Preclinical Development

BNP7787-Mediated Modulation of Paclitaxel- and Cisplatin-Induced Aberrant Microtubule Protein Polymerization In vitro

Aulma R. Parker, Pavankumar N. Petluru, Meizhen Wu, Min Zhao, Harry Kochat, and Frederick H. Hausheer

Abstract

Taxane and platinum drugs are important agents in the treatment of cancer and have shown activity against a variety of tumors, including ovarian, breast, and lung cancer, either as single agents or in combination with other chemotherapy drugs. However, a serious and prevalent side effect of taxane (docetaxel and all formulations/derivatives of paclitaxel) and platinum (cisplatin, carboplatin, and oxaliplatin) agents is dose-limiting chemotherapy-induced peripheral neuropathy (CIPN). CIPN can result in treatment delays, dose modifications, and, in severe cases, discontinuation of chemotherapy. Consequently, effective treatments for CIPN are needed. Dimesna (BNP7787; Tavocept™; disodium 2,2′-dithio-bis-ethanesulfonate) is an investigational drug that is undergoing international clinical development as a treatment that is coadministered with first-line taxane and platinum combination chemotherapy in patients with inoperable advanced primary adenocarcinoma of the lung. BNP7787 is currently being developed with the objective of increasing the survival of cancer patients receiving taxane- and/or cisplatin-based chemotherapy. Additional data indicate that BNP7787 may also protect against common and serious chemotherapy-induced toxicities, including chemotherapy-induced anemia, nausea, emesis, nephrotoxicity, and neuropathy, without interfering with antitumor activity of the chemotherapeutic agent(s). Studies herein show that BNP7787 prevents aberrant microtubule protein (MTP) polymerization that is caused by exposure of MTP to paclitaxel or cisplatin. BNP7787 modulates paclitaxel-induced hyperpolymerization of MTP in a dose-dependent manner, and mesna, an in vivo metabolite of BNP7787, protects against time-dependent cisplatin-induced inactivation of MTP. We propose that interactions between BNP7787 and MTP may play a role in BNP7787-mediated protection against CIPN.

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Introduction

Taxane and platinum drugs are important chemotherapeutic compounds in the treatment of cancer and have shown activity against a variety of tumors either as single agents or in combination with other drugs (1, 2) but, unfortunately, are accompanied by neurotoxicity, manifested primarily as peripheral neuropathy (3–5). Taxane and platinum chemotherapy-induced peripheral neuropathy (CIPN) is a serious problem that can be dose limiting or result in discontinuation of treatment (6). Mechanisms behind CIPN are complex and involve damage to the peripheral nerves and include axonopathy and neuropathy as the two major clinical forms of neurotoxicity associated with chemotherapeutic agents (7, 8). Amifostine, glutathione, glutamine/glutamate, calcium/magnesium infusions, neurotrophic factors, nerve growth factor, gabapentin, vitamin E, N-acetylcysteine, diethyl-dithiocarbamate, recombinant erythropoietin, and carbamazepine are among the many agents that have been evaluated for use as potential neuroprotective agents (9–11). Despite promising results in some clinical trials, none of the therapies that have been evaluated thus far have become a standard of care, or have otherwise provided definitive evidence of benefit in the prevention, mitigation, or treatment of CIPN (7, 12, 13). Additionally, many of these therapies have adverse side effects, which may limit their utility in patients, and in many cases, it is presently unknown if there is significant concurrent potential interference with the antitumor activity.

Whereas significant information is available about mechanisms of paclitaxel and cisplatin antitumor activity (2, 8, 14), less information about precise mechanisms involved in paclitaxel and cisplatin CIPN exist. The antitumor effect of taxanes are due to altered cytoskeletal microtubule protein (MTP) functioning that can result in activation of apoptotic pathways, whereas...
taxane-induced CIPN is believed to be a consequence of damage to nerves that are rich in MTP (4–6, 15–25). Paclitaxel (Fig. 1B) binds to several regions on β-tubulin, a major protein component of both cytoskeletal and nerve MTP. The paclitaxel-binding site on β-tubulin includes portions of the NH2-terminal Val23, Asp26 amino acids, and the amino acid residues 217 to 236 and 272 to 278 (26–29). Paclitaxel (in the presence or absence of GTP) has been shown in vitro to induce polymerization of MTP beyond levels observed in control reactions (30–32). This phenomenon is referred to as hyperpolymerization or enhanced tubulin polymerization.

Cisplatin exerts its antitumor effect by forming platinum-DNA adducts with preference for two adjacent GG residues on the same DNA strand (1,2-intrastrand adducts; Fig. 1C; refs. 1, 33). These platinum-DNA adducts interfere with DNA replication and cause DNA damage that triggers activation of intracellular apoptotic pathways (34). Unlike paclitaxel, cisplatin interactions with MTP are not thought to be important for its antitumor activity. However, interactions between cisplatin and MTP have been reported (21–24), and these interactions might adversely affect the structural integrity of axons and impair axonal transport, thereby contributing to peripheral neuropathy accompanying cisplatin chemotherapy (6, 19, 20, 35).

Although much effort has been expended toward the development of a clinically useful neuroprotective agent that does not interfere with taxane- or cisplatin-mediated antitumor activity, the prevention of CIPN remains an important unmet need (9–11). Dimesna (BNP7787; Tavocept; disodium 2,2′-dithio-bis-ethanesulfonate; Fig. 1A) is a water-soluble disulfide currently undergoing international phase III clinical testing in patients with advanced non–small cell lung cancer (11, 36–40). In previous clinical studies, BNP7787 showed the potential to increase overall and 1-year survival in patients with non–small cell lung cancer receiving taxane and platinum combination chemotherapy (36). These improved survival outcomes were accompanied by medically and statistically significant reductions in favor of BNP7787 treatment in the incidence and severity of side effects commonly observed with taxane and platinum combination chemotherapy, including reductions in kidney toxicity, anemia, nausea, and vomiting, and significantly reduced treatment discontinuations due to CIPN.

BNP7787 seems to protect against neurotoxicity that traditionally accompanies taxane and platinum chemotherapeutic regimens (11, 38, 41), and importantly, we have previously reported that BNP7787 does not interfere with paclitaxel-induced apoptosis or with taxane-, platinum-, Vinca alkaloid-, or epothilone-induced cytotoxicity in human cancer cell lines (11). Although the mechanism(s) for potential BNP7787-mediated neuroprotective benefits is not fully elucidated, it may involve interactions between BNP7787 and MTP. The objectives of the experiments described herein were to examine the effect of BNP7787 (Fig. 1A) in vitro on aberrant MTP polymerization, induced by paclitaxel and cisplatin, to begin to elucidate the mechanisms involved in BNP7787-mediated neuroprotection.

**Materials and Methods**

Mesna (mercaptoethanesulfonic acid, sodium salt), GTP, cytochrome c, and sucrose were purchased from Sigma-Aldrich. BNP7787 and monoaquocisplatin were

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**Figure 1.** A, BNP7787 (Tavocept) structure. B, paclitaxel structure. C, cisplatin structure and subsequent aquation leading to formation of cisplatin-DNA adducts that interfere with DNA replication and DNA damage.
synthesized at BioNumerik Pharmaceuticals, Inc. Paclitaxel was purchased from Hauser Chemicals. Cisplatin (Platinol-AQ, Bristol-Myers Squibb) was obtained from the Cancer Therapy Research Center (San Antonio, TX). Glutamine was purchased from ICN Biochemical. Ureyl acetate was purchased from Polysciences, Inc. Electron micrograph grids (400 mesh, formvar/carbon film on specimen gvi) were purchased from Electron Microscopy Sciences. Other buffers, metal ion salts, and glycerol used in experiments described herein were purchased from VWR or Fisher. NAP G-25 columns were purchased from Amersham/GE Healthcare.

MTP preparation

MTP was purified from bovine brain cerebrum as described in the literature (42). Meninges were removed from fresh bovine cerebrum, and the cerebrum was placed into a 1-L beaker containing ~300 mL of ice-cold buffer A (0.1 mol/L MES, 1 mmol/L EGTA, 0.5 mmol/L MgCl₂, 0.1 mmol/L EDTA (pH 6.5)). Grey matter (100 g) was carefully cut from the cerebrum and placed in a chilled blender to which buffer A (100 mL), GTP (2.2 mL of 100 mmol/L stock), and β-mercaptoethanol (β-ME; 7 μL of a 14.3 mol/L stock) were added. This heterogeneous mixture was homogenized at high speed in a Waring blender (3 × 15 seconds). The resulting thick, homogeneous mixture was poured into high-speed polycarbonate centrifuge tubes (26.9 mL volume) and centrifuged at 25°C for 90 minutes (RCFav = 118,747 × g). The light red supernatant was brought to 750 μL final volume with buffer P. This supernatant was incubated with platinum alone, mesna alone, or platinum plus mesna, or mesna plus monoaquocisplatin, or (f) cysteine plus monoaquocisplatin. Typically, each sample was 1.25 mL of an ~7.8 μg/μL protein sample plus 76 μL of a preincubated mixture of mesna, cysteine, monoaquocisplatin, mesna plus monoaquocisplatin, or cysteine plus monoaquocisplatin. The final assay concentrations of mesna, cysteine, and monoaquocisplatin were 200, 200, and 36 μmol/L, respectively. At selected time intervals (e.g., 4, 8, 12, 16, and 24 hours), an aliquot (typically 186 μL) from the various incubation reactions was removed and brought to 750 μL final volume with buffer P. Final tubulin concentration was ~10 μmol/L. From this 750-μL sample, three 196-μL aliquots were transferred to microtiter plate wells, and the baseline at A₅₆₀ was monitored for 1 to 3 minutes. MTP polymerization was initiated at 37°C by addition of GTP (1 mmol/L) and MgSO₄ (0.5 mmol/L) to wells using an automatic pipetman. For these monoaquoplatinum experiments, MgSO₄ was used instead of MgCl₂ to avoid chloride-mediated complications (note section above describing that chloride ion will replace the aquo adduct of monoaquocisplatin for these monoaquoplatinum experiments, MgSO₄ was used instead of MgCl₂ to avoid chloride-mediated complications). The polymerization reaction was followed by monitoring the increase in absorbance at 350 nm (A₃₅₀) on a Cary 100 UV-Vis spectrometer using the Cary 100 Kinetics application (Varian Instruments) or on a SpectraMax Plus microtiter UV-Vis plate reader using SpectraMax Pro software (Molecular Devices).

GTP-catalyzed MTP polymerization assays of MTP incubated with platinum alone, mesna alone, or platinum and mesna

G-25-chromatographed MTP (~9.7 mg total protein per incubation) was incubated with (a) buffer only, (b) mesna only, (c) cysteine only, (d) monoaquocisplatin only, (e) mesna plus monoaquocisplatin, or (f) cysteine plus monoaquocisplatin. Typically, each sample was 1.25 mL of an ~7.8 μg/μL protein sample plus 76 μL of a preincubated mixture of mesna, cysteine, monoaquocisplatin, mesna plus monoaquocisplatin, or cysteine plus monoaquocisplatin. The final assay concentrations of mesna, cysteine, and monoaquocisplatin were 200, 200, and 36 μmol/L, respectively. At selected time intervals (e.g., 4, 8, 12, 16, and 24 hours), an aliquot (typically 186 μL) from the various incubation reactions was removed and brought to 750 μL final volume with buffer P. Final tubulin concentration was ~10 μmol/L. From this 750-μL sample, three 196-μL aliquots were transferred to microtiter plate wells, and the baseline at A₃₅₀ was monitored for 1 to 3 minutes. MTP polymerization was initiated at 37°C by addition of GTP (1 mmol/L) and MgSO₄ (0.5 mmol/L) to wells using an automatic pipetman. For these monoaquoplatinum experiments, MgSO₄ was used instead of MgCl₂ to avoid chloride-mediated complications (note section above describing that chloride ion was removed from solutions for these platinum-mediated experiments using G-25 size exclusion chromatography; chloride ion will replace the aquo adduct of monoaquocisplatin reforming cisplatin but monoaquocisplatin is the putative reactive species in vivo). The polymerization reaction was followed by monitoring the increase in
A350 in a microtiter plate format using the SpectraMax Plus plate reader.

**MTP polymerization assays of MTP protein incubated with paclitaxel, BNP7787, or mesna alone or in combination**

MTP (10 μmol/L) was preincubated with BNP7787 (0–16 μmol/L), mesna (200 μmol/L), or NaCl (32 mmol/L; each mole of BNP7787 contains 2 moles of sodium; NaCl was used as a control) in microcentrifuge tubes, on ice, for 20 minutes before initiation of MTP assays. Reactions were transferred from the microcentrifuge tubes to cuvettes or to 96-well plates; polymerization was initiated by addition of (a) GTP/MgCl2 (1 mmol/L/1 mmol/L final concentration), (b) paclitaxel alone (10 μmol/L), or (c) paclitaxel (10 μmol/L) and GTP/MgCl2 (1 mmol/L/1 mmol/L final concentration), and microtubule formation was monitored at A350 using UV-Vis spectroscopy.

**Electron microscopy analysis**

MTP was preincubated with BNP7787 (6 mmol/L) for 20 minutes in buffer P on ice. After this preincubation, MTP polymerization reactions were initiated with GTP/MgCl2 (1 mmol/L/1 mmol/L) or GTP/MgCl2/paclitaxel (1 mmol/L/1 mmol/L/6 μmol/L). Electron micrograph samples were prepared by gently mixing samples of reactions with an equal volume of 50% sucrose in buffer P [0.1 mol/L PIPES, 1 mmol/L EGTA (pH 6.5)] and mounting on carbon-coated grids (400 mesh formvar/carbon; Electron Microscopy Sciences). Grids were washed with cytochrome c (1%) and water and stained with uranyl acetate (1%). Electron microscopy was done using a Philips 2088 electron microscope (Philips Instruments) at an accelerating voltage of 60 kV. Micrographs were taken at ×36,000 and ×7,000 magnifications.

**Normalization of data from platinum-related MTP experiments**

MTP loses its ability to polymerize over time (referred to as decay), and a decay profile of MTP polymerization is shown in Fig. 2A. All data from the extended exposures of MTP to monoaquocisplatin with and without mesna or cysteine were normalized by setting the % polymerization values for the pH buffer control to 100% and normalizing the % polymerization values from the other reactions to this 100% buffer control value (assays were run in triplicate). Decay, or decreases in polymerization, occurs over time most likely because tubulin denatures and precipitates over time. This denatured and/or precipitated tubulin cannot assemble into microtubules, and a decrease in total polymerization, as monitored by turbidity at A350, occurs.

**Results**

**Effect of extended exposure to monoaquocisplatin on MTP polymerization in vitro**

The aquated form of cisplatin, monoaquocisplatin, is believed to be the chemotherapeutically active form of cisplatin (Fig. 1C; ref. 1). The equilibrium between cisplatin and monoaquocisplatin is affected by the prevailing chloride concentration in plasma and inside the cell. At high chloride concentrations (e.g., 100 mmol/L in

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**Figure 2.** A, example of time-dependent decay of the ability of MTP to polymerize into microtubules (control sample with no drug treatment; note that individual MTP preparations vary slightly in terms of decay profiles). B, time-dependent loss of the ability of MTP to polymerize when MTP is incubated with monoaquocisplatin before initiation of the polymerization assay. Mesna protects MTP from this loss of the ability to polymerize. Final assay concentrations were mesna (200 μmol/L) and monoaquocisplatin (36 μmol/L). B, all data, where MTP was subjected to extended exposure (0–25 h) to monoaquocisplatin with and without mesna, were normalized by setting the percentage of polymerization values from the pH buffer control (at each exposure time point) to 100% and normalizing the percentage of polymerization values from the other reactions to this 100% buffer control value (assays were run in triplicate). Percent polymerization values are A350 readings 30 min after polymerization was initiated.
plasma), cisplatin predominates over monoaquocisplatin. However, chloride concentration in most cells is very low (in some cases essentially zero), and once cisplatin enters the cell, the low chloride environment facilitates formation of the highly reactive monoaquocisplatin (1). Several groups have reported that extended exposure of MTP to platinum compounds results in the loss of the ability of MTP to polymerize into microtubules, a phenomenon called decay (21–23). Consistent with these reports, we observed that when monoaquocisplatin was incubated with MTP before initiation of MTP polymerization assays, with increasing incubation time, there was increased protein denaturation/precipitation. This was reflected in increased background A350 readings (before initiation of polymerization assays) for samples from longer incubation times and a smaller net change in A350 when polymerization was initiated (Fig. 2A). This denaturation/precipitation was especially prominent in samples where incubation times before initiation of polymerization were ≥8 hours and resulted in a starting absorbance that was higher (due to denaturation/precipitation of MTP over time) and a smaller total absorbance change when polymerization was initiated (Fig. 2A). Despite the complications of this decay phenomenon, studies described herein show that extended exposure of MTP to monoaquocisplatin resulted in the notable and reproducible loss of the ability of MTP to polymerize (i.e., loss of ability to polymerize that is beyond the well-documented tubulin decay phenomenon; Fig. 2B).

Experiments also showed that short preincubation (30 minutes) of mesna with monoaquocisplatin prevented the monoaquocisplatin-induced loss of the ability of MTP to polymerize (Fig. 2B, black columns). Mesna is a metabolite of BNP7787 (Fig. 3A) and is postulated to displace the aquo group from monoaquocisplatin, resulting in the formation of a possible sulfur-platinum adduct that may prevent monoaquocisplatin from forming an adduct with surface cysteine residues on tubulin.

Figure 3. A, postulated SN2 route of nonenzymatic reduction of BNP7787 to mesna in the kidney. B, mesna may displace the aquo group from monoaquocisplatin and the formation of a possible sulfur-platinum adduct may prevent monoaquocisplatin from forming an adduct with surface cysteine residues on tubulin.
nor cysteine alone (in the absence of monoaquocisplatin) had any effect on MTP polymerization.

**Effect of BNP7787 and mesna on GTP-catalyzed MTP polymerization**

BNP7787 (4, 6, 8, and 10 mmol/L), preincubated with MTP on ice (20 minutes), inhibited GTP-catalyzed polymerization of MTP at 37°C in a dose-dependent manner (Fig. 4A). These data trends were reproducible using different MTP preparations and different lots of BNP7787. The *in vivo* metabolite of BNP7787, mesna, did not affect MTP polymerization in an appreciable manner even at very high levels that are not physiologically achievable (e.g., 10 mmol/L; Fig. 4B). At lower mesna concentrations that correspond more closely with peak plasma levels observed in patients, there was no effect on *in vitro* MTP polymerization. MTP polymerization [at 41 g/m² BNP7787 (~14 mmol/L BNP7787 in plasma), the C<sub>max</sub> for mesna was ~323 μmol/L; ref. 38]. MTP polymerization was unchanged in assays where MTP was preincubated with clinically achievable concentrations of mesna (up to 300 μmol/L) before initiation of polymerization, and higher concentrations of mesna also had no effect (Fig. 4B).

**Effect of BNP7787 on paclitaxel-catalyzed MTP hyperpolymerization**

BNP7787 (1, 4, 8, 12, and 16 mmol/L), preincubated with MTP on ice (20 minutes), inhibited paclitaxel-promoted MTP hyperpolymerization in a dose-dependent manner (Fig. 4D). Plasma concentrations equivalent to 10 mmol/L for BNP7787 are achieved in clinical trials (11, 37, 38). Paclitaxel peak plasma concentrations can be as high as

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**Figure 4.** Effects of BNP7787 and mesna on MTP polymerization under various assay conditions. A, BNP7787 has a dose-dependent inhibitory effect on GTP-promoted MTP polymerization. B, mesna does not affect GTP-promoted MTP polymerization (data in A were obtained using a Cary 100 UV-Vis cuvette-based spectrometer, whereas data in B were obtained using a SpectraMax Plus microtiter plate UV-Vis spectrophotometer). C, BNP7787 modulates GTP/paclitaxel-promoted MTP polymerization. The line with open squares is an MTP polymerization assay promoted only by GTP, a GTP-only control. D, BNP7787 modulates paclitaxel-promoted MTP polymerization (no GTP present) and this effect is not due to the two sodium counterions of BNP7787, as 32 mmol/L NaCl alone had no effect (data not shown).
10 μmol/L (8). In our studies, paclitaxel (10 μmol/L) was used to achieve a 1:1 (drug:tubulin) subunit ratio and optimal hyperpolymerization effects in the in vitro MTP polymerization assay. Paclitaxel (10 μmol/L)-promoted MTP hyperpolymerization was reproducibly inhibited by BNP7787 at concentrations of >1 mmol/L (Fig. 4C and D). Although all data herein should be interpreted qualitatively, we observed ~50% inhibition of paclitaxel-promoted MTP polymerization by BNP7787 at concentrations of 12 mmol/L (Fig. 4D), and final polymerization levels of MTP exposed to paclitaxel were essentially equal to controls that lacked BNP7787 and paclitaxel (GTP control; Fig. 4C) when ≥6 mmol/L BNP7787 was present (Fig. 4C).

Effect of BNP7787 on paclitaxel/GTP/MgCl2-catalyzed MTP hyperpolymerization

BNP7787 (6, 8, 10, and 12 mmol/L), preincubated with MTP on ice (20 minutes), inhibited paclitaxel/GTP/MgCl2-catalyzed MTP hyperpolymerization in a dose-dependent manner (Fig. 4C). This dose-dependent inhibitory trend was reproducible using different MTP preparations and different lots of BNP7787 (data not shown). When MTP polymerization was initiated by a paclitaxel/GTP/MgCl2 mixture (10 μmol/L/1 mmol/L/1 mmol/L, respectively), BNP7787 concentrations of 6 to 8 mmol/L resulted in a net paclitaxel/GTP/MgCl2-catalyzed polymerization of MTP equivalent to that observed for a control reaction where polymerization is initiated with GTP/MgCl2 only. Because 6 to 10 mmol/L concentrations of BNP7787 are pharmacologically achievable, the in vitro inhibitory effects of BNP7787 on the MTP polymerization catalyzed by paclitaxel/GTP may be achieved in vivo as well. At lower paclitaxel concentrations, correspondingly lower levels of BNP7787 antagonized the effect of paclitaxel on MTP polymerization (data not shown). These data trends were reproducible using different MTP preparations and different lots of BNP7787. Furthermore, sodium chloride (32 mmol/L; Fig. 4D) does not inhibit MTP polymerization; therefore, the sodium in BNP7787 does not exert the inhibitory effect, and the inhibitory/protective effects on MTP polymerization under these experimental conditions are attributed solely to BNP7787.

Qualitative evaluation of the effect of BNP7787 on MTP morphology in the presence and absence of paclitaxel

Qualitative evaluation of electron microscopy grids indicated that BNP7787, preincubated with MTP on ice (20 minutes) before initiation of MTP polymerization using GTP/MgCl2, resulted in a reduction in the abundance of microtubules visible in sectors of the grids in the presence and absence of paclitaxel (Fig. 5B and D). This corresponded well with the decrease in $A_{355}$ observed in reactions containing BNP7787 in the presence and absence of paclitaxel. There were no effects on overall gross microtubule morphology by BNP7787 that were discernible using this approach, and the effect of BNP7787 on MTP polymerization in the presence of paclitaxel does not result in the formation of morphologically or structurally aberrant microtubules. However, it seems that when BNP7787 and paclitaxel (Fig. 5D) are both present, that fewer microtubules (qualitative assessment) are formed compared with when BNP7787 is not present (Fig. 5C).

Discussion and Conclusions

Physicians often underreport and underestimate the severity of the CIPN that patients experience, and there is an important, unmet need for agents that reduce the discomfort, severity, and long-term side effects of CIPN (44, 45). Paclitaxel-induced CIPN often initially presents as numbness and tingling in the hands and feet or impaired deep tendon reflexes (8) and can include axonopathy, myelinopathy, and/or neuropathy (7, 8). Cisplatin-induced CIPN is a frequent and often dose-limiting toxicity that often manifests itself in patients as numbness and paresthesia in the hands and feet and becomes progressively more debilitating as chemotherapy continues (1, 14).

BNP7787 is an investigational drug that is undergoing clinical development to increase survival and prevent or mitigate serious chemotherapy-induced toxicities in patients with inoperable advanced primary adenocarcinoma of the lung receiving platinum and taxane chemotherapy (11, 37–40, 46, 47). BNP7787 has the potential to protect against neurotoxicity that traditionally accompanies taxane and platinum chemotherapeutic regimens (11, 38, 41). BNP7787 substantially differs in pharmacology and mechanisms from other sulfur-containing small molecules that have been evaluated for the potential...
to neuroprotect against platinum- and taxane-induced neuropathy. BNP7787 has the potential to be administered to patients in large doses (e.g., 18.4 g/m²) without toxicity and without significant perturbations in plasma disulfide proportions or in the strongly oxidizing extracellular environment (Fig. 2; ref. 11) and thereby provides substantial pharmacologically available thiol and disulfide augmentation, which in turn may affect numerous physiologic processes, including the oxidative state of the plasma, interstitial space, and intracellularly, as well as by targeting thioredoxin and glutaredoxin systems (36). These characteristics make BNP7787 distinct from previous sulfur-containing small molecules that have been examined for neuroprotective potential (9–11). Although BNP7787 is expected to remain predominantly in the disulfide form in the plasma (11, 48), the intracellular environment and the interstitial space are likely venues for BNP7787 metabolism to a key metabolite, mesna (Fig. 6A), and we have reported in detail on thiol-disulfide exchange reactions that occur between BNP7787 and physiologic thiols previously (11, 37, 48).

Herein, we report that the BNP7787 metabolite mesna completely protected MTP from monoaquocisplatin-induced losses of MTP polymerization activity (Fig. 2B). In the absence of a protecting agent such as BNP7787-derived mesna, monoaquocisplatin is highly reactive and interacts most probably with surface-accessible

![Figure 6. A, BNP7787 thiol disulfide exchange reactions may occur intracellularly and/or in the interstitial space (11, 37, 48). B, proposed BNP7787-mediated oxidation of cysteine residues on the αβ-tubulin heterodimer. BNP7787 may undergo thiol-disulfide exchange with surface-accessible cysteine residues on αβ-tubulin, forming a mesna-cysteine adduct close to the paclitaxel-binding site. The structure was generated using 1JFF coordinates and the Accelrys Discovery Studio program.](image-url)
sulfur-containing groups (e.g., cysteine residues) on tubulin and MAPs. In contrast, an inactivated mesna-platinum species (Fig. 3B) would not be reactive with these moieties on tubulin or MAPs. Cisplatin interactions with MTP are not thought to contribute to its antitumor effects but may be one contributing mechanism behind cisplatin-induced neurotoxicity (6, 21–24). Mesna, a metabolite of BNP7787, was able to protect against monoaquocisplatin-induced perturbation of MTP polymerization, and we propose that, in vivo, at specific locations BNP7787 may undergo reduction to mesna (Fig. 3A) and/or form BNP7787-derived mesna-disulfide heteroconjugates (Fig. 6A), and these BNP7787-derived species could provide significant protection against cisplatin-induced neurotoxicity. In contrast, we observed no detectable effect of mesna alone on MTP polymerization (Fig. 4B) or on paclitaxel-induced hyperpolymerization of MTP (data not shown). Furthermore, we observed (Fig. 4C) that BNP7787 normalizes the well-characterized paclitaxel-induced hyperpolymerization of MTP (30–32), and because plasma levels of 8 mmol/L BNP7787 and higher are pharmacologically achievable at doses of 18.4 g/m² and higher (39), BNP7787-mediated protection against paclitaxel-induced hyperpolymerization of MTP observed in vitro may potentially occur in patients receiving paclitaxel as well. For example, BNP7787-mediated oxidation of surface-accessible cysteine residues on tubulin, resulting in formation of mesna-cysteine disulfides, may be one mechanism behind BNP7787-mediated inhibition of paclitaxel-induced hyperpolymerization of MTP. Computational studies to examine this hypothesis are underway (Fig. 6B).

Many agents have been evaluated for potential neuroprotective benefits in patients receiving paclitaxel and cisplatin chemotherapy, but none are currently approved to treat CIPN (9–11). As noted earlier, BNP7787 does not interfere with paclitaxel-induced apoptosis or with taxane-, platinum-, *Vinca* alkaloid-, or epothilone-induced cytotoxicity in human cancer cell lines (11). Further, in animal models, BNP7787 did not exhibit tumor protection when given with taxane and platinum agents and concurrently prevented lethal chemotherapy-induced toxicity from paclitaxel, cisplatin, carboplatin, and oxaliplatin (11, 37, 38, 40, 46). In phase I and III clinical trials, BNP7787 was not observed to interfere with the antitumor activity of cisplatin and paclitaxel (11, 47). In an additional phase III clinical trial, treatment with BNP7787 seemed to significantly improve 1-year survival of patients with advanced primary adenocarcinoma of the lung who were being treated with cisplatin and paclitaxel regimens in the first-line setting (36). Previous studies have suggested that BNP7787 seems to have significant cisplatin nephroprotective benefits (11, 37, 38, 40, 46, 49, 50). Based on data presented herein, we propose that BNP7787 interactions with MTP may be one mechanism through which BNP7787 may act to protect against taxane- or platinum-induced neurotoxicity by normalizing paclitaxel-induced hyperpolymerization of MTP and by forming BNP7787-derived mesna in *vivo*, which reacts with and inactivates the highly reactive monoaquocisplatin species.

**Disclosure of Potential Conflicts of Interest**

F.H. Hausheer is the Chairman, Chief Executive Officer, and a substantial shareholder of BioNumerik Pharmaceuticals, Inc., a private pharmaceutical company that owns the proprietary rights and patents relating to BNP7787 and provided funding relating to the research described in the publication. A.R. Parker, P.N. Petliaru, M. Zhao, and H. Kochat are employees and shareholders and/or option holders of BioNumerik Pharmaceuticals, Inc. M. Wu is a former employee of BioNumerik Pharmaceuticals, Inc.

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**References**


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