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ORIGINAL ARTICLE

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Modulation of plasma thiols and mixed disulfides by BNP7787 in patients receiving paclitaxel/cisplatin therapy

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Abstract *Background*: BNP7787 (disodium 2,2′-dithio-bis-ethane sulfonate) was evaluated in a phase I clinical trial with paclitaxel and cisplatin to assess the safety and potential efficacy for preventing or reducing cisplatin- and paclitaxel-induced toxicities. During this trial the effects of BNP7787 administration on the total concentrations (oxidized plus free) of cysteine, homocysteine and GSH in plasma, free and total GSH in WBC and rate of urinary excretion of cysteine were studied. The pharmacokinetics of ultrafilterable (free, non-protein bound) platinum were also determined after cisplatin (75 mg/m^2) treatment which followed paclitaxel (175 mg/m^2) and BNP7787 (8.2 to 27.6 g/m^2). *Methods*: Plasma thiols were measured by HPLC with fluorescence detection and platinum was measured by atomic absorption spectrophotometry. *Results*: BNP7787 administration produced a significant depletion of all plasma thiols in all the patients studied. Differences were noted in the kinetics of BNP7787-induced depletion of cysteine and other thiols. A significant depletion of cysteine occurred with a time lag of about 2 h after the end of BNP7787 infusion, while a reversible depletion of GSH and homocysteine occurred immediately following the start of BNP7787 infusion, with the plasma thiol/disulfide nadir corresponding to the end of infusion. The mean half-life of cysteine depletion following BNP7787 administration was 2.2 h, significantly longer than for homocysteine (0.23 h), or GSH (0.18 h; $P < 0.05$ for both). A several-fold increase in the urinary excretion of cysteine occurred following BNP7787 administration in all patients. The BNP7787-induced thiol/disulfide depletion in plasma was not affected by cisplatin administration ($P > 0.05$). BNP7787 administration had no effect on the ultrafilterable platinum pharmacokinetics. The 2-h lag in the depletion of cysteine, the most abundant thiol in plasma, suggests that the process may be related to the formation of free mesna from BNP7787 and that increased levels of mesna are not in circulation until after 2 h after BNP7787 administration. No effect of BNP7787 was seen on the GSH concentration in WBC, possibly reflecting the inability of these cells to take up BNP7787. *Conclusion*: The results suggest that BNP7787 has the potential to enhance cisplatin antitumor activity by depleting the reactive thiols in plasma.

Keywords BNP7787 · Thiols · Disulfides · Cysteine · Homocysteine · Glutathione

Abbreviations *BSO* Buthionine sulfoximine · *DTT* Dithiothreitol · *GSH* Glutathione · *Mesna* 2-Mercapto ethane sulfonate sodium · *MRT* Mean residence time · *PUF* Plasma ultrafiltrate · *WBC* White blood cells

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Introduction

BNP7787 (disodium 2,2′-dithio-bis-ethanesulfonate) is a new chemoprotective agent undergoing clinical development for the prevention and/or reduction of chemotherapy-induced toxicities. It is a water-soluble disulfide that lacks a free thiol or sulfate moiety [17]. This agent has been developed with the hypothesis that administration of a water-soluble disulfide drug molecule would

be much less toxic and much less likely to have unfavorable drug-drug interactions than its corresponding free thiol species. A proportion of the disulfide is expected to undergo metabolism in the cell by the action of reductases and thiol transferases into 2-mercaptoethane sulfonate, a free thiol [8, 12, 39]. Such reactions in key target organs such as kidney, bone marrow and neuronal cells would be expected to provide protection from toxicity of chemotherapeutic agents such as platinum drugs and alkylating agents. The safety and efficacy data from the preclinical studies and those emerging from clinical trials indicate that BNP7787 protects against several common and serious chemotherapy-induced toxicities [17, 38]. Studies in rats and beagle dogs have shown that BNP7787 protects against lethal and sublethal toxicities associated with cisplatin, including nephrotoxicity, emesis, myelosuppression and neurotoxicity [17, 18]. Similar protective effects have been observed for toxicities associated with paclitaxel [6], carboplatin [20] and oxaliplatin (Hausheer FH, unpublished laboratory observations). These and other studies have demonstrated that BNP7787 improves the therapeutic index of cisplatin, carboplatin and paclitaxel and does not interfere with their antitumor activity [4, 6, 17, 18, 19, 20, 21]. It has also been shown that BNP7787 treatment does not result in tumor protection in a variety of human cancer cell lines and in tumor-bearing rat and mouse xenograft models [4, 6, 17, 18, 19, 20, 21].

BNP7787 is stable and chemically inert in plasma due to the presence of the disulfide linkage, the high pO_2 concentration, and the absence of enzymatic metabolism of disulfides in plasma. Several mechanisms of action for BNP7787 have been characterized. The drug is a direct modulator of tubulin polymerization and protects against taxane-induced hyperpolymerization of tubulin [6, 19, 21]. The free thiol form of BNP7787 has been shown to block tubulin toxicity induced by hydrated platinum species [19, 21]. The cytoprotective properties against nephrotoxicity, neurotoxicity and myelosuppression noted with this agent suggest that BNP7787 is taken up by certain tissues such as the kidney, bone marrow and neuronal cells, and is metabolized to the free thiol form to elicit its protective function.

Glutathione (γ-glutamyl-cysteinyl-glycine, GSH) is a tripeptide present in cells at millimolar concentrations [9]. GSH has many essential functions in the cell such as the maintenance of the thiol status of proteins, reduction of ribonucleotides to deoxyribonucleotides and participation in leukotriene and prostaglandin metabolism [9]. GSH is an important antioxidant and protects cells against oxidative damage from drugs and free radicals [36]. GSH reacts with electrophiles either directly or through glutathione-S-transferase-mediated reactions [40]. In addition, the multidrug-resistance-associated protein (MRP) is either a GSH-S-conjugate carrier [43], or requires GSH to pump the drugs out of cells [41]. GSH counteracts the antitumor activity of a number of chemotherapeutic agents used in cancer treatment, thereby leading to drug resistance. It has been shown that in many cancers the cellular GSH levels are much higher than in the adjacent normal tissues. Elevation of GSH has been demonstrated in many platinum drug-resistant cell lines and is considered to be an independent resistance factor for chemotherapy [3, 11, 16]. Depletion of GSH results in an enhancement of the activities of many platinum agents in the experimental drug resistance models [28, 32]. GSH also protects normal cells from drug toxicity. It has been shown that GSH protects cardiac and skeletal muscles from cyclophosphamide-induced toxicity in a nude mouse model [14].

GSH is produced in a two-step synthesis [10]. The first is the formation of γ-glutamylcysteine from cysteine and glutamic acid catalyzed by intracellular γ-glutamylcysteine synthetase. The second step is the addition of glycine to γ-glutamylcysteine with the formation of GSH. Cysteine is the rate-limiting substrate for GSH biosynthesis [10]. GSH production can be increased by agents such as N-acetylcysteine which produce an elevation in cysteine [31] or decreased by inhibitors of γ-glutamylcysteine synthetase such as BSO [2, 26]. Cysteine in cells is either produced through the cystathionine pathway with homocysteine as the intermediate or is taken up by cells from extracellular sources [9, 10]. The depletion of GSH and cysteine in tumors is an important therapeutic objective of cancer therapy.

In an earlier phase I clinical study in which patients received escalating doses of ifosfamide and mesna followed by a fixed dose of carboplatin, we found a significant depletion of total cysteine and homocysteine levels in plasma in all patients [33]. A moderate reversible depletion of plasma GSH was seen only in 60% of the patients and the kinetics of GSH depletion suggested that this effect may be related to reactions of GSH mostly with ifosfamide metabolites [33]. We describe here the effects of BNP7787 on the total cysteine, homocysteine and GSH levels in plasma and GSH in WBC in patients receiving this drug alone and a week later in combination with paclitaxel and cisplatin chemotherapy.

Materials and methods

Drug administration, patients and sampling for thiol measurements

BNP7787 was given as a single agent on day −7 (1 week prior to chemotherapy) and again on day 1 following paclitaxel (175 mg/m^2) administered i.v. over 3 h and prior to cisplatin (75 mg/m^2 over 1 h). Patients received 1 l of 0.9% NaCl over 2 h just prior to BNP7787. BNP7787 was administered as a single 15–30 min i.v. infusion. Thiol modulation was studied in a total of 12 patients at the doses of 8.2, 12.3, 18.4 and 27.6 g/m^2 ($n=5$, 2, 1 and 4, respectively). Blood samples were obtained immediately preceding the BNP7787 treatment, 5 min before the end of the BNP7787 infusion, at the end of the infusion, and at 0.5, 2.0 and 6.0 h after infusion on day −7 and day 1 of chemotherapy. On day 1, one additional blood sample was taken prior to the paclitaxel infusion. A 12-h pooled urine sample prior to the BNP7787 treatment and a 6-h sample following the treatment were obtained on day −7. On day 1, a 12-h pooled urine sample was obtained prior to the initiation of chemotherapy and a 6-h sample was collected following BNP7787 treatment. Samples were obtained for pharmacokinetic

measurements of ultrafilterable platinum at 0.5, 1.0, 1.25, 1.5, 2.0, 3.0, 4.0 and 6.0 h after cisplatin administration. All blood samples were collected into heparinized tubes. The blood and urine were transported to the laboratory on ice. Plasma and cells were separated and collected immediately from the samples. Ultrafiltrates from plasma were obtained immediately following plasma collection. All plasma, PUF and urine samples were stored frozen at −20°C until analysis. The WBC were collected by dextran sedimentation [33]. Plasma samples for GSH measurements were stored with 50 μl of L-serine/Na borate to inhibit λ-glutamyltranspeptidase activity, which would otherwise have destroyed GSH [5]. Cells were stored, frozen in 5% sulfosalicylic acid to prevent oxidation of GSH [1].

Thiol measurements

The total cysteine, total homocysteine and total GSH levels were measured in plasma. The levels of free and total GSH were measured in WBC. Total cysteine was measured in urine. 'Total' refers to the free 'SH' form plus mixed disulfides including protein disulfides of each of the thiols.

A previously validated reversed-phase HPLC procedure with fluorescence detection was used to quantitate all plasma thiols, following derivatization with monobromobimane (thiolyte) as described previously [33]. Mixed disulfides were reduced to the free thiol form by incubation with DTT prior to derivatization with thiolyte [1]. The free GSH in WBC was measured without prior reduction by DTT. The derivatization reaction was carried out for 20 min in the dark at pH 8.0 [13]. The reaction was stopped by the addition of 50% sulfosalicylic acid and after the removal of precipitated protein by centrifugation, the derivatized thiols in the supernatant were subjected to HPLC. Quantitation of the free thiols was based on identically prepared analytical standards. The concentration of thiols was expressed on a per milliliter basis for plasma, and on a per milligram protein basis for the cells. Protein was measured by the assay of Lowry et al. [24]. The excretion rate of cysteine in urine was calculated as follows: [cysteine concentration in urine (mg/ml) × total volume of urine (ml) in the collection period]/urine collection period (h).

Platinum measurements

PUF was obtained from plasma by centrifugal ultrafiltration using Amicon Centrifree Micropartition Systems and platinum measured by atomic absorption spectrophotometry (PE ZL4100) equipped with an autosampler. PUF was diluted 1:1 in 0.1% nitric acid plus 0.2% Triton X-100 prior to a 20-μl injection. The standards were identically prepared in the same matrix. The method had been validated previously in our laboratory [37] and included the determination of limits of quantitation (LOQ, 20 ng/ml), linear range (20 to 400 ng/ml), precision (inter- and intraday assay variability), accuracy of the measurement of quality controls of known concentrations and stability of samples stored at −20°C. The quality controls prepared at three different concentrations representing the high end, middle and low end of the standard curve range were assayed along with the patient samples. In order to accept the patient data, the observed QC values had to be $<15\%$ of the expected for the high and mid QC values and $<20\%$ for the QC values near the LOQ; otherwise the assays were rerun.

Cisplatin pharmacokinetic and thiol kinetic data analysis

The pharmacokinetics of free platinum concentrations in plasma were evaluated using standard noncompartmental methods, as implemented in LAGRAN [35]. Briefly, the area under the concentration-time curve was calculated by the log-linear trapezoidal rule. Half-life was calculated as ln 2/ke, where ke is the terminal elimination rate constant, computed by least squares linear regression of the data points in the terminal elimination phase. The resultant parameters were compared with those in previous reports of cisplatin pharmacokinetics; however, no formal statistical comparisons were made.

The time to nadir for plasma thiols was calculated from the beginning of the BNP7787 infusion until the time of the minimum observed concentration. The half-life of thiol depletion was computed from the baseline thiol concentration, just prior to drug infusion, until the time of nadir. The magnitude of thiol depletion was defined as the percent difference between baseline and nadir concentrations [(baseline−nadir)/baseline × 100].

Statistics

Related group statistical comparisons were evaluated using the Wilcoxon signed-ranks test, and independent sample comparisons by the Mann-Whitney test. The relationship between BNP7787 dose and thiol kinetics was evaluated by simple linear regression. All descriptive statistics and statistical analyses were conducted in SYSTAT (Version 10; SPSS, Chicago, Ill.). A P-value less than 0.05 was required for a declaration of statistical significance.

Results

Thiols in plasma

Changes in total cysteine, homocysteine and GSH in plasma following BNP7787 alone on day −7 are shown in Figs. 1A, 2A and 3A, respectively, and following BNP7787 with chemotherapy (paclitaxel and cisplatin) on day 1 are shown in Figs. 1B, 2B and 3B, respectively).

As shown in Figs. 1, 2 and 3, the levels of all measured plasma thiols/disulfides decreased significantly from baseline following BNP7787 administration ($P<0.01$). However, the kinetic characteristics of these changes were quite different between plasma cysteine and the other thiols (Table 1). While a significant decline in total cysteine was not evident until 2 h after administration of BNP7787 (Fig. 1), a rapid decline in total homocysteine and GSH were clearly evident after only a few minutes from initiation of the BNP7787 infusions (Figs. 2 and 3). The time to nadir and half-life of depletion were both greater for cysteine than for the other thiols ($P<0.01$ for all comparisons). The greatest effect on homocysteine and GSH levels was observed at the end of BNP7787 infusion, when the peak plasma level of BNP7787 would be expected. Following the end of BNP7787 infusion, the GSH and homocysteine levels in plasma started to recover and approach their respective pretreatment levels by 6 h after infusion. However, at the highest dose of BNP7787 studied (27.6 g/m^2), the homocysteine levels in plasma remained 20 to 30% of the pretreatment level at 6 h. The magnitude and rate of decline in plasma cysteine concentration appeared to be dependent on BNP7787 dose ($P<0.01$ for both), while no dose dependence was found for either homocysteine or GSH ($P>0.05$). This relationship between dose and plasma cysteine depletion and half-life is illustrated in Fig. 4.

A comparison of the data between A and B of Figs. 1, 2 and 3 for each of the thiols indicates that cisplatin had no effect on the BNP7787-induced depletion of plasma thiols/disulfides ($P>0.05$). While the

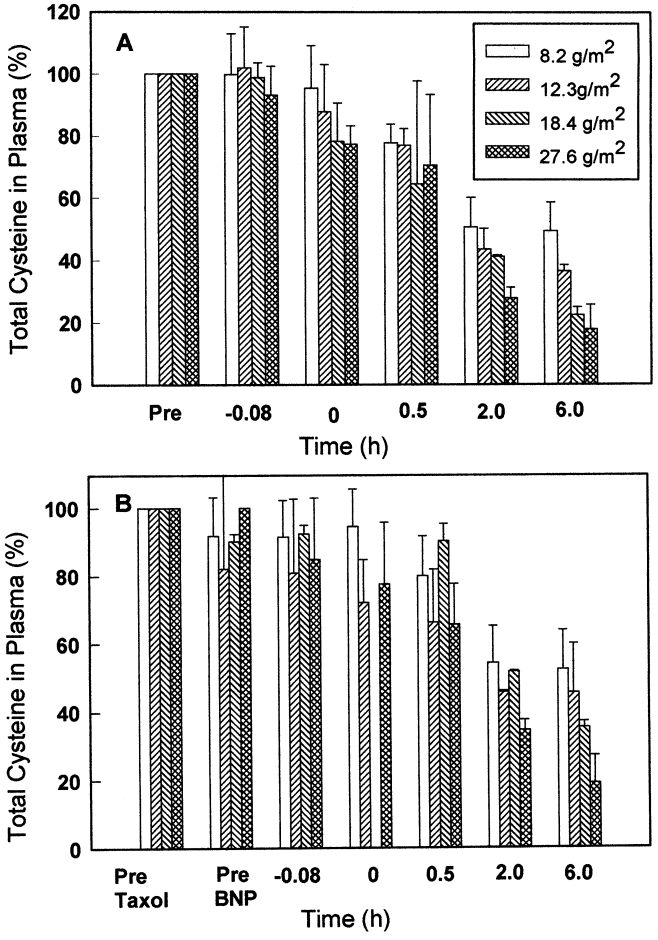

Fig. 1A, B Changes in total cysteine in plasma with time during and after BNP7787. **A** Day −7, BNP7787 administered alone. **B** Day 1, BNP7787 followed by a 1-h infusion of cisplatin (75 mg/m^2). Each bar represents the mean ± SD of the change in all the patients treated at that BNP7787 dose (*Pre* pretreatment samples; for day 1 the *pre* paclitaxel concentrations are used to demonstrate the changes in subsequent samples). On the *x*-axis, −0.08 h and 0 h represent 5 min before the end of the infusion and the end of infusion, respectively. The mean pretreatment concentrations (all micromolar) at the four doses were as follows: *8.2 g/m^2* day −7 423.5 ± 46.8, day 1 522.2 ± 99.5; *12.3 g/m^2* day −7 351.9 ± 34.6, day 1 392.4 ± 140; *18.4 g/m^2* day −7 283.5, day 1 308.5; *27.6 mg/m^2* day −7 249.8 ± 49.6, day 1 291.6 ± 70.6

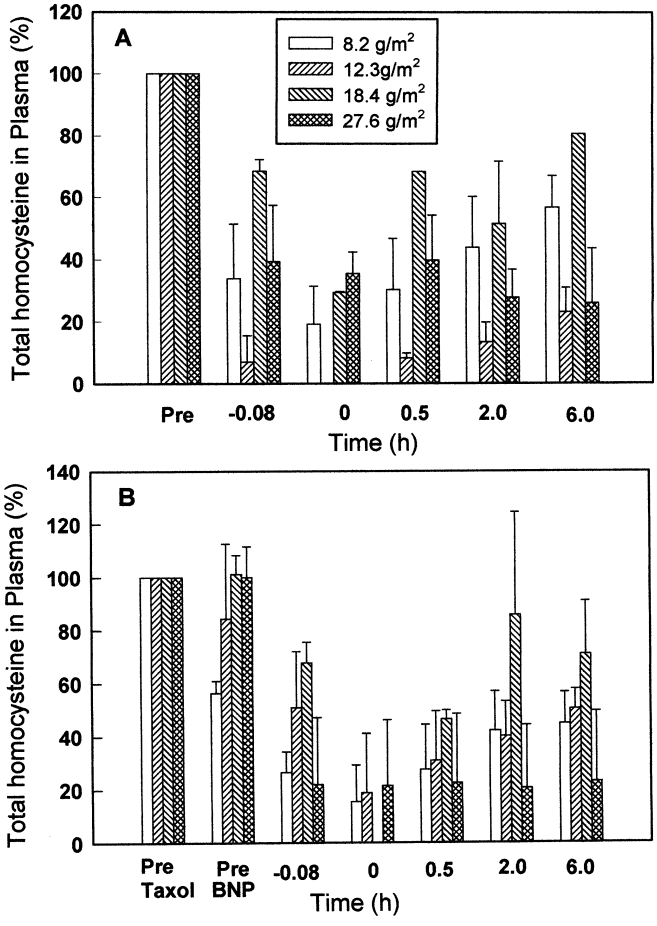

Fig. 2A, B Changes in total homocysteine in plasma with time during and after BNP7787. **A** Day −7, BNP7787 administered alone. **B** Day 1, BNP7787 followed by a 1-h infusion of cisplatin (75 mg/m^2). Each bar represents the mean ± SD of the change in all the patients entered at that BNP7787 dose (*Pre* pretreatment samples; for day 1 the *pre* paclitaxel concentrations are used to demonstrate the changes in subsequent samples). On the *x*-axis, −0.08 h and 0 h represent 5 min before the end of the infusion and the end of infusion, respectively. The mean pretreatment concentrations (all micromolar) at the four doses were as follows: *8.2 g/m^2* day −7 12.2 ± 5.1, day 1 10.7 ± 4.5; *12.3 g/m^2* day −7 11.8 ± 5.2, day 1 6.0 ± 1.9; *18.4 g/m^2* day −7 1.2, day 1 1.6; *27.6 mg/m^2* day −7 15.2 ± 7.2, day 1 10.4 ± 1.7

decline in cysteine was highly consistent in every patient studied and showed clear dose dependence, some variability occurred in the decline and recovery of homocysteine and GSH between patients and doses. One patient treated with a dose of 18.4 mg/m^2 BNP7787 showed less of an effect on both homocysteine and GSH than the others (Figs. 2 and 3A) and this patient's values contributed most to the observed variability. With the exception of this patient, the data for GSH and homocysteine from the remaining 11 patients suggest that the low doses of 8.2 to 12.3 g/m^2 were quite effective in producing a significant decline in homocysteine and GSH to levels of less than 20% of their pretreatment levels. These levels remained significantly below pretreatment levels for 2 h following the BNP7787 infusion, a period during which cisplatin was administered ($P < 0.05$).

The free GSH levels in WBC did not decrease during or after BNP7787 administration ($P > 0.05$), as illustrated in Fig. 5. GSH levels did not decline in these cells when BNP7787 was administered alone (Fig. 5A) or in combination with paclitaxel and cisplatin (Fig. 5B). The total GSH in white blood cells followed the same pattern, and no effect of BNP7787 was observed (data not shown).

Urinary excretion of cysteine

The rate of cysteine urinary excretion increased approximately tenfold during the 6-h period following BNP7787 administration in all patients studied (Fig. 6).

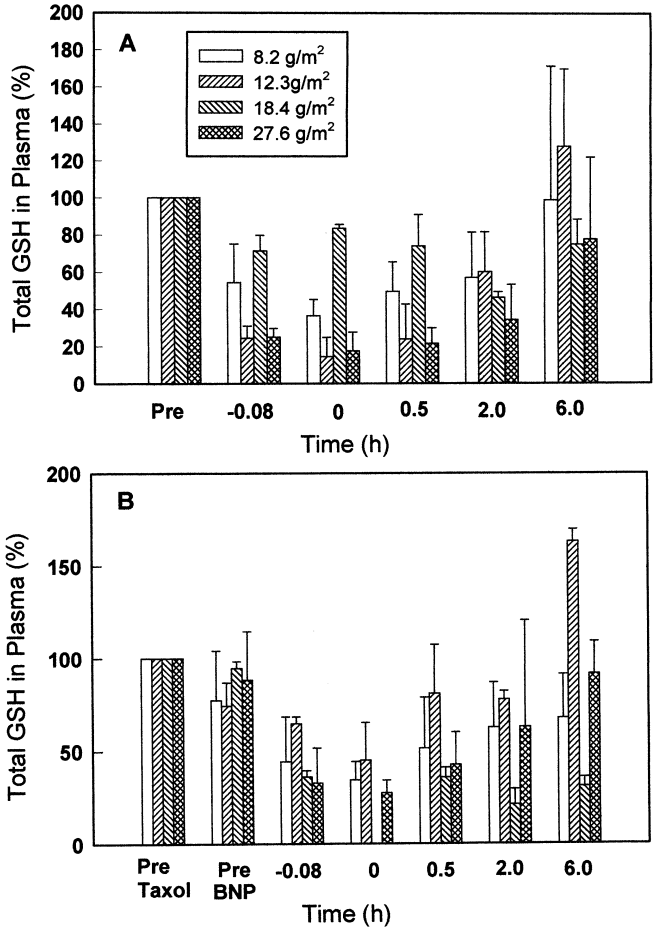

Fig. 3A, B Changes in total GSH in plasma with time during and after BNP7787. **A** Day −7, BNP7787 administered alone. **B** Day 1, a 1 h infusion of cisplatin (75 mg/m^2) followed BNP7787. Each bar represents the mean ± SD of the change in all the patients entered at that BNP7787 dose (*Pre* pretreatment samples; for day 1 the *pre* paclitaxel concentrations are used to demonstrate the changes in subsequent samples). On the *x*-axis, −0.08 h and 0 h represent 5 min before the end of the infusion and the end of infusion, respectively. The mean pretreatment concentrations (all micromolar) at the four doses were as follows: *8.2 g/m^2* day −7 4.1 ± 1.7, day 1 4.0 ± 2.7; *12.3 g/m^2* day −7 3.4 ± 1.3, day 1 3.3 ± 1.7; *18.4 g/m^2* day −7 4.3, day 1 5.8; *27.6 mg/m^2* day −7 4.3 ± 0.9, day 1 3.5 ± 1.9

Excretion rates during the 6-h period following BNP7787 administration during the initial study period (BNP7787 alone) were approximately half those observed during the second study period (BNP7787 coadministered with cisplatin and paclitaxel; $P < 0.01$.). Most of this difference between study periods was from four patients, whose rate of cysteine excretion increased four- to eightfold (Fig. 7).

Pharmacokinetics of ultrafilterable platinum after cisplatin

The pharmacokinetic parameters of ultrafilterable platinum following cisplatin administration at the different doses of BNP7787 are shown in Table 2. All the observed pharmacokinetic parameters are consistent with those published for cisplatin [34].

Table 1 Kinetics of plasma thiol depletion following administration of BNP7787

		Cysteine	Homocysteine	GSH
Depletion half-life (h)	Mean	2.2	0.23	0.18
	Median	2	0.15	0.28
	CV%	3.4	91	97
Time to nadir (h)	Mean	5.1	0.6	0.81
	Median	6	0.25	0.25
	CV%	38	313	170
Decrease from baseline to nadir (%)	Mean	64.1	80.5	71.9
	Median	63.5	78.4	74.3
	CV%	44	76	56

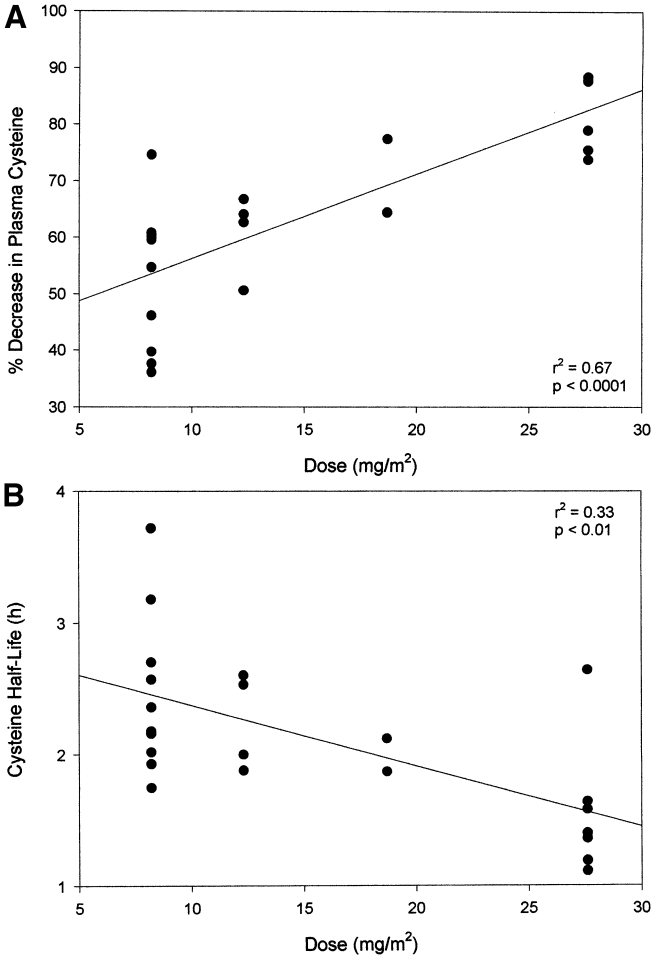

Fig. 4A, B Relationships between BNP7787 dose and plasma cysteine concentrations (**A**) and half-life (**B**)

Discussion

There is a wealth of literature that suggests that GSH plays a key role in resistance to a variety of chemo-

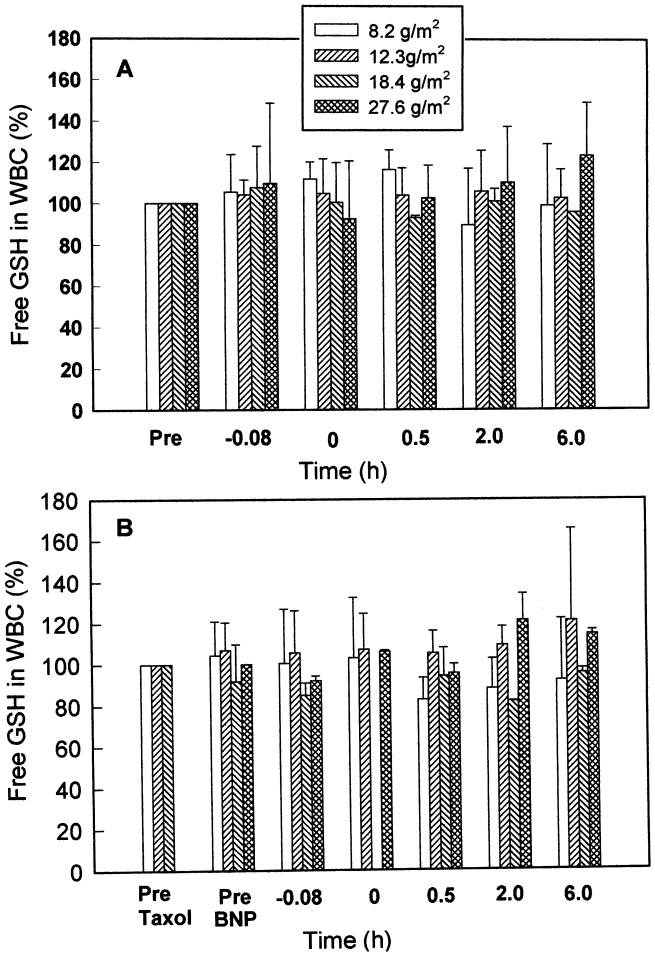

Fig. 5A, B GSH in WBC during and after BNP7787. **A** Day −7, BNP7787 administered alone. **B** Day 1, BNP7787 followed by a 1-h infusion of cisplatin (75 mg/m^2). Each bar represents the mean ± SD of the change in all the patients entered at that BNP7787 dose (*Pre* pretreatment samples; for day 1 the *pre* paclitaxel concentrations are used to demonstrate the changes in subsequent samples). On the *x*-axis, −0.08 h and 0 h represent 5 min before the end of the infusion and the end of infusion, respectively. The mean pretreatment values (all nanomoles per milligram protein) at the four doses were as follows: *8.2 g/m^2* day −7 14.7 ± 2.9, day 1 14.2 ± 3.1; *12.3 g/m^2* day −7 15.0 ± 0.8, day 1 15.6 ± 2.0; *18.4 g/m^2* day −7 18.1, day 1 22.85; *27.6 mg/m^2* day −7 10.7 ± 3.1, day 1 10.2 ± 3.3

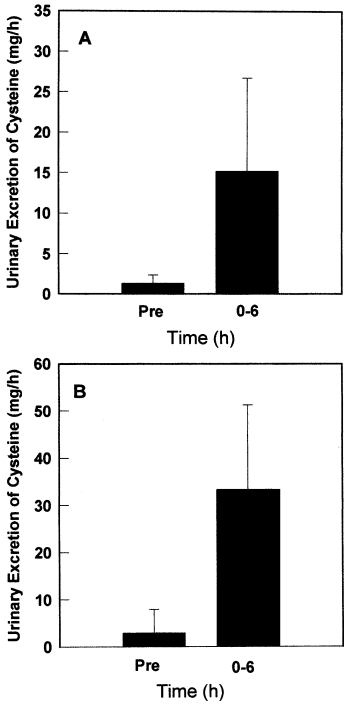

Fig. 6A, B Urinary excretion of cysteine pretreatment and during a 6-h period after BNP7787 administration (**A** day −7, **B** day 1)

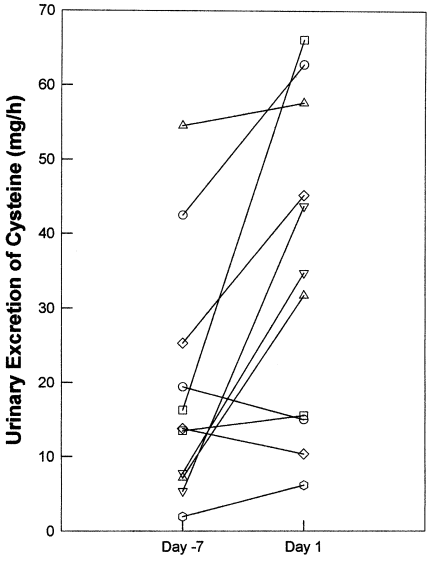

Fig. 7 The rate of urinary excretion of cysteine from day −7 to day 1 in individual patients

therapeutic agents, especially platinum drugs and electrophilic alkylating agents [15, 16, 29]. Many studies have demonstrated that inhibiting GSH synthesis with BSO potentiates the activities of platinum agents [27, 28, 32]. Studies have shown that the biotransformation products of platinum agents in plasma include many thiol-Pt conjugates, indicating that circulating thiols inactivate platinum agents [7, 25]. Lowering of circulating thiols during platinum administration is therefore desirable. We have shown that mesna depletes thiols in plasma [33]. However, free thiol cytoprotective agents have been controversial because of their reactivity and potential inactivation of the circulating platinum agents in direct reactions [17]. The free thiol-containing or thiol-generating cytoprotective agents that have undergone clinical evaluation (e.g. sodium thiosulfate, diethyl dithiocarbamate, reduced GSH and WR2721) have demonstrated tumor protection as well as their own intrinsic toxicities following administration [17].

We have shown in a previous study that in patients receiving mesna with ifosfamide followed by carboplatin, total plasma cysteine and homocysteine are depleted significantly in a dose-dependent manner [33]. At the highest dose administered (8 g/m^2 per day) there was

Table 2 Pharmacokinetics of ultrafilterable platinum following BNP7787 administration.Values are means ± SD

BNP7787 (g/m^2)	Cisplatin (mg/m^2)	Number of patients	$t_{1/2}$ (h)	CL(l/h)	AUC ($\mu g/ml \cdot h$)	Vss (l)
8.2	75	5	1.6 ± 0.42	22.4 ± 4.7	4.06 ± 0.7	29.16 ± 8.2
12.3	75	2	2.2 ± 0.7	28.1 ± 4.5	3.0 ± 0.4	43.6 ± 20.0
18.4	75	1	3.2	16.9	4.1	43.5
27.6	75	3	2.5 ± 1.3	29.0 ± 4.9	2.9 ± 0.1	54.5 ± 16.4

a 99% depletion. Total GSH was depleted only moderately (by about 50%) with no dose relationship and only in 60% of the patients [33]. The current study with BNP7787 clearly shows that this drug is different from mesna in terms of its ability to induce a significant depletion of all plasma thiols studied, including GSH. In the current study, the depletion of cysteine increased with escalating doses of BNP7787 and occurred with a significant time lag of 2 h following BNP7787 administration. A significant depletion of GSH and homocysteine occurred immediately after the start of BNP7787 infusion at all dose levels, including the lowest doses, with no demonstrable effect of dose. The GSH and homocysteine levels in plasma remained significantly lower than the pretreatment values for 2 h following BNP7787, during which time cisplatin was administered. Unlike free mesna, the disulfide BNP7787 would not be expected to react with any of the plasma thiols, disulfides, platinum agents or platinum metabolites directly in plasma, so the thiol/disulfide depletion caused by BNP7787 administration would be expected to provide greater efficacy for cisplatin and other electrophilic chemotherapeutic agents that react with GSH and other thiols.

The differences in the kinetics of depletion between cysteine and the other thiols/disulfides suggest that the depletion process is related to the relative plasma concentrations of the thiols/disulfides. Total homocysteine and total GSH exist in plasma in low micromolar concentrations, while total cysteine concentrations are in the order of 0.25 m*M* and above. In addition, while about 65% of GSH in plasma is in its free thiol form, only about 5% of cysteine and homocysteine are in the free thiol form, and the rest in all combinations of cysteine, homocysteine and GSH, in the form of mixed disulfides including disulfides with proteins [22, 23]. Our previous in vitro studies have demonstrated that free mesna is a reducing agent which is capable of directly reducing the disulfides cystine and homocystine to their free thiol forms [33]. Cysteine is the most abundant plasma thiol and least sterically hindered disulfide and thus a higher concentration of the reducing free thiol form of BNP7787 would be required to deplete it. The delay in significant cysteine depletion in plasma in the first 2 h after BNP7787 (8.2 to 27.6 g/m^2) administration suggests that BNP7787 is the predominant circulating species during this time. After this time a small fraction of mesna formed by the reduction of BNP7787 in some tissues such as kidney, intestine, neuronal cells and bone marrow may be entering the circulation leading to plasma cysteine depletion. Separate pharmacokinetic studies of BNP7787 in patients (and in animals) indicate that indeed a small fraction of mesna is formed (about 1–3%) relative to the total concentration of BNP7787 at this time (Hausheer F, unpublished observations). The half-life and MRT of mesna are approximately 2.8 and 4.8 h as opposed to the half-life and MRT of BNP7787, which are 1.4 and 1.9 h. These findings support the view that a slow generation of mesna is taking place and that it is outside the plasma compartment.

In every patient studied, a dose-dependent depletion of plasma cysteine was observed only 2 h after the end of BNP7787 infusion, suggesting that the free mesna entry into plasma is a slow and dose-dependent process. The fact that we infused 8.2 to 27.6 g/m^2 of BNP7787 which corresponds to about 14 to 48 g of the total agent over 15 to 30 min and found such a delayed and relatively moderate reductive clearance of total cysteine indicates that the entry of the reactive free thiol form into plasma is slow and not likely to be clinically significant. These results support the argument that unlike mesna, BNP7787 does not contribute to direct chemical reactions with platinum species to any appreciable degree, since the average half-life of free platinum in the 12 patients studied was only 2.1 ± 0.9 h and the entry of free mesna into the circulation appears to be ≥2 h.

The increase in urinary excretion of cysteine is consistent with the depletion of plasma cysteine. It is unclear, however, why the rate of urinary excretion of cysteine had further increased in four patients after BNP7787 on day 1, when it was administered following paclitaxel. The lack of change in plasma cysteine levels after paclitaxel alone, but prior to administering BNP7787 (Fig. 1B) suggest that it could not be an effect of paclitaxel. In fact there are no significant differences in the magnitude of plasma cysteine depletion between day −7 when BNP7787 was administered alone and day 1 when it was administered following paclitaxel but prior to cisplatin. It is possible that the apparent increase in the rate of cysteine in these four patients could have been due, in part, to problems inherent in urine collections for pharmacological studies such as accurate documentation of collection period, total urine volume and/or storing of a homogeneous urine aliquot.

In the current study the observation that the nadir for GSH and homocysteine in plasma coincided with the time of peak plasma concentration for BNP7787, and that the plasma levels of these physiological thiols/disulfides recovered following the end of BNP7787 infusion suggests that the effect is directly related to the infusion of BNP7787. At the highest dose of BNP7787 administered in four patients, the total homocysteine concentrations at 6 h remained at 20 to 30% of pretreatment levels suggesting that, like the total cysteine,

the free thiol form of BNP7787 also produces the continued reductive clearance for homocysteine.

Similar to the findings of our previous study of free mesna [33], we saw no effect of BNP7787 on GSH in WBC. This may have been related to the lack of uptake of BNP7787 into these cells. BNP7787 is a highly water-soluble dianionic molecule and is apparently not taken up by most cells including WBC. However, the drug appears to be taken up by the renal tubular epithelial cells, intestine, bone marrow, and the dorsal root ganglia where the protective effects of this drug are observed.

The pharmacokinetics of ultrafilterable platinum following BNP7787 and cisplatin administration were similar to those found previously [34]. It has also been observed in other phase I trials that BNP7787 administration has no effect upon the plasma levels of total platinum, ultrafilterable intact cisplatin, monohydrated platinum or the free platinum in all patients [42].

The phase I studies of BNP7787 showed major differences in the safety profiles of BNP7787 and mesna. High doses of BNP7787 (up to 41.0 g/m^2 i.v. over 15 to 30 min) were nontoxic [38], whereas doses of mesna of up to 2.4 g/m^2 are associated with substantial toxicity such as diarrhea, nausea, vomiting, pain and hypotension in more than 80% of patients [30]. The studies presented here, together with the excellent safety profile of the drug, suggest that BNP7787 has the potential to enhance cisplatin activity by depleting plasma thiols without concurrently producing free mesna.

References

1. Anderson ME (1985) Determination of glutathione and glutathione disulfide in biological samples. Methods Enzymol 113:548
2. Bailey HH, Mulcahy RT, Tutsch KD, Arzoomanian RZ, Alberti D, Tombes MB, Wilding G, Pomplun M, Spriggs DR (1994). Phase I clinical trial of intravenous L-buthionine sulfoximine and melphalan: an attempt at modulation of glutathione. J Clin Oncol 12:194
3. Behrens BC, Hamilton TC, Masuda H, Grotzinger KR, Whang-Peng J, et al (1987) Characterization of a cis-diamminedichloroplatinum (II) resistant human ovarian cancer cell line and its use in evaluation of platinum analogues. Cancer Res 47:414
4. Boven E, Hulscher S, Schluper HM, Erkelens C, van der Vijgh WJ (1998) BNP7787, a new protector against platinum induced toxicities, does not influence the antitumor efficacy of cisplatin or carboplatin. Proc Am Assoc Cancer Res 39:158
5. Burgunder JM, Varriale A, Lauterburg BH (1989) Effect of N-acetylcysteine on plasma cysteine and glutathione following paracetamol administration. Eur J Clin Pharmacol 36:127
6. Cavalletti E, Cavaletti G, Tredici G, Oggioni N, Spinelli S, Reddy D, Zhao M, Wu M, Hausheer FH (1999) Oral and intravenous BNP7787 protects against paclitaxel-mediated neurotoxicity in Wistar rats. Proc Am Assoc Cancer Res 40:398
7. Daley-Yates PT, McBrien DCH (1984) Cisplatin metabolites in plasma, a study of their pharmacokinetics and importance in the nephrotoxic and antitumor activity of cisplatin. Biochem Pharmacol 33:3063
8. Dechant KL, Brodgen RN, Pilkington T, Faulds D (1991) Ifosfamide/mesna. A review of its antineoplastic activity, pharmacokinetic properties and therapeutic efficacy in cancer. Drugs 42:428
9. Deleve LD, Kaplowitz N (1991) Glutathione metabolism and its role in hepatotoxicity. Pharmacol Ther 52:287
10. Deneke SM, Fanburg BL (1989) Regulation of cellular glutathione. Am J Physiol 257:L163
11. El-akawi Z, Abu-hadid M, Perez R, Glavy J, Zdanowicz J, Creaven PJ, Pendyala L (1996) Altered glutathione metabolism in oxaliplatin resistant A2780 ovarian carcinoma cells. Cancer Lett 105:5
12. El-Yazigin A, Ernst P, Al-Rawithi S, Legayada E, Raines DA (1997) Pharmacokinetics of mesna and dimesna after simultaneous intravenous bolus and infusion administration in patients undergoing bone marrow transplantation. J Clin Pharmacol 37:618
13. Fahey RC, Newton GL (1987) Determination of low molecular weight thiols using monobromobimane fluorescent labelling and high performance liquid chromatography. Methods Enzymol 143:85
14. Friedman HS, Colvin M, Aisaka K, Popp J, Bossen EH, Reimer KA, Powell JB, Hilton J, Gross SS, Levi R, Bigner DD, Griffith OW (1990) Glutathione protects cardiac and skeletal muscle from cyclophosphamide-induced toxicity. Cancer Res 50:2455
15. Gosland M, Lum B, Schimmelpfennig J, Baker J, Doukas M (1996) Insights into mechanisms of cisplatin resistance and potential for its clinical reversal. Pharmacotherapy 16:16
16. Hamaguchi K, Godwin AK, Yakushiji M, O'Dwyer PJ, Ozols RF, Hamilton TC (1993) Cross-resistance to diverse drugs is associated with primary cisplatin resistance in ovarian cancer cell lines. Cancer Res 53:5225
17. Hausheer FH, Kanter P, Cao S, Haridas K, Seetharamulu P, Reddy D, Petluru P, Zhao M, Murali D, Saxe JD, Yao S, Martinez N, Zukowski A, Rustum YM (1998) Modulation of platinum induced toxicities and therapeutic index: mechanistic insights and first and second-generation protecting agents. Semin Oncol 25:584–599
18. Hausheer F, Cavaletti G, Tredici G, Oggioni N, Spinelli S, Pezzoni G, Manzotti C, Haridas K, Reddy D, Zhao M, Seetharamulu P, Yao S, Pavankumar P, Murali D, Wu M, Saxe J, Cavalletti E (1999) Oral and intravenous BNP7787 protects against platinum neurotoxicity without in vitro or in vivo tumor protection. Proc Am Assoc Cancer Res 40:398
19. Hausheer FH, Kochat H, Reddy D, Zhao M, Seetharamulu P, Yao S, Pavankumar P, Murali D, Wu M, Saxe J, Parker A, Hamilton S (2000) BNP7787: a novel chemoprotecting agent for platinum and taxane toxicity. Proc Am Assoc Cancer Res 41:768
20. Hausheer F, Kanter P, Rustum Y, Cao S, Haridas K, Reddy D, Seetharamulu P, Zhao M, Yao S, Pavankumar P, Murali D (2001) BNP7787: a novel antitumor potentiating drug which protects against cisplatin and carboplatin toxicities. Proc Am Assoc Cancer Res 38:311
21. Hausheer FH, Kochat H, Zhao M, Seetharamulu P, Huang Q, Berghorn E (2001) BNP7787, a novel neuroprotective agent in taxane and platinum regimens, does not interfere with antitumor activity. Proc Am Assoc Cancer Res 42:370
22. Kleinman WA, Richie PJ (2000) Status of glutathione and other thiols and disulfides in human plasma. Biochem Pharmacol 60:19–29
23. Lauterburg BH, Nguyen T, Hartmann B, Junker E, Küpfer A, Cerny T (1994) Depletion of total cysteine, glutathione, and homocysteine in plasma by ifosfamide/mesna therapy. Cancer Chemother Pharmacol 35:132
24. Lowry OH, Rosenbrough MT, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. J Biol Chem 193:265
25. Luo FR, Wyrick SD, Chaney SG (1999) Pharmacokinetics and biotransformations of oxaliplatin in comparison with ormaplatin following a single bolus intravenous injection in rats. Cancer Chemother Pharmacol 44:19
26. Meister A (1991) Glutathione deficiency produced by inhibition of its synthesis, and its reversal; applications in research and therapy. Pharmacol Ther 51:155

27. Mistry P, Harrap KR (1991) Historical aspects of glutathione and cancer chemotherapy. Pharmacol Ther 49:125
28. Mistry P, Loh SY, Kelland LR, Harrap KR (1993) Effect of buthionine sulfoximine on PtII and PtIV drug accumulation and the formation of glutathione conjugates in human ovarian-carcinoma cell lines. Int J Cancer 55:848
29. Muggia FM, Los G (1993) Platinum resistance: laboratory findings and clinical implications. Stem Cells 11:182
30. PDR (2001) Mesnex Injection. Physicians desk reference (PDR electronic library). Medical Economics Company, Montvale, NJ
31. Pendyala L, Creaven PJ (1995) Pharmacokinetic and pharmacodynamic studies of N-acetylcysteine, a potential chemopreventive agent during a phase I trial. Cancer Epidemiol Biomarkers Prev 4:245
32. Pendyala L, Perez R, Weinstein A, Zdanowicz J, Creaven PJ (1997) Effect of glutathione depletion on the cytotoxicity of cisplatin and iproplatin in a human melanoma cell line. Cancer Chemother Pharmacol 40:38
33. Pendyala L, Creaven PJ, Schwartz G, Meropol NJ, Bolanowska-Higdon W, Zdanowicz J, Murphy M, Perez R (2000) Intravenous ifosfamide/mesna is associated with depletion of plasma thiols without depletion of leukocyte glutathione. Clin Cancer Res 6:1314
34. Perez R, Pendyala L, Meropol NJ, Jones V, Kindler H, Loewen G, Schwartz G, Noel D, Proefrock A, Berghorn E, Mahew E, Raghavan D, Creaven PJ (2003) Initial clinical and pharmacokinetic trials of N-phosphonoacetyl-L-aspartate (PALA) plus cisplatin and carboplatin. Cancer (in press)
35. Rocci ML Jr, Jusko WJ (1983) Lagran program for area and moments in pharmacokinetic analysis. Comput Programs Biomed 16:203
36. Schulz JB, Lindenau J, Seyfried J, Dichgans J (2000) Glutathione, oxidative stress and neurodegeneration. Eur J Biochem 267:4904
37. Schwartz GN, Pendyala L, Amantea MA, Humphriss E, Creaven PJ (1998) Pharmacokinetics of platinum (Pt) in a phase I trial of liposomal cisplatin in patients with advanced cancer. Proc Am Assoc Cancer Res 39:364
38. Schwartz GN, Schilsky RL, Pendyala L, Berghorn E, Bertucci D, Ratain MJ, Hausheer FH (2000) Phase I trial of escalating doses of BNP7787 in patients receiving paclitaxel (TAX) and cisplatin (CDDP). Proc ASCO 19:218a
39. Shaw IC, Weeks MS (1987) Excretion of disodium Bis-2 mercaptoethanesulphonate (dimesna) in the urine of volunteers after oral dosing. Eur J Cancer Clin Oncol 23:933–935
40. Tew KD (1994) Glutathione-associated enzymes in anticancer drug resistance. Cancer Res 54:4313
41. Vanhoefer U, Cao SS, Minderman H, Toth K, Skenderis BS, Slovak ML, Rustum YM (1996) D,L-Buthionine-(S,R)-sulfoximine potentiates in vivo the therapeutic efficacy of doxorubicin against multidrug resistance protein-expressing tumors. Clin Cancer Res 2:1961
42. Verschraagen M, Boven E, Westerman M, Ruijter R, Hausheer FH, Reddy D, Pinedo HM, van der Vijgh WJ (2000) The pharmacokinetic behavior of BNP7787 (dimesna) and its metabolite mesna and the influence of BNP7787 on the pharmacokinetics of (hydrated) cisplatin in cancer patients. Proc Am Assoc Cancer Res 41:606
43. Zaman GJR, Lankelma J, Tellingen OV, Beijnen J, Dekker H, Paulusma C, Oude Elferink RPJ, Bass F, Borst P (1995) Role of glutathione in the export of compounds from cells by the multidrug-resistance-associated protein. Proc Natl Acad Sci U S A 92:7690