

Nitric oxide prodrugs and metallochemotherapeutics: JS-K and CB-3-100 enhance arsenic and cisplatin cytolethality by increasing cellular accumulation

Jie Liu,¹ Chengxiu Li,¹ Wei Qu,¹ Elaine Leslie,¹ Challice L. Bonifant,² Gregory S. Buzard,³ Joseph E. Saavedra,³ Larry K. Keefer,² and Michael P. Waalkes¹

¹Inorganic Carcinogenesis Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute at NIEHS, Research Triangle Park, North Carolina; ²Chemistry Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute at Frederick, Frederick, Maryland; and ³Basic Research Program, Science Applications International Corporation-Frederick, Frederick, Maryland

Abstract

Development of chemotherapeutic resistance is a major cause of pharmacologic failure in cancer treatment. One mechanism of resistance in tumor cells is the overexpression of glutathione *S*-transferases (GSTs) that serve two distinct roles in the development of drug resistance via the formation of glutathione conjugates with drugs for their cellular efflux, and the inhibition of the mitogen-activated protein kinase pathway. To target GST-based resistance to chemotherapeutics, a series of nitric oxide (NO)-releasing diazeniumdiolates was synthesized and shown to release NO on reaction with GST and/or glutathione. Two diazeniumdiolates, JS-K [*O*²-(2,4-dinitrophenyl) 1-[(4-ethoxycarbonyl)piperazin-1-yl]diazene-1-ium-1,2-diolate] and CB-3-100 [*O*²-(2,4-dinitrophenyl) 1-[4-(*N,N*-diethylcarboxamido)piperazin-1-yl]diazene-1-ium-1,2-diolate], were studied on their ability in reversing arsenic and cisplatin resistance in a rat liver cell line that is tumorigenic and shows acquired tolerance to arsenic and cisplatin, with overexpression of GSTs. The enhanced cytolethality produced by the NO donors was accompanied by increased accumulation of arsenic and platinum within cells and by

enhanced activation of mitogen-activated protein kinase members *c-jun*-NH-kinase and extracellular signal-regulated kinase. Our data indicate that JS-K and CB-3-100 are promising lead compounds for the possible development of a novel class of adjuvant chemotherapeutic agents potentially capable of reversing arsenic and cisplatin resistance in certain tumor cells. [Mol Cancer Ther 2004;3(6):709–14]

Introduction

Resistance to anticancer agents is a common reason for treatment failures. One important component of resistance can be the overexpression of glutathione *S*-transferases (GSTs; refs. 1-3). GSTs are the enzymes that catalyze the conjugation of xenobiotics with cellular reduced glutathione (GSH). Overexpression of GSTs, particularly GST- π , often allows tumor cells to gain a selective survival advantage over normal cells by enhanced detoxification through GSH conjugation and/or efflux of the conjugate through the multidrug resistance protein (MRP) pumps (2-5). Overexpression of GSTs can also act as inhibitors of mitogen-activated protein kinases (MAPKs; ref. 3), thereby reducing the cell killing by anticancer drugs. This selection becomes critical in the development of multidrug chemotherapeutic resistance.

To target GST-based chemotherapeutic resistance in GST-overexpressing tumors, nitric oxide (NO) prodrugs of the *O*²-aryl diazeniumdiolate class were designed to act as substrates for GST- π . One of these is JS-K [*O*²-(2,4-dinitrophenyl) 1-[(4-ethoxycarbonyl)piperazin-1-yl]diazene-1-ium-1,2-diolate], already showed to interact with GSTs *in vitro* and within cells to release NO (6) and to activate MAPK pathways (7). In this study, we studied two *O*²-aryl diazeniumdiolates, JS-K and CB-3-100 [*O*²-(2,4-dinitrophenyl) 1-[4-(*N,N*-diethylcarboxamido)piperazin-1-yl]diazene-1-ium-1,2-diolate], on their ability to overcome drug resistance in the CAsE (Chronic As-Exposed cells) cell line; this is a tumorigenic line derived from rat liver that has been shown to overexpress GST- π , MRP1, MRP2, and P-glycoprotein and to have acquired resistance to arsenite, cisplatin, and other anticancer agents, such as doxorubicin, vinblastine, and actinomycin D (8, 9). We have found that both JS-K and CB-3-100 were effective in increasing cisplatin and arsenic cytolethality, likely by increasing their intracellular accumulation and by enhancing MAPK pathways.

Materials and Methods

Chemicals

JS-K (10) and CB-3-100 (11) were synthesized as detailed below, and their structures are shown in Fig. 1. CB-3-100

Received 12/16/03; revised 3/18/04; accepted 4/2/04.

Grant support: National Cancer Institute/NIH under contract NO1-CO-12400.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: Dr. Elaine Leslie is the recipient of a Canadian Institutes of Health Fellowship and the Davis Charitable Foundation Research Fellowship Award.

Requests for reprints: Michael P. Waalkes, Inorganic Carcinogenesis Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute at NIEHS, mail drop FO-09, 111 Alexander Drive, Research Triangle Park, NC 27709. Phone: 919-541-2328; Fax: 919-541-3970. E-mail: Waalkes@niehs.nih.gov

Copyright © 2004 American Association for Cancer Research.

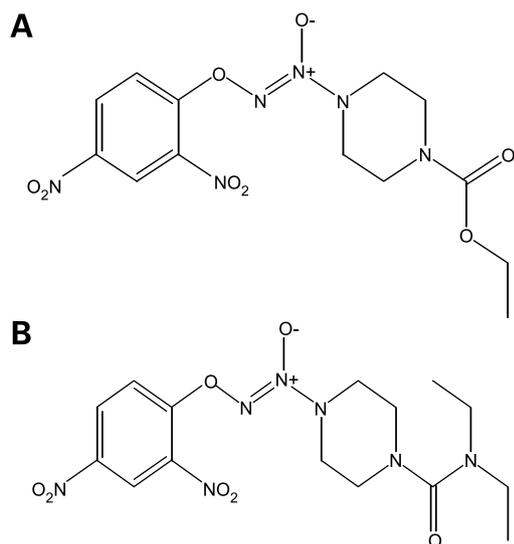


Figure 1. Structures of JS-K (A) and CB-3-100 (B).

was prepared as outlined below. All other chemicals were of reagent grade and commercially available. NO was purchased from Matheson Gas Products (Montgomeryville, PA). UV spectra were recorded on a Hewlett-Packard Model 8452A diode array spectrophotometer. Nuclear magnetic resonance (NMR) spectra were collected with a Varian XL-200 NMR Spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Elemental analyses were done by Atlantic Microlab, Inc. (Norcross, GA).

JS-K

A solution of 5.3 g (0.0286 mol) of *N,N*-diethylcarbamoyl piperazine in 15 mL of methanol was placed in a Parr bottle and treated with 6.7 mL (0.0306 mol) of methanolic sodium methoxide. The solution was purged with 40 psi of NO and stirred at 25°C for 72 hours. The solid precipitate was filtered, washed with ether, and dried to give 4.54 g (60%) of diazeniumdiolate: UV (0.1 mol/L NaOH) λ_{\max} (ϵ) 250 nm (9.8 mmol/L⁻¹ cm⁻¹); ¹H NMR (0.1 NaOD in D₂O) δ 1.13 (t, 6 H), 3.15 (m, 4 H), 3.27 (d, 4 H), 3.45 (m, 4 H).

CB-3-100

The diazeniumdiolate salt of the previous paragraph (1.48 g; 5.8 mmol) was dissolved in 10 mL of 5% aqueous sodium bicarbonate solution and cooled to 0°C under argon. To the cold solution was added a solution of 608 μ L (4.84 mmol) of 2,4-dinitrofluorobenzene in 9.5 mL of *t*-butyl alcohol. The resulting mixture was allowed to warm to room temperature and stirred for 24 hours. The yellow precipitate that formed was collected by filtration and washed with water to give 1.75 g of crude product. Recrystallization from ethanol gave 1.5 g of analytically pure crystals: M.P. 74°C to 78°C; UV (ethanol) λ_{\max} (ϵ) 300 nm (12.7 mmol/L⁻¹ cm⁻¹); ¹H NMR (CDCl₃) δ 1.15 (t, 6 H), 3.25 (q, 4 H), 3.44 (t, 4 H), 3.67 (t, 4 H), 7.68 (d, 1 H, J = 9.2 Hz), 8.46 (m, 1 H), 8.89 (d, 1 H, J = 2.7 Hz); ¹³C NMR

(CDCl₃) δ 13.17, 41.78, 45.66, 50.33, 117.65, 122.17, 129.08, 137.29, 142.36, 153.81, 163.59.

C₁₅H₂₁N₇O₇

Calculated : C43.80, H5.15, N23.83

Found : C43.83, H5.18, N23.82

Cell Culture and Treatments

CAsE cells, a rat liver epithelial cell line developed by chronic exposure of rat liver TRL 1215 cells to 500 nmol/L arsenite for 24 weeks, have been described previously (8). These cells form malignant tumors on inoculation into nude mice. Cells were cultured in William's E medium containing 10% fetal bovine serum in the absence of arsenic for at least 2 weeks before the experiments. Cells were cultured in 96-well plates for cytotoxicity evaluation or in 6-well plates for cellular accumulation studies. At 90% confluence, cells were treated with JS-K or CB-3-100 (0, 3, 10, 30, and 100 μ mol/L, dissolved in DMSO and diluted in medium), together with various concentrations of arsenic (0 to 200 μ mol/L sodium arsenite, dissolved in distilled water and diluted in medium) or cisplatin (0 to 1000 μ mol/L, dissolved in saline and diluted in medium) for up to 24 hours.

Chemotherapeutic Lethality

The Promega non-radioactive cell proliferation assay was used to determine acute cytotoxicity as defined by metabolic integrity. This assay measures the amount of formazan produced by metabolic conversion of Owen's reagent [(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt, MTS)] by dehydrogenase enzymes in the mitochondria of metabolically active, and, therefore, viable, cells. The quantity of formazan product, as measured by absorbance at 490 nm, is directly proportional to the number of living cells. Data are expressed as percentage of control untreated cells.

Cellular Accumulation of Arsenic and Cisplatin

The CAsE cells were grown to 90% confluence, and then incubated with medium containing 25 μ mol/L arsenite or 100 μ mol/L cisplatin, in the presence or absence of JS-K (10 and 30 μ mol/L) or CB-3-100 (10 and 30 μ mol/L) for 2, 4, 8, and 24 hours. At the end of incubation, medium was removed and cells were thoroughly washed 4 times with PBS and harvested. After sonication, cellular protein was determined by the Bradford dye-binding assay (Bio-Rad, Hercules, CA). The cell suspensions were then completely digested in nitric acid, heated at 70°C overnight to dryness, and redissolved in distilled water. Total arsenic, which includes both inorganic and organic forms, and platinum were determined using graphite furnace atomic absorption spectrometry (Perkin-Elmer AAnalyst100, Norwalk, CT), and normalized with cellular protein content.

Western Blot Analysis

Protein samples (30 μ g) derived from the various cell preparations were subjected to SDS-PAGE and transferred

onto nitrocellulose membranes. The membranes were blocked with 5% nonfat dry milk in TBS containing 0.05% Tween 20 (TBST) and probed with antibodies against phosphorylated *c-jun*-NH-kinase 1/2 (JNK1/2) and phosphorylated extracellular signal-regulated kinase 1/2 (ERK1/2; Cell Signaling Technology, Beverly, MA). After incubation with secondary antibodies, immunoblots were visualized with the LumiGlo detection method (Cell Signaling Technology).

Statistics

Data are means \pm SE of triplicate wells from three experiments using separate cell preparations. For comparison between two groups, the Student *t* test was used; for comparisons among three or more groups, the data were analyzed by ANOVA, followed by a Ducann's multiple-range test.

Results

Toxicity of NO Donors

Preliminary experiments indicated that incubating cells with JS-K or CB-3-100 at concentrations below 100 $\mu\text{mol/L}$ for 24 hours did not have significant effects on cell viability (also see Fig. 2 at the zero-arsenite NO-donor-alone points). Thus, 100 $\mu\text{mol/L}$ concentrations or less were used in all subsequent studies. The solubility of JS-K is limited in aqueous solution (≈ 10 $\mu\text{mol/L}$) (10); however, this solubility was improved in media containing serum to ≈ 100 $\mu\text{mol/L}$ without visible precipitation for several hours. In cells cultured at 37°C with serum (10%)-containing media, no apparent precipitation was noted up to 24 hours.

Increasing Arsenite Toxicity with Enhanced Arsenic Accumulation

The effects of JS-K and CB-3-100 on arsenic cytotoxicity are shown in Fig. 2. Arsenic (as sodium arsenite) induced a concentration-dependent reduction in cell survival. JS-K and CB-3-100 enhanced arsenite cytotoxicity in a concentration-dependent manner, particularly at concentrations above 30 $\mu\text{mol/L}$. The LC_{50} for arsenite in the absence of the NO donors was 200 $\mu\text{mol/L}$, and this was decreased to 60 and 50 $\mu\text{mol/L}$ in the presence of 100 $\mu\text{mol/L}$ JS-K and CB-3-100, respectively. To determine whether JS-K and CB-3-100 enhance arsenite toxicity by increasing cellular arsenic accumulation, the concentration of arsenic in CAsE cells was determined at 2, 4, 8, and 24 hours after incubation with a non-toxic concentration of arsenic (25 $\mu\text{mol/L}$) with or without non-toxic concentrations of JS-K or CB-3-100 (10 to 30 $\mu\text{mol/L}$). The results clearly showed that co-treatment of cells with the NO donors do not have apparent effects on cellular arsenic uptake at earlier times (2 to 4 hours), but increased cellular arsenic accumulation thereafter (8 and 24 hours) in a concentration-dependent manner (Fig. 3). At 30 $\mu\text{mol/L}$ of NO donors, cellular arsenic levels were elevated 2.8- and 2.6-fold by JS-K and CB-3-100, respectively, at 24 hours.

Enhancing Arsenic Activation of MAPKs

Effect of the NO donors on acute arsenic activation of phosphorylated MAPKs was further examined. The JNK

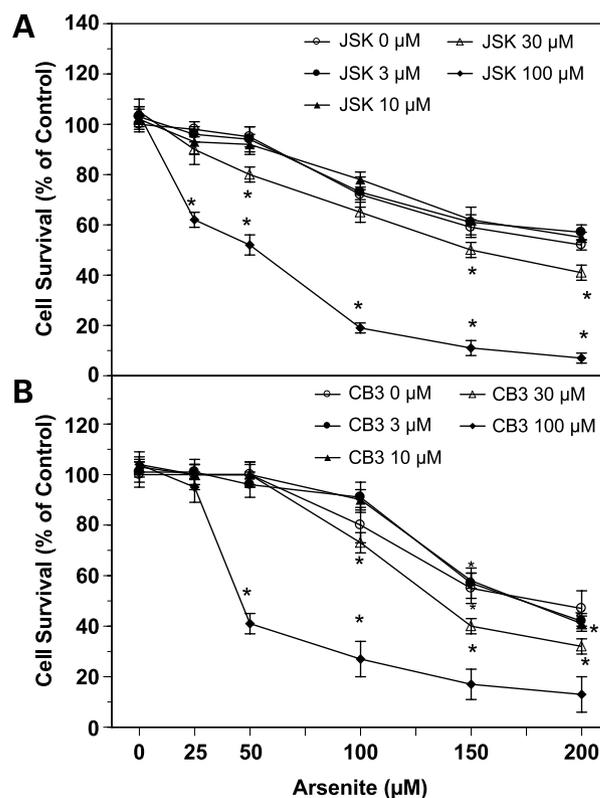


Figure 2. Effects of JS-K (A) and CB-3-100 (B) on arsenic toxicity. CAsE cells were incubated with various concentrations of sodium arsenite (0 to 200 $\mu\text{mol/L}$), together with various concentrations of JS-K or CB-3-100 (0 to 100 $\mu\text{mol/L}$). Cell viability was assessed via MTS assay 24 hours after treatment. Points, means of three separate cell preparations; bars, SE. *, significantly different from controls, $P < 0.05$.

and ERK pathways have been implicated in the regulation of tumor cell killing by various chemotherapeutics, and were suppressed by GST overexpression (3). The levels of phosphorylated JNK1/2 and ERK1/2 were determined by Western blot analysis (Fig. 4). Both JS-K and CB-3-100 treatment enhanced arsenic activation of phosphorylated JNK1/2 and ERK1/2.

Increasing Cisplatin Toxicity with Increased Platinum Accumulation

The effects of JS-K and CB-3-100 on cisplatin cytotoxicity are shown in Fig. 5. Cisplatin induced a concentration-dependent reduction in cell survival. Both JS-K and CB-3-100 significantly enhanced cisplatin cytotoxicity in a concentration-dependent manner, particularly at concentrations greater than 30 $\mu\text{mol/L}$. The LC_{50} for cisplatin was 580 $\mu\text{mol/L}$, and this was decreased to 150 and 100 $\mu\text{mol/L}$ in the presence of 100 $\mu\text{mol/L}$ JS-K and CB-3-100, respectively. To determine whether JS-K and CB-3-100 enhance cisplatin toxicity by increasing cellular platinum accumulation, the concentration of platinum in cells was determined 2, 4, 8, and 24 hours after incubation with a non-cytotoxic concentration of cisplatin (100 $\mu\text{mol/L}$)

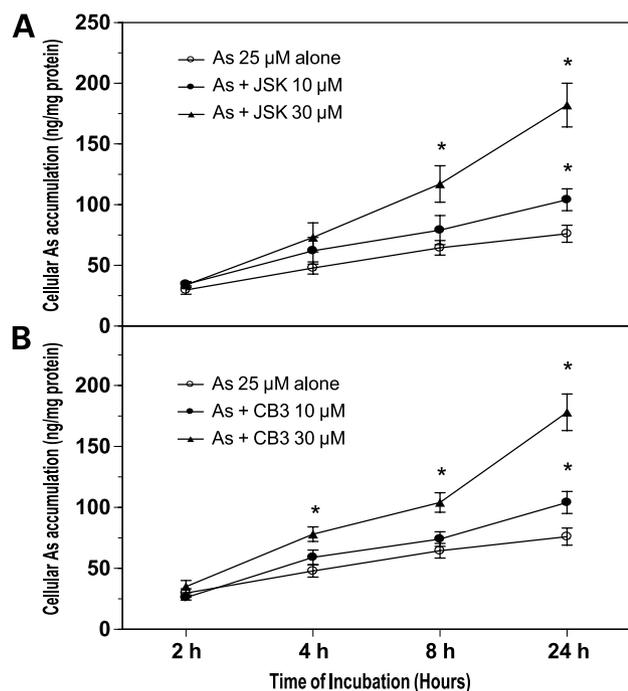


Figure 3. Effects of JS-K (A) and CB-3-100 (B) on cellular arsenic accumulation. CASe cells were incubated with 12.5 $\mu\text{mol/L}$ sodium arsenite together with 10 and 30 $\mu\text{mol/L}$ JS-K or CB-3-100 for 2, 4, 8, and 24 hours. The cellular arsenic concentration was determined by graphite furnace atomic absorption spectrometry. Points, means of three separate cell preparations; bars, SE. *, significantly different from arsenic-only controls, $P < 0.05$.

together with non-toxic concentrations of JS-K or CB-3-100 (10 to 30 $\mu\text{mol/L}$; Fig. 6). The results clearly showed that co-treatment of cells with JS-K and CB-3-100 resulted in concentration-dependent increases in cellular platinum accumulation. Cellular platinum content showed maximal increases of 3.6- and 3.1-fold, respectively, with JS-K and CB-3-100 co-exposure, as compared with cisplatin alone. Again, the effects of NO donors on the uptake of cisplatin at early time points (2 to 4 hours) were not appreciable.

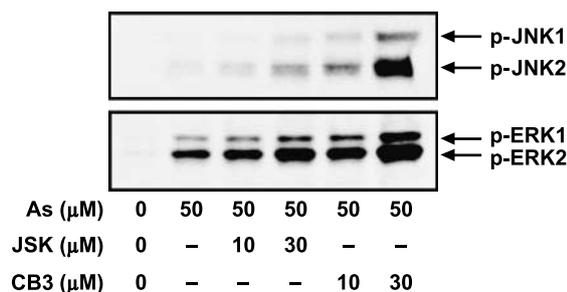


Figure 4. Effect of JS-K and CB-3-100 on arsenic activation of MAPKs. CASe cells were incubated with 50 $\mu\text{mol/L}$ sodium arsenite together with 10 and 30 $\mu\text{mol/L}$ JS-K or CB-3-100 for 3 hours. The levels of phosphorylated JNK1/2 and ERK1/2 were determined by Western blot analysis as detailed in Materials and Methods. Blots, typical result of three independent experiments.

Discussion

The present study clearly shows that JS-K and CB-3-100 are effective in increasing arsenic and cisplatin toxicity in tumorigenic CASe cells, apparently by increasing cellular accumulation of arsenic and platinum, and by enhancing activation of MAPK pathways, probably through their interactions with GSTs.

GSTs have long been known to release NO from certain alkyl nitrates (12). JS-K (10) and CB-3-100 were both designed for their interactions with GST. Increased NO release has been shown *in vitro* using purified GSTs and the molecular interactions of JS-K with GSTs to produce NO have been shown (6, 10). In this regard, it has been shown that NO carriers *S*-nitrosoglutathione and dinitrosyl-dithiol-iron complexes are capable of inhibiting GSTs (13, 14) in the cells, and GSTs are also susceptible to inactivation by NO-derived oxidants (15). GSTs may even act as NO storage systems (16, 17). The NO donor, DEA/NO, has been shown to inhibit cytosolic GST activities by *S*-nitrosylation, particularly for GST- π (18). Thus, targeting GST by designing NO donors is a plausible and feasible strategy for drug development.

Arsenic and arsenic-containing compounds have re-emerged as remarkably effective cancer chemotherapeutic

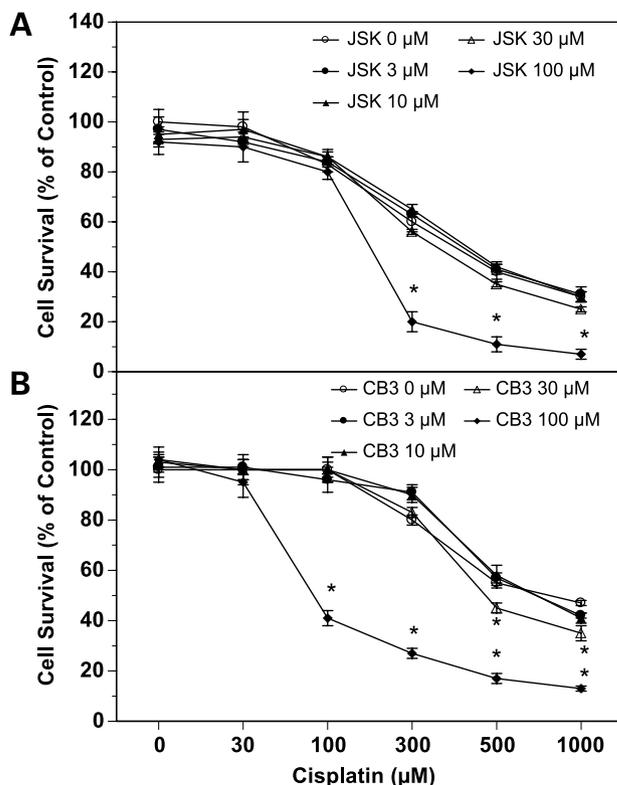


Figure 5. Effects of JS-K (A) and CB-3-100 (B) on cisplatin toxicity. CASe cells were incubated with various concentrations of cisplatin (0 to 1000 $\mu\text{mol/L}$), together with various concentrations of JS-K or CB-3-100 (0 to 100 $\mu\text{mol/L}$). The cell viability was assessed 36 hours after treatment. Points, means of three separate cell preparations; bars, SE. *, significantly different from controls, $P < 0.05$.

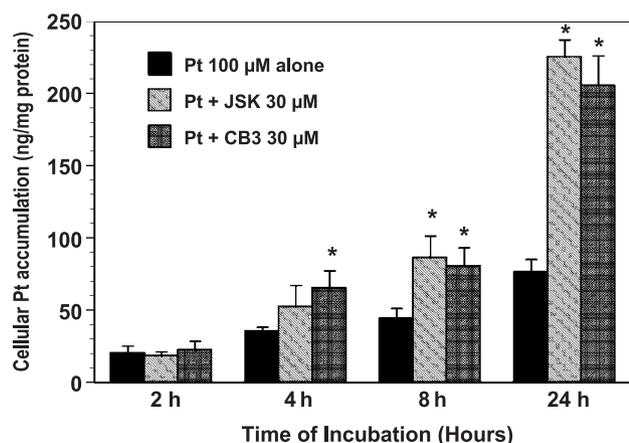


Figure 6. Effects of JS-K and CB-3-100 on cellular platinum (Pt) accumulation. CAsE cells were incubated with 100 μ mol/L cisplatin together with 30 μ mol/L JS-K or CB-3-100 for 2, 4, 8, and 24 hours. The cellular platinum concentration was determined by graphite furnace atomic absorption spectrometry. Points, means of three separate cell preparations; bars, SE. *, significantly different from arsenic-only controls, $P < 0.05$.

agents (19). In particular, arsenicals are very effective in the treatment of acute promyelocytic leukemia (20). There are also some promising efforts to expand the use of arsenicals to solid tumors. There is, however, the potential for resistance to arsenic chemotherapy to develop after the long-term use of the metalloid, as it does in human and rodent cells chronically exposed to arsenic (9, 21). In this regard, GSH depletion coupled with arsenic exposure reverses acquired arsenic resistance (21-23). Recent studies indicate that conjugation of arsenic with GSH is an important step for hepatobiliary arsenic efflux via MRP2 pumps (24, 25). Inhibition of GST- π increases cellular arsenic accumulation and cytotoxicity in a variety of cultured cells (9, 21, 26, 27). Thus, it is likely that increased arsenic cytotoxicity with JS-K or CB-3-100 co-treatment could be coupled to increased cellular accumulation of arsenic, possibly through the inactivation of GSTs and the decreased formation of GSH-arsenic conjugates for cellular efflux. GSTs can also act as inhibitors of MAPKs (3), and the activation of MAPKs is an important mechanism for hepatoma cell killing by JS-K (7). In the present study, both JS-K and CB-3-100 enhanced activation of phosphorylated JNK1/2 and ERK1/2, supporting the role of GST inhibition on MAPK activation (3). In any event, these NO prodrugs are clearly effective in increasing arsenic retention and MAPKs activation in tumorous cells, and, thereby, increasing the rate of cell killing.

Cisplatin is used extensively as a single chemotherapeutic agent or in combination with other drugs and/or radiation (28). Acquired resistance to cisplatin is a major problem in cancer treatment. A number of mechanisms have been proposed for the development of cisplatin resistance, but the most probable one involves the enhancement of a GSH-Pt adduct for cellular efflux (29, 30). Thus, inactivation of GST and the resulting blockage of the

formation of the GSH-Pt complex could reverse cisplatin resistance in tumor cells (31). In a fashion similar to arsenic, the increased sensitivity to cisplatin cytotoxicity by JS-K and CB-3-100 observed in the present study could also be due, at least in part, to the increased accumulation of the platinum compound within cells, as well as activation of the MAPK pathways (data not shown).

The diazeniumdiolate NO donor JS-K has also been shown to induce apoptosis in the HL-60 human myeloid leukemia cells (6), as well as in solid tumor cells, such as the prostate tumor cell line PPC-1, colon tumor cell line DLA-1, the mammary tumor cell line MethA, and Hep 3B hepatoma cells (6, 7). JS-K inhibits xenograft growth of HL-60 and PPC-1 cells when implanted in mice (6). In addition, other NO donors were shown to exert cytostatic effects against leukemia P388 and L1210 cells, and inhibit the development of drug resistance to cyclophosphamide (32). The diazeniumdiolate NO donor DETA/NO improves cisplatin efficacy against head and neck squamous cell carcinoma (33). In this regard, CAsE cells are malignantly transformed cells, producing aggressive, malignant tumors on inoculation into nude mice (8). Thus, evidence from a variety of systems with diazeniumdiolate NO donors forecasts potential utility for them as adjuvant to chemotherapeutic attack against actual tumors.

In summary, this study shows that two novel diazeniumdiolates JS-K and CB-3-100 are effective in increasing arsenic and cisplatin toxicity in previously highly resistant cells, apparently by increasing cellular accumulation of the metal and by enhancing activation of the MAPK pathways. Thus, the diazeniumdiolates JS-K and CB-3-100 could be promising candidates for possible development of a new class of adjuvant prodrugs for cancer chemotherapy, particular against drug-resistant tumors.

Acknowledgments

We thank Dr. Jingbo Pi and Yaxiong Xie for critical review and Catherine Harris for technical assistance.

References

1. Tew KD, Ronai Z. GST function in drug and stress response. *Drug Resist Updat* 1999;2:143-7.
2. Gate L, Tew KD. Glutathione S-transferases as emerging therapeutic targets. *Exp Opin Ther Targets* 2001;5:477-89.
3. Townsend DM, Tew KD. The role of glutathione-S-transferase in anticancer drug resistance. *Oncogene* 2003;22:7369-75.
4. Leslie EM, Deeley RG, Cole SP. Toxicological relevance of the multidrug resistance protein 1, MRP1 (ABCC1) and related transporters. *Toxicology* 2001;167:3-23.
5. Borst P, Elferink PO. Mammalian ABC transporters in health and disease. *Annu Rev Biochem* 2002;71:537-92.
6. Shami PJ, Saavedra JE, Wang LY, et al. JS-K, a glutathione/glutathione S-transferase-activated nitric oxide donor of the diazeniumdiolate class with potent antineoplastic activity. *Mol Cancer Ther* 2003;2:409-17.
7. Ren Z, Kar S, Wang Z, Wang M, Saavedra JE, Carr BI. JS-K, a novel non-ionic diazeniumdiolate derivative, inhibits Hep 3B hepatoma cell growth and induces c-Jun phosphorylation via multiple MAP kinase pathways. *J Cell Physiol* 2003;197:426-34.
8. Zhao CQ, Young MR, Diwan BA, Coogan TP, Waalkes MP. Association

- of arsenic-induced malignant transformation with DNA hypomethylation and aberrant gene expression. *Proc Natl Acad Sci USA* 1997;94:10907-12.
9. Liu J, Chen H, Miller DS, et al. Overexpression of glutathione *S*-transferase π and multidrug resistance transport proteins is associated with acquired tolerance to inorganic arsenic. *Mol Pharmacol* 2001; 60:302-9.
 10. Saavedra JE, Srinivasan A, Bonifant CL, et al. The secondary amine/nitric oxide complex ion $R_2N[N(O)NO]^-$ as nucleophile and leaving group in SNAr reactions. *J Org Chem* 2001;66:3090-8.
 11. Kushner S, Brancone LM, Flewitt RI, et al. Experimental chemotherapy of filariasis. V. The preparation of derivatives of piperazine. *J Org Chem* 1948;13:144-53.
 12. Singhal SS, Piper JT, Srivastava SK, et al. Rabbit aorta glutathione *S*-transferases and their role in bioactivation of trinitroglycerin. *Toxicol Appl Pharmacol* 1996;140:378-86.
 13. Keese MA, Bose M, Mulsch A, Schirmer RH, Becker K. Dinitrosyl-dithiol-iron complexes, nitric oxide (NO) carriers *in vivo*, as potent inhibitors of human glutathione reductase and glutathione-*S*-transferase. *Biochem Pharmacol* 1997;54:1307-13.
 14. De Maria F, Pedersen JZ, Caccuri AM, et al. The specific interaction of dinitrosyl-diglutathionyl-iron complex, a natural NO carrier, with the glutathione transferase superfamily: suggestion for an evolutionary pressure in the direction of the storage of nitric oxide. *J Biol Chem* 2003;278:42283-93.
 15. Wong PS, Eiserich JP, Reddy S, Lopez CL, Cross CE, van der Vliet V. Inactivation of glutathione *S*-transferases by nitric oxide-derived oxidants: exploring a role for tyrosine nitration. *Arch Biochem Biophys* 2001; 394:216-28.
 16. Lo BM, Nuccetelli M, Caccuri AM, et al. Human glutathione transferase P1-1 and nitric oxide carriers; a new role for an old enzyme. *J Biol Chem* 2001;276:42138-45.
 17. Turella P, Pedersen JZ, Caccuri AM, et al. Glutathione transferase superfamily behaves like storage proteins for dinitrosyl-diglutathionyl-iron complex in heterogeneous systems. *J Biol Chem* 2003;278:42294-9.
 18. Ji Y, Toader V, Bennett BM. Regulation of microsomal and cytosolic glutathione *S*-transferase activities by *S*-nitrosylation. *Biochem Pharmacol* 2002;63:1397-404.
 19. Wang ZY. Arsenic compounds as anticancer agents. *Cancer Chemother Pharmacol* 2001;48 Suppl 1:S72-6.
 20. Waxman S, Anderson KC. History of the development of arsenic derivatives in cancer therapy. *Oncologist* 2001;6 Suppl 2:3-10.
 21. Brambila EM, Achanzar WE, Qu W, Webber MM, Waalkes MP. Chronic arsenic-exposed human prostate epithelial cells exhibit stable arsenic tolerance: mechanistic implications of altered cellular glutathione and glutathione *S*-transferase. *Toxicol Appl Pharmacol* 2002;183:99-107.
 22. Davison K, Cote S, Mader S, Miller WH. Glutathione depletion overcomes resistance to arsenic trioxide in arsenic-resistant cell lines. *Leukemia* 2003;17:931-40.
 23. Shimizu M, Hochadel JF, Fulmer BA, Waalkes MP. Effect of glutathione depletion and metallothionein gene expression on arsenic-induced cytotoxicity and *c-myc* expression *in vitro*. *Toxicol Sci* 1998;45:204-11.
 24. Gregus Z, Gyurasics A. Role of glutathione in the biliary excretion of the arsenical drugs trimelarsan and melarsoprol. *Biochem Pharmacol* 2000;59:1375-85.
 25. Kala SV, Neely MW, Kala G, et al. The MRP2/cMOAT transporter and arsenic-glutathione complex formation are required for biliary excretion of arsenic. *J Biol Chem* 2000;275:33404-8.
 26. Wang HF, Lee TC. Glutathione *S*-transferase π facilitates the excretion of arsenic from arsenic-resistant Chinese hamster ovary cells. *Biochem Biophys Res Commun* 1993;192:1093-9.
 27. Wang Z, Dey S, Rosen BP, Rossman TG. Efflux-mediated resistance to arsenicals in arsenic-resistant and -hypersensitive Chinese hamster cells. *Toxicol Appl Pharmacol* 1996;137:112-9.
 28. Trimmer EE, Essigmann JM. Cisplatin. *Essays Biochem* 1999;34:191-211.
 29. Goto S, Yoshida K, Morikawa T, Urata Y, Suzuki K, Kondo T. Augmentation of transport for cisplatin-glutathione adduct in cisplatin-resistant cancer cells. *Cancer Res* 1995;55:4297-301.
 30. Akiyama S, Chen ZS, Sumizawa T, Furukawa T. Resistance to cisplatin. *Anticancer Drug Des* 1999;14:143-51.
 31. Gosland M, Lum B, Schimmelpfennig J, Baker J, Doukas M. Insights into mechanisms of cisplatin resistance and potential for its clinical reversal. *Pharmacotherapy* 1996;16:16-39.
 32. Kononova NP, Goncharova SA, Volkova LM, Raevskaia TA, Eremlenko LT, Korolev AM. Nitric oxide donor increases the effectiveness of cytostatic therapy and inhibits the development of drug resistance. *Vopr Onkol* 2003;49:71-5. (English Abstract PMID: 12715374)
 33. Azizzadeh B, Yip HT, Blackwell KE, et al. Nitric oxide improves cisplatin cytotoxicity in head and neck squamous cell carcinoma. *Laryngoscope* 2001;111:1896-900.

Molecular Cancer Therapeutics

Nitric oxide prodrugs and metallochemotherapeutics: JS-K and CB-3-100 enhance arsenic and cisplatin cytolethality by increasing cellular accumulation

Jie Liu, Chengxiu Li, Wei Qu, et al.

Mol Cancer Ther 2004;3:709-714.

Updated version Access the most recent version of this article at:
<http://mct.aacrjournals.org/content/3/6/709>

Cited articles This article cites 31 articles, 9 of which you can access for free at:
<http://mct.aacrjournals.org/content/3/6/709.full#ref-list-1>

Citing articles This article has been cited by 1 HighWire-hosted articles. Access the articles at:
<http://mct.aacrjournals.org/content/3/6/709.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://mct.aacrjournals.org/content/3/6/709>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.