and dose-dependent accumulation of both BBR 3464 and cisplatin occurred. Platinum accumulation following BBR 3464 exposure was threefold higher than following cisplatin exposure in BE(2)M17 and SK-N-DZ cells, and reached a ninefold higher concentration in LAN-1 cells (Fig. 3).

DNA platination

Platinum binding to DNA after a 1-h exposure to the drugs was nonsaturable and dose-dependent, resembling intracellular platinum accumulation. Following BBR 3464 exposure the rate of platinum adduct formation in all three cell lines was higher than that obtained with cisplatin at equimolar drug concentrations. Dose-DNA platination curves (Fig. 4) showed a 12- and 15-fold difference in platinum adduct formation in SK-N-DZ and BE(2)M17 cells, respectively, and a much higher rate of platination was noted in LAN-1 cells (about 54-fold). In BE(2)M17

Table 1 IC_{50} values (μ*M*) assessed by cell count at 72 h after a 1-h treatment with cisplatin or BBR 3464

Cell line	Cisplatin	BBR 3464	Cisplatin/BBR 3464
SK-N-DZ	5.40 ± 1.90	0.37 ± 0.10	15
BE(2)Ml7	6.97 ± 0.75	0.13 ± 0.05	54
LAN-1	6.67 ± 2.21	0.067 ± 0.02	100

Table 2 IC_{50} values (μ*M*) assessed by colony forming assay after a 1-h treatment with cisplatin or BBR 3464

Cell line	Cisplatin	BBR 3464	Cisplatin/BBR 3464
SK-N-DZ	6.37 ± 1.28	0.12 ± 0.025	53
BE(2)Ml7	5.50 ± 0.82	0.017 ± 0.003	323
LAN-1	7.40 ± 1.64	0.0017 ± 0.0005	4353

cells, cisplatin induced platinum-DNA adduct formation to a higher extent than in the other two cell lines, according to the intracellular platinum accumulation.

In vivo evaluation in human tumor xenograft models

The antitumor activity of BBR 3464 was evaluated in nude mice against two human neuroblastoma xenografts, SK-N-DZ and BE(2)M17 and compared with that of cisplatin. The highest dose of each drug was approximately the LD_{10} estimated in non-tumor-bearing mice following the corresponding schedules for testing antitumor activity. The data are reported in Table 4 and growth curves are shown in Fig. 5. BBR 3464 showed an antitumor effect at both doses (0.30 and 0.35 mg/kg) with a TWI of >80% in both tumor xenografts. At 0.35 mg/kg, the LCK value was >1 in both xenografts.

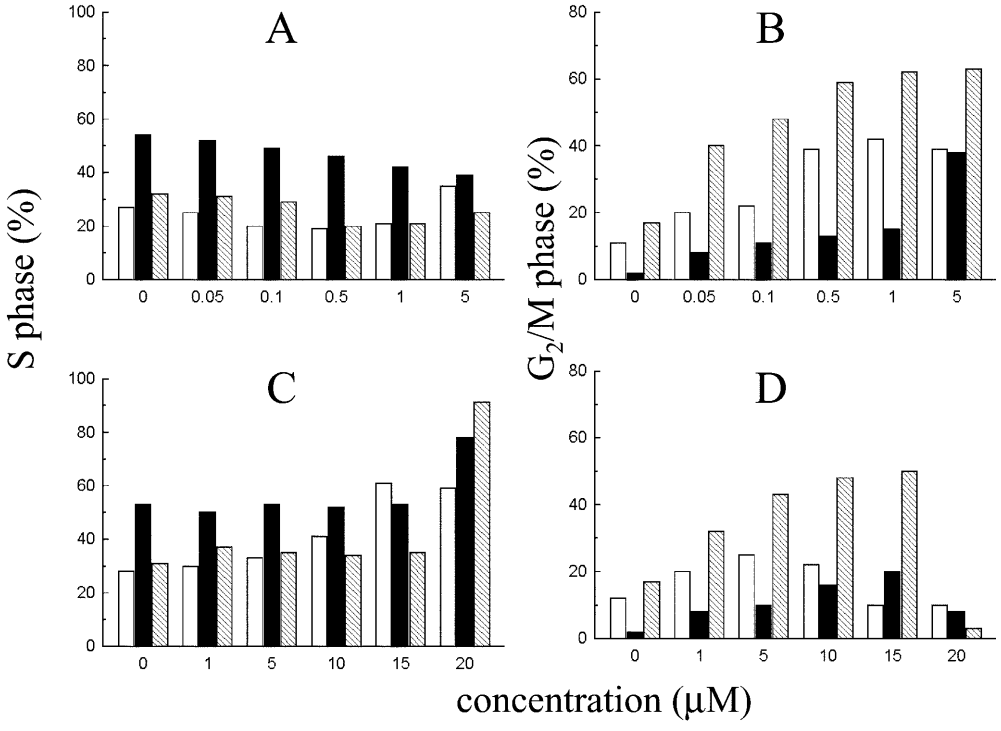

Fig. 1A–D Cell cycle analysis performed 24 h after a 1-h exposure to BBR 3464 (**A**, **B**) or to cisplatin (**C**, **D**) on the three neuroblastoma cell lines BE(2)M17 (*open bars*), SK-N-DZ (*filled bars*) and LAN-1 (*hatched bars*). This is a representative of three experiments

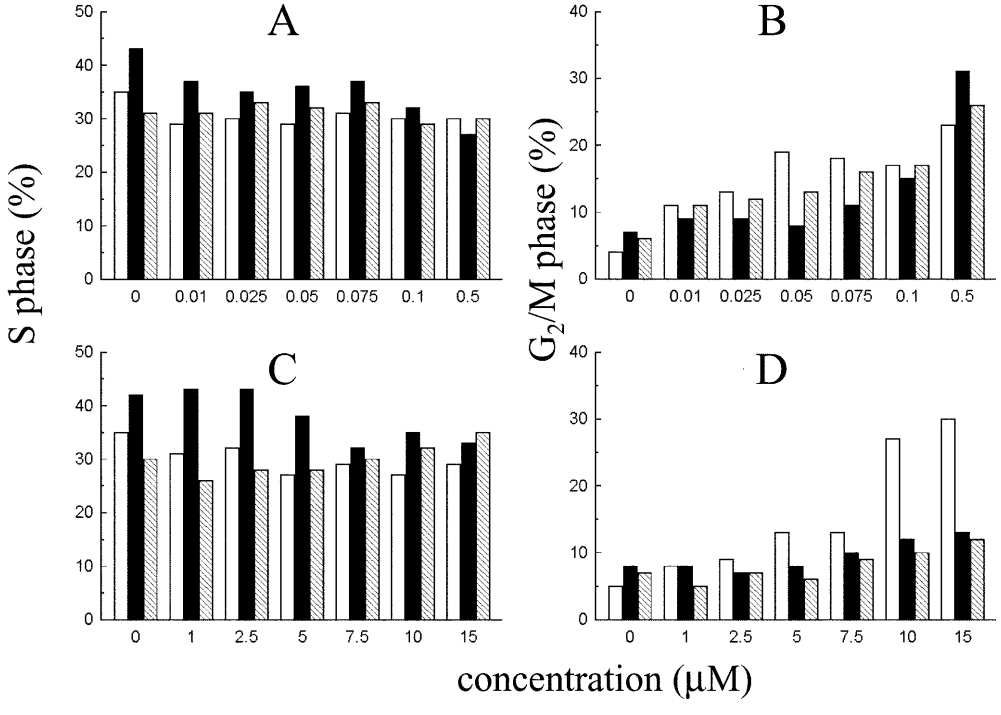

Fig. 2A–D Cell cycle analysis performed 72 h after a 1-h exposure to BBR 3464 (**A**, **B**) and to cisplatin (**C**, **D**) on the three neuroblastoma cell lines BE(2)M17 (*open bars*), SK-N-DZ (*filled bars*) and LAN-1 (*hatched bars*). This is a representative of three experiments

Table 3 Induction of apoptosis following drug treatment at three different inhibitory concentrations. Apoptosis level is calculated as the percentage of apoptotic cells in the whole cell population

Cell line	IC values	Drug concentration (μM)		Percent apoptosis[a]	
		BBR 3464	Cisplatin	BBR 3464	Cisplatin
SK-N-DZ	IC_{30}	0.1	2.6	3.5	3.5
	IC_{50}	0.37	5.4	11.0	10.0
	IC_{80}	3.00	14.0	24.5	38.0
BE(2)M17	IC_{30}	0.1	2.0	1.2	1.0
	IC_{50}	0.13	7.0	1.4	2.1
	IC_{80}	0.77	17.0	3.2	16.8
LAN-1	IC_{30}	0.014	2.73	0.2	2.0
	IC_{50}	0.067	6.67	0.3	4.5
	IC_{80}	0.8	27.0	23.0	26.0

[a] Treated cells over untreated cells

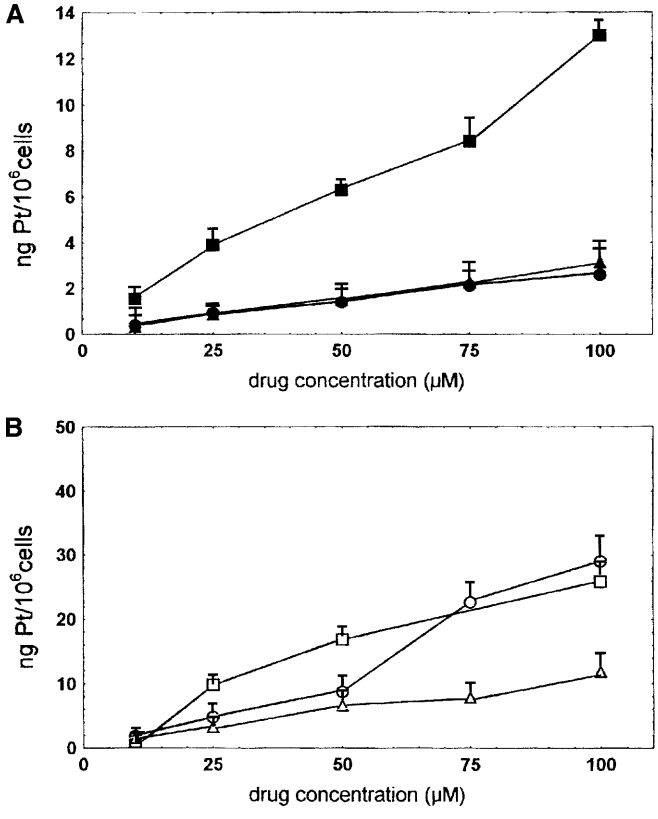

Fig. 3A, B Intracellular platinum accumulation in the three neuroblastoma cell lines following a 1-h exposure (**A**) to cisplatin (● LAN-1, ■ BE(2)M17, ▲ SK-N-DZ), and (**B**) to BBR 3464 (○ LAN-1, □ BE(2)M17, Δ SK-N-DZ). The mean values from two separate experiments are shown

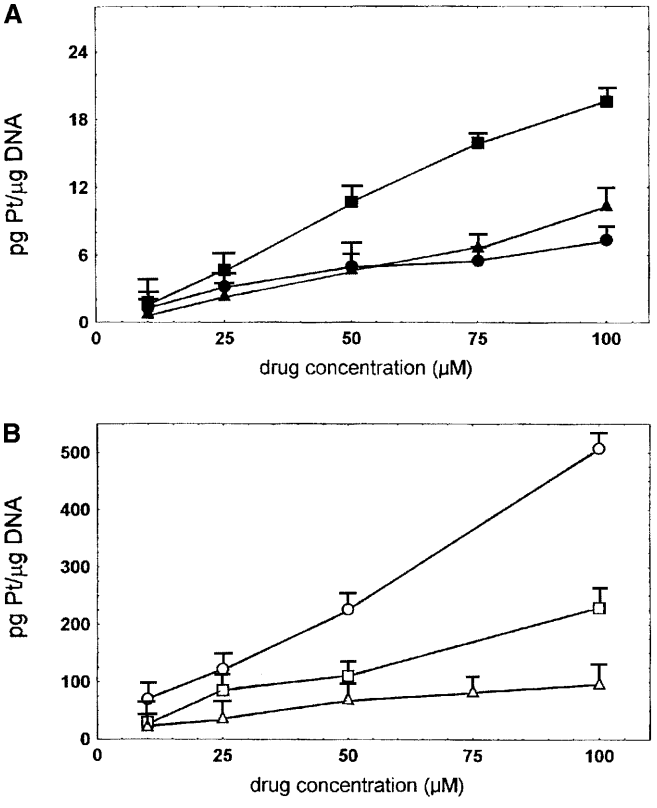

Fig. 4A, B Platinum DNA adduct formation following a 1-h exposure (**A**) to cisplatin (● LAN-1, ■ BE(2)M17, ▲ SK-N-DZ), and (**B**) to BBR 3464 (○ LAN-1, □ BE(2)M17, Δ SK-N-DZ). The mean values from two separate experiments are shown

Interestingly, very high TWI values (90%) were maintained up to the end of the observation time (33 and 42 days) in both SK-N-DZ and BE(2)M17 xenografts, respectively, treated with 0.35 mg/kg BBR 3464. Cisplatin administered in the q4d×3 schedule had no effect on tumor growth, while the single-dose cisplatin schedule was active in both tumor xenografts at the 12 mg/kg dose (TWI 90% and LCK 1.00 in SK-N-DZ, TWI 86% and LCK 1.09 in BE(2)M17).

Discussion

Neuroblastoma is one of the most common pediatric solid tumors and cisplatin is among the most active drugs utilized in the treatment of this disease. Although an initial response to a multiagent treatment protocol is a relatively frequent event, the development of drug resistance and tumor recurrence almost invariably follow. In this context, new platinum analogues non-cross-

Table 4 Antitumor activity of cisplatin and BBR 3464 against human tumor xenografts (*TWI* tumor weight inhibition, *LCK* $\log_{10}$ cell kill)

Tumor	Drug	Dose (mg/kg)[a]	Schedule	TWI (%)	LCK	Comments
SK-N-DZ	BBR 3464	0.30	q4d×3	81*	0.85	Active
		0.35	q4d×3	89**	1.30	Active
	Cisplatin	2	q4d×3	63	0.20	Inactive
		4	q4d×3	72	0.50	Inactive
		6	Single dose	73	0.55	Inactive
		12	Single dose	90	1.00	Active
BE(2)M17	BBR 3464	0.30	q4d×3	80*	0.68	Active
		0.35	q4d×3	90**	1.00	Active
	Cisplatin	2	q4d×3	50	0.20	Inactive
		4	q4d×3	68	0.30	Inactive
		6	Single dose	38	0.20	Inactive
		12	Single dose	86	1.09	Active

*$P < 0.05$, **$P < 0.01$, Student's *t*-test, BBR 3464 vs cisplatin (4 mg/kg)
[a]Maximum tolerated dose with the indicated schedule

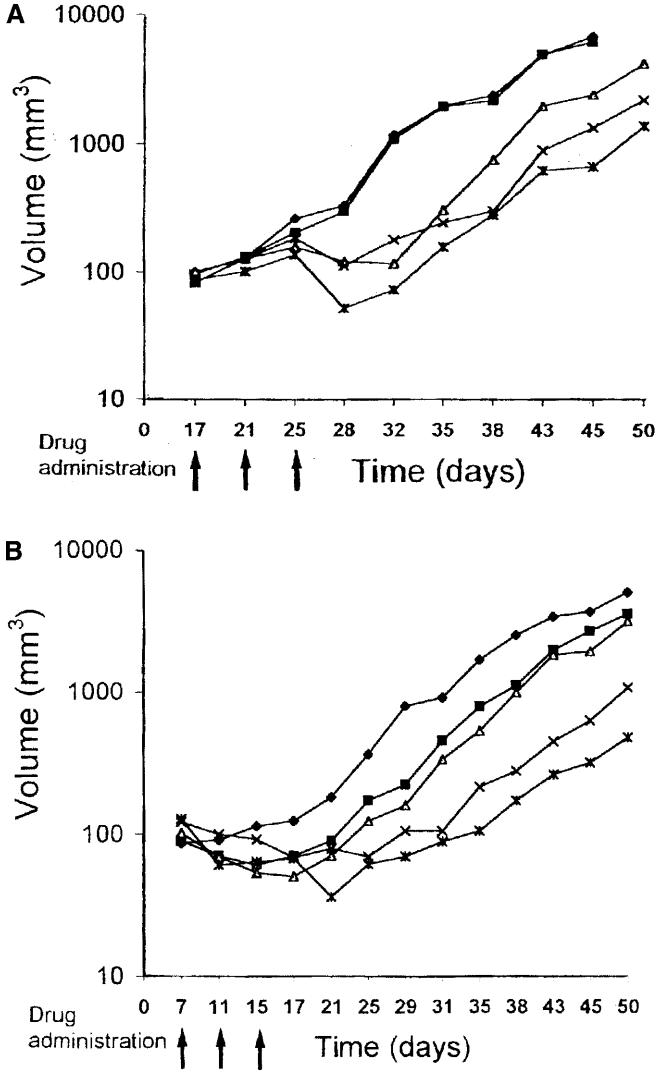

Fig. 5A, B Growth curves of SK-N-DZ (**A**) and BE(2)M17 (**B**) in nude mice treated with CDDP or BBR 3464. Drug treatment was administered three times at 4-day intervals (◆ untreated control, ■ cisplatin 2 mg/kg, Δ cisplatin 4 mg/kg, × BBR 3464 0.30 mg/kg, BBR 3464 0.35 mg/kg)

resistant with cisplatin may be a new and effective therapeutic tool to improve disease-free survival. Among the new platinum coordination compounds, the trinuclear platinum complex BBR 3464 appears the most promising and has been able to circumvent the inherent or acquired cisplatin-resistance in vitro and in vivo in a panel of human adult tumor models [8, 12].

In the present study we evaluated in vivo and in vitro the antitumor activity of BBR 3464 in human neuroblastoma cell lines. Our findings indicate that this compound is endowed with a definite antitumor effect in neuroblastoma. As compared to cisplatin, BBR 3464 appears more active although with differing degrees of antitumor effect depending on the cell line tested and the assay utilized. In the short-term growth inhibition assay, BBR 3464 was up to 100-fold more potent than cisplatin and even more potent than cisplatin when the antitumor effect was tested in the clonogenic assay. The difference in the antitumor effect of BBR 3464 on the different cell lines was evident in both assays, while cisplatin exerted a comparable antitumor activity in all cell lines. In addition, the antitumor effect of cisplatin, as evaluated in the clonogenic assay, produced IC_{50} values very close to those obtained in the short-term assay. The possible mechanism underlying the discrepancy between the two assays has recently been reviewed by Brown and Wouters [2]. It is evident that the clonogenic assay is the best way to assess the overall level of cell killing, while the short-term assay may underestimate the drug cytotoxic effect since this assay is mainly influenced by mechanisms causing early death, such as apoptosis or necrosis. Indeed, the induction of apoptosis by BBR 3464 in the dose range tested appeared to be lower than or at most comparable with that induced by cisplatin, so that the increased antitumor activity of BBR3464 appears not to be related to an apoptotic mechanism.

The higher antitumor effect of BBR 3464 was mainly related to delayed cytotoxicity, suggesting a different mechanism of action that may be at the basis of the lack of cross-resistance with cisplatin. The results

of other studies and our study indicate that BBR 3464 exposure results in higher DNA platinum binding than exposure to cisplatin and, as shown by Brabec et al. [1], in long-range intra- and interstrand crosslinks. Perego et al. [10] and Brabec et al. [1] have suggested that the different pattern of DNA lesions rather than the higher level of DNA-bound platinum might be responsible for the difference in the antitumor activity of BBR 3464. Cisplatin-induced lesions are repaired both by nucleotide excision and DNA mismatch repair systems. Most probably these mechanisms are not involved in repairing BBR 3464 genotoxic insults, since alterations in these mechanisms have been reported not to affect BBR 3464 activity [9]. A possible explanation is that, if DNA lesions produced by BBR 3464 are not restored by either of these DNA repair systems, progression through the cell cycle is hampered for a long time; the results of the cell cycle analysis appear to support this hypothesis.

The antitumor activity of BBR 3464 was confirmed in neuroblastoma xenografts obtained by injecting nude mice with SK-N-DZ and BE(2)M17 cells. The BBR 3464 MTD in nude mice bearing tumors was determined as 0.35 mg/kg administered i.v. in a q4d×3 schedule. BBR 3464 at this dose and in this schedule exerted an antitumor activity comparable to that of 12 mg/kg cisplatin administered i.v. as a single dose, with an approximately 11-fold cisplatin/BBR 3464 dose ratio. The longer-lasting growth-inhibitory effect of BBR 3464 (TWI 90% up to the end of the observation time) as compared to cisplatin was consistent with our in vitro data.

BBR 3464 entered clinical trials in June 1998 and phase I studies in adult patients are in progress [3]. Grade 3–4 neutropenia and diarrhea have been observed (15 patients) but no drug-related renal, neurological or lung toxicity. Doses administered in clinical trials (0.2–1.1 mg/m^2) are from 50- to 70-fold lower than cisplatin doses currently utilized in adult tumors. The encouraging results obtained in these studies in neuroblastoma experimental models and the manageable toxicity observed in adult studies could support a phase I clinical study to test BBR 3464 safety and efficacy in pediatric patients affected by advanced neuroblastoma. We hope that our preclinical results will be confirmed in the clinical setting and that children with neuroblastoma may benefit from this new compound.

Acknowledgements This work was partially supported by the Associazione Italiana Ricerca sul Cancro and by the Fondazione per l'Oncologia Pediatrica.

References

1. Brabec V, Kasparkova J, Vrana O, Novakova O, Cox JW, Qu Y, Farrell N (1999) DNA modifications by a novel bifunctional trinuclear platinum phase I anticancer agent. Biochemistry 38(21):6781
2. Brown JM, Wouters BG (1999) Apoptosis, p53, and tumor cell sensitivity to anticancer agents. Cancer Res 59:1391
3. Calvert PM, Hughes A, Azzabi A, Verrill M, Camboni G, Verdi E, Bernareggi A, Zucchetti M, Minoia C, Sessa C, Carmichael J, Calvert AH (2000) Phase I clinical and pharmacokinetic study of BBR 3464, a novel bifunctional platinum analogue, in patients with advanced solid tumours (abstract). Proc Am Soc Clin Oncol 19:921F
4. Cooper MJ, Hutchins GM, Cohen PS, Helman LJ, Israel MA (1991) Neuroblastoma cell lines mimic chromaffin neuroblastoma maturation. Prog Clin Biol Res 366:343
5. Evans AE, D'Angio GJ, Propert K, Anderson J, Hann HW (1987) Prognostic factors in neuroblastoma. Cancer 59:1853
6. Ferlini C, Di Cesare S, Rainaldi G, Malorni W, Samoggia P, Biselli R, Fattorossi A (1996) A flow cytometric analysis of the early phases of apoptosis by cellular and nuclear techniques. Cytometry 24:106
7. Kelland LR, Murrer BA, Abel G, Giandomenico CM, Mistry P, Harrap KR (1992) Ammine/amine platinum (IV) dicarboxylates: a novel class of platinum complex exhibiting selective cytotoxicity to intrinsically cisplatin-resistant human ovarian carcinoma cell lines. Cancer Res 52:822
8. Manzotti C, Pratesi G, Menta E, Di Domenico R, Cavalletti E, Fiebig HH, Kelland LR, Farrell N, Polizzi D, Supino R, Pezzoni G, Zunino F (2000) BBR 3464: a novel triplatinum complex exhibiting a preclinical profile of antitumor efficacy different from cisplatin. Clin Cancer Res 6:2626
9. Perego P, Zunino F, Carenini N, Giuliani F, Spinelli S, Howell SB (1998) Sensitivity to cisplatin and platinum-containing compounds of *Schizosaccharomyces plombe* rad *mutans*. Mol Pharmacol 54:213
10. Perego P, Caserini C, Gatti L, Carenini N, Romanelli S, Supino R, Colangelo D, Viano I, Leone R, Spinelli S, Pezzoni G, Manzotti C, Farrell N, Zunino F (1999) A novel trinuclear platinum complex overcomes cisplatin resistance in an osteosarcoma cell system. Mol Pharmacol 55:528
11. Pratesi G, De Cesare M, Caserini C, Perego P, Dal Bo L, Polizzi D, Supino R, Bigioni M, Manzini S, Iafrate E, Salvatore C, Casazza A, Arcamone F, Zunino F (1998) Improved efficacy and enlarged spectrum of activity of a novel anthracycline disaccharide analogue of doxorubicin against human tumor xenografts. Clin Cancer Res 4:2833
12. Pratesi G, Perego P, Polizzi D, Righetti SC, Supino R, Caserini C, Manzotti C, Giuliani FC, Pezzoni G, Tognella S, Spinelli S, Farrell N, Zunino F (1999) A novel trinuclear platinum complex effective against cisplatin-resistant tumours: hypersensitivity of p53-mutant human tumour xenografts. Br J Cancer 80:1912
13. Stram DO, Matthay KK, O'Leary M, Reynols CP, Haase GM, Atkinson JB, Brodeur GM, Seeger RC (1996) Consolidation chemoradiotherapy and autologous bone marrow transplantation versus continued chemotherapy for metastatic neuroblastoma: a report of two concurrent children's cancer group studies. J Clin Oncol 14:2417