Accumulation of BNP7787 in Human Renal Proximal Tubule Cells

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ABSTRACT: BNP7787, an investigational drug undergoing global Phase III development, appears to have potential advantages over other cytoprotective compounds that have been evaluated for preventing and mitigating cisplatin-induced nephrotoxicity. Herein, we characterized the *in vitro* accumulation of BNP7787 in human renal proximal tubule cells (HK-2) in which cisplatin is known to be taken up and accumulate. HK-2 cells were incubated with pharmacological concentrations of BNP7787 for varying times. Temperature-dependent accumulation of BNP7787 in cells was observed and the BNP7787-derived metabolite, mesna, formed intracellularly was directly monitored. The peak level of BNP7787-derived mesna measured in HK-2 cells was approximately 0.6 nmol/10^6 cells; this is pharmacologically similar to reported platinum concentrations in kidney cells and may be sufficient to afford nephroprotection. Therefore, in addition to previously suggested glomerular filtration, the cellular accumulation of BNP7787 by HK-2 cells is a plausible newly identified mechanism by which BNP7787 may accumulate in renal tubular cells, where it can exert its pharmacological effects to protect against cisplatin-induced nephrotoxicity by direct covalent conjugation of mesna with cisplatin, or by the formation of BNP7787-derived mesna–disulfide heteroconjugates that exert nephroprotective effects by inhibition of the key toxification enzyme targets γ-glutamyltranspeptidase and aminopeptidase N. © 2011 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:3977–3984, 2011

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INTRODUCTION

Nephrotoxicity of the clinically important chemotherapeutic drug, *cis*-diamminedichloroplatinum (cisplatin), limits the utility and convenience of cisplatin-containing regimens.[1–4] The kidney appears to take up and retain platinum to a greater extent than other organs.[5] In the clinic, therapeutic interventions aimed at preventing and mitigating cisplatin-induced nephrotoxicity include saline hydration and forced diuresis with furosemide and mannitol.[2,3,6] Although such therapeutic interventions may result in shorter tubular contact and transit time, and dilution of cisplatin concentration in the tubule, they do not completely protect against cisplatin renal toxicity, and substantially add to the cost, complexity, and time required to administer cisplatin relative to carboplatin and oxaliplatin. A number of small molecule protective agents have also been studied for prevention or management of cisplatin-induced nephrotoxicity and a wide range of biological targets have been evaluated for their potential to exert renal protective effects and/or for their role in cisplatin-induced nephrotoxicity.[1,3,7] Many of the small molecule protective agents have limited utility in the clinic for treating cisplatin-induced nephrotoxicity due to adverse side effects, attenuation of antitumor activity, and/or disruption of the physiological thiol–disulfide ratios in plasma and intracellularly.[8,9] Accordingly, saline hydration and/or forced diuresis remains the standard of care in the clinic.[2,3]

BNP7787 (disodium 2,2′-dithio-bis ethane sulfonate; Tavocept™ (BioNumerik Pharmaceuticals, Inc., San Antonio, TX, USA); Fig. 1) is a water-soluble investigational agent that is undergoing clinical development.[8–13] In rats, BNP7787 (intravenous)

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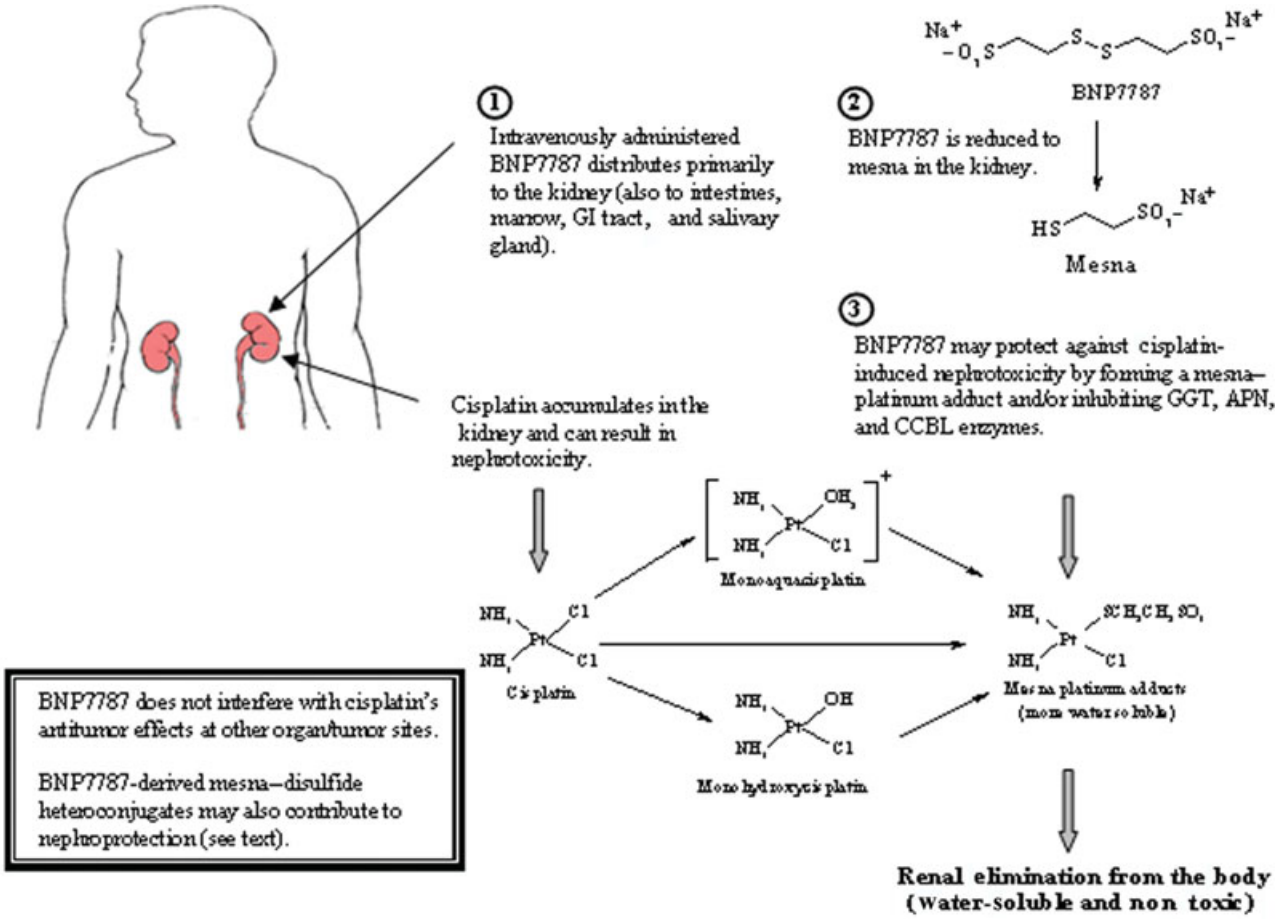

Figure 1. When cisplatin is administered, it enters the kidney cells by transporter-mediated or via free diffusion mechanisms and accumulates specifically in S3 segment of proximal tubule. Both BNP7787 and its metabolites are preferentially taken up by the kidney cells in which BNP7787-derived mesna reacts with cisplatin, resulting in the formation of BNP7787-derived mesna–cisplatin conjugates. The BNP7787-derived mesna–cisplatin conjugate, in contrast to cisplatin alone, is not nephrotoxic

is predominantly distributed in the proximal renal tubular cells of the kidney, the intestine, marrow, and salivary gland (Fig. 1).[13,14] BNP7787 does not appear to interfere with the antitumor activity of cisplatin, carboplatin, oxaliplatin, docetaxel, or paclitaxel either *in vitro* or *in vivo*.[8,10] BNP7787 metabolism appears to involve nonenzymatically mediated thiol and disulfide transfer reactions that yield BNP7787-derived mesna–disulfide heteroconjugates.[8,9,15,16]

Transport-mediated uptake is likely the major pathway of cisplatin uptake in renal tubular cells, although passive uptake also occurs.[17,18] Possible routes for uptake of BNP7787 or BNP7787-derived mesna–disulfide heteroconjugates in the kidney include transport into the cell,[19] uptake by organic anion transporters 1, 3, and 4,[20] and free glomerular filtration (BNP7787 is a small molecular weight anion and is expected to be freely filtered by the glomeruli). Additionally, BNP7787 has the potential to reduce the concentration of plasma thiols and thus potentially may enhance the antitumor activity of cisplatin.[19] In order for BNP7787 to exert nephroprotective effects against cisplatin *in vivo*, it is required to either act *in situ* upon and/or be taken up by the renal tubular cells. We hypothesize that BNP7787 exerts a localized nephroprotective effect in the kidney without interfering with or inhibiting cisplatin-induced cytotoxicity at other *in vivo* locations (Fig. 1) and that both cellular uptake and free glomerular filtration, distribution and *in situ* reduction of BNP7787 may be important for the BNP7787-mediated protection against cisplatin-induced nephrotoxicity. Herein, identification of selective temperature-dependent accumulation conditions and quantitative high-performance liquid chromatography–electrochemical detection (HPLC–EC) techniques are used to estimate protein-free and protein-bound BNP7787 by detecting the BNP7787-derived metabolite, mesna, in all forms in cultured human proximal tubule cells.