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Original article

## Protective effects of a glutathione disulfide mimetic (NOV-002) against cisplatin induced kidney toxicity

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### ABSTRACT

NOV-002 is a glutathione disulfide (GSSG) mimetic with chemoprotective activity. Previous and ongoing clinical studies demonstrate a significantly improved 1-year survival and decreased tumor progression rates in non-small cell lung (NSCLC) and ovarian cancer patients when NOV-002 was included in cisplatin containing regimens. In order to understand this chemoprotective property, we employed as an animal model of kidney toxicity, 8-week-old BL6 mice that were treated with a single nephrotoxic dose of cisplatin (15 mg/kg, ip) and sacrificed on Day 5. One group of animals was treated with NOV-002 (15 mg/kg, im) daily. NOV-002-treated mice had significantly lower levels of plasma creatinine compared to mice treated with cisplatin alone (4.7 vs 2.9 mg/dL, respectively). Moreover, NOV-002 protected the kidneys from cisplatin mediated proximal tubule damage, including dilation of tubules and the presence of protein casts. Since cisplatin-induced nephrotoxicity can be mediated by a glutathione-platinum conjugate catalyzed by  $\gamma$ -glutamyl-transpeptidase (GGT) and glutathione is an endogenous substrate of GGT, the protective effect of NOV-002 in the kidney may be attributed to its ability to act as a competitive substrate for the enzyme.

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## 1. Introduction

NOV-002 is a mimetic of oxidized glutathione (GSSG) and has impact on cellular redox balance. This balance is critical to the maintenance of cell viability. In particular, thiol homeostasis is one important part of redox homeostasis and is subject to pharmacological manipulation via NOV-002. NOV-002 is currently in pivotal Phase III clinical trials in advanced non-small cell lung cancer. In trials conducted to date, NOV-002 administered in combination with standard chemotherapeutic regimens has resulted in increased efficacy (survival, tumor response) and reduced toxicity in tandem with enhanced tolerance to standard chemotherapeutic therapies [1,2]. The structure of NOV-002 is shown in Fig. 1. It is oxidized glutathione (GSSG) with cis-platinum at an approximate 1000:1 ratio. The cisplatin may serve to stabilize the GSSG and does not contribute substantially to the pharmacology of the compound and indeed, the preclinical data confirm that GSSG is the active component of NOV-002 [3].

Cisplatin remains a front line drug for the treatment of solid epithelial tumors, but causes nephrotoxicity. This toxicity is

mediated through effects on quiescent proximal tubule cells that contain high levels of GGT and cysteine conjugate beta-lyase. GGT hydrolyzes glutathione, glutathione disulfide and glutathione-conjugates (exemplified by the glutathione conjugate of cisplatin). Nephrotoxicity is dependent upon catalysis via GGT and beta-lyase where it has been shown that both pharmacologic and genetic modulation abolished nephrotoxicity in rodent models [4–6]. NOV-002 is a substrate of GGT and alters cellular redox status. The studies reported here were designed to further elucidate its pharmacologic profile in terms of mitigating kidney toxicity.

## 2. Materials and methods

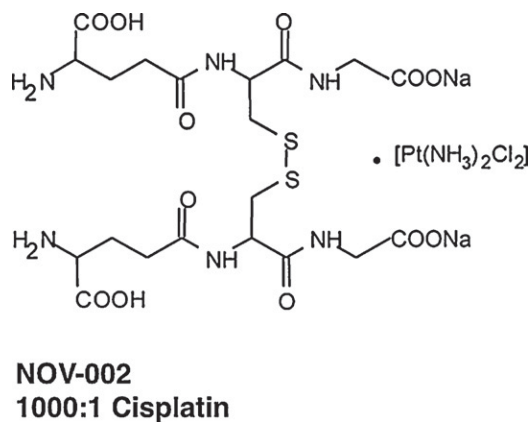
### 2.1. Animals

Female C57/BL6 mice were housed in the Animal Resource Facility of the Medical University of South Carolina. Animal care and all treatment protocols were approved by the MUSC IACUC Committee. Mice 8–12 weeks old were used for these studies. Mice (6 to 9 per group) were weighed and treated with:

- (a) 0.9% saline (ip);
- (b) 25 mg/kg NOV-002 (ip daily  $\times$  5 days);
- (c) 15 mg/kg cisplatin (ip);

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**Fig. 1.** Structure of NOV-002. NOV-002 is the disodium salt of glutathione disulfide in a 1000:1 ratio with cisplatin.

- (d) 15 mg/kg cisplatin (ip)  
(e) 25 mg/kg NOV-002 (ip daily  $\times$  5 days).

Five days after treatment, the mice were weighed and blood was collected in heparin-coated tubes by orbital bleed. The mice were sacrificed and the kidneys were fixed for histologic analysis.

## 2.2. Hematology and creatinine

Complete blood counts and blood chemistry analysis were performed by the Drug Metabolism and Pharmacokinetics Facility at the Hollings Cancer Center, MUSC. Serum creatinine levels were determined with an automated Abaxis VetScan.

## 2.3. Histology

Kidneys were fixed in paraformaldehyde, embedded in paraffin, sectioned at 4  $\mu$ m, and stained with hematoxylin and eosin. Damage was assessed on blinded slides independently by three observers (D.M.T., K.D.T. and L.H.).

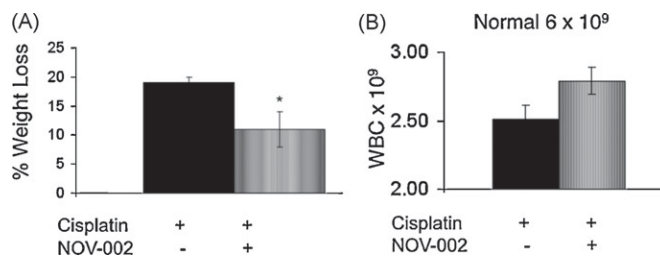
## 2.4. Data analysis

The mean and S.D. were computed for each treatment group. Statistically significant differences between treatment groups were detected by the One Way Anova Test using the saline or NOV-002 treatment as the control and with Tukey's Multiple Comparison Test for all pairwise multiple comparisons [7].

## 3. Results

### 3.1. Effect of cisplatin on body weight and mortality

Animals were weighed prior to treatment on Day 1 and prior to sacrifice on Day 5. On Day 1, mice weighed an average of 19 g. The percent change in body weight was calculated for each animal and shown in Fig. 2A. In the saline- and NOV-002-treated control groups, there were no statistically relevant changes in the initial and final average weight. However, mice in the cisplatin treatment group showed a  $19 \pm 5\%$  decrease whereas NOV-002 plus cisplatin treated animals lost  $11 \pm 3\%$  ( $p < 0.05$ ). While only one of the cisplatin animals died, morbidity in this group was high with all animals displaying lethargy and lack of grooming. When NOV-002 was administered in combination with cisplatin, there was an alleviation of such symptoms and no animal deaths ( $p < 0.01$ ) (Fig. 3).

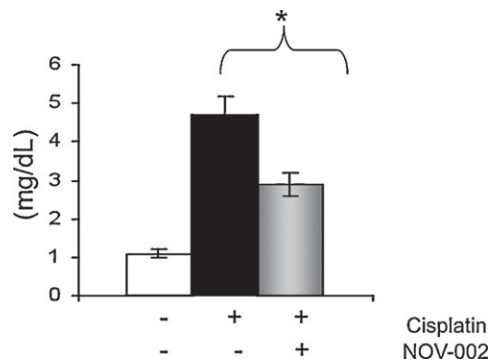


**Fig. 2.** The effect of NOV-002 treatment on weight loss and white blood counts (WBC). A. Mice were weighed on Days 1 and 5. The average % weight loss was calculated for each animal. The average body weight of saline and NOV-002 treated animals did not change. B. Blood was collected immediately before sacrifice on Day 5. The average white blood cell counts for saline and control treated animals were  $6 \times 10^9$ . Cisplatin and NOV-002 plus cisplatin groups were significantly decreased, suggesting bone marrow toxicity,  $p < 0.01$ .

Bone marrow suppression is observed in patients treated with cisplatin. Patients receiving NOV-002 in combination with chemotherapy have long-term myeloprotective effects [2]. We evaluated the white blood cell counts in animals immediately prior to sacrifice on Day 5. The saline and NOV-002 treated animals had average values of  $\sim 6 \times 10^9$  cells/mL. Cisplatin and NOV-002 plus cisplatin treatment groups showed significant decrease in this population,  $1.9$  and  $2.5 \times 10^9$  cells/mL, respectively. These data suggest that the myeloproliferative effects observed in NOV-002 treated patients may be related to recovery rather than acute protection.

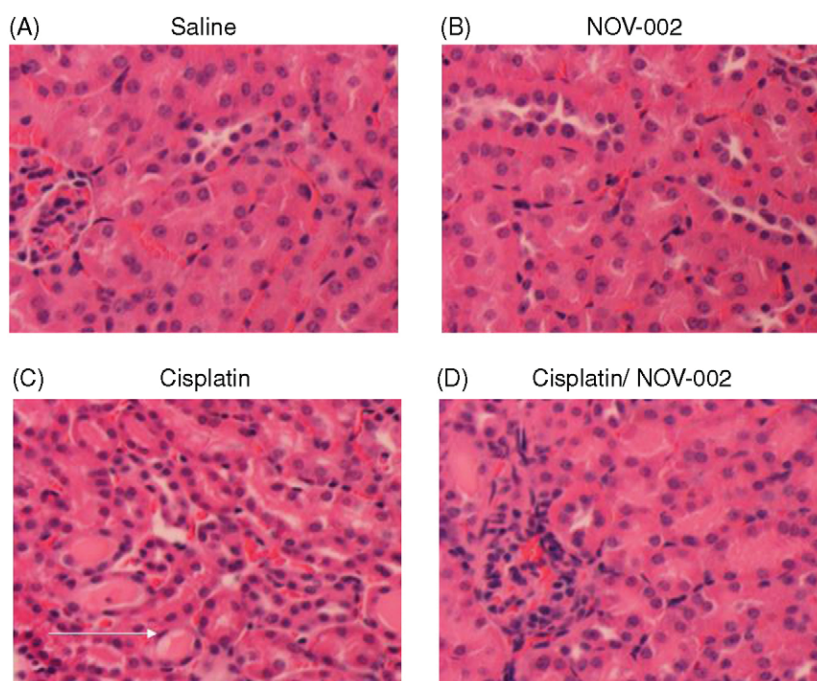
### 3.2. Effect of cisplatin on nephrotoxicity and renal morphology

Kidney damage was assessed by analyzing serum creatinine and histopathology. Immediately, prior to sacrifice on Day 5, blood was collected and analyzed using an automated VetScan. Creatinine levels in the saline and NOV-002 treatment group were normal,  $1.1 \pm 0.1$  mg/dL. Nephrotoxicity in the cisplatin treatment group was severe,  $4.7 \pm 0.5$  mg/dL whereas NOV-002 treatment was nephroprotective,  $2.9 \pm 0.3$  mg/dL, ( $p < 0.01$ ). Histologic analysis was utilized to confirm the nephrotoxicity of cisplatin (Fig. 4). The criteria for histopathology was assessed based on changes in tubular morphology, the presence/absence of an intact epithelial brush-border and the presence of protein casts, an indicator of extensive tubular damage. Normal renal morphology as determined by a clear lumen and an intact-brush border was observed in the saline and NOV-002 treated animals. The histological morphology of



**Fig. 3.** The effect of NOV-002 treatment on renal function. Mice were treated with saline (ip); 25 mg/kg NOV-002 (ip daily  $\times$  5 days); 15 mg/kg cisplatin (ip) on Day 1; or 25 mg/kg NOV-002 (ip daily  $\times$  5 days) plus 15 mg/kg cisplatin (ip) on Day 1. Five days after treatment blood was collected and creatinine was measured using an automated VetScan. Cisplatin-treated mice had significantly higher levels of creatinine than NOV-002 plus Cisplatin treatment.

\* Signifies significantly different from all other groups  $p < 0.05$ .



**Fig. 4.** The effect of NOV-002 treatment on renal morphology. Mice were treated with: (A) saline (ip); (B) 25 mg/kg NOV-002 (ip daily  $\times$  5 days); (C) 15 mg/kg cisplatin (ip) on Day 1; or (D) 25 mg/kg NOV-002 (ip daily  $\times$  5 days) plus 15 mg/kg cisplatin (ip) on Day 1. Five days after treatment mice were sacrificed and their kidneys were paraffin embedded and stained with hematoxylin and eosin. Cisplatin-treated mice had extensive proximal tubule damage including the presence of protein casts (arrow) and dilated tubules.

**Table 1**  
Mortality and nephrotoxicity of cisplatin in mice.

	Mortality	Elevated creatinine	Abnormal histology
Saline	0/6 <sup>a</sup>	0/6	0/6
NOV-002	0/6	0/6	0/6
Cisplatin	1/8 <sup>b</sup>	6/7 <sup>b</sup>	7/7 <sup>b</sup>
Cisplatin/ NOV-002	0/9	3/9	3/9

<sup>a</sup> Data are the number of mice who died, had elevated plasma creatinine (>0.2 mg/dL) or abnormal renal histology over the number of mice treated.

<sup>b</sup> Statistically significant differences between treatment groups were detected with a Fisher Exact Test,  $p < 0.01$ .

cisplatin- and NOV-002 plus cisplatin treated animals mirrored the results in the blood chemistry analysis. Extensive tubular necrosis, loss of the epithelial brush-border and the presence of protein casts in the lumen was observed in all animals in the cisplatin treated group (Table 1). Only three out of nine animals in the NOV-002 plus cisplatin treatment group showed either elevated creatinine or abnormal histology. Additionally, the damage was less extensive and the presence of protein casts was not detected.

#### 4. Discussion

Previously, reduced glutathione has been used clinically to ameliorate cisplatin toxicities in patients [8–10]. Since NOV-002 is already in clinical trial as a general chemoprotective drug, our interest lay in its, as yet undefined, capacity to protect against kidney damage. Our preclinical results show that NOV-002 enacts protection against cisplatin-induced nephrotoxicity and that this is manifest through improved *in vivo* kidney functions and reduced morbidity and mortality in the mice. These results will serve to alleviate any concerns that the low cisplatin concentrations present in the NOV-002 formulation would manifest additional toxicities when administered with standard therapeutic regimens

that contain cisplatin. In general, the pleiotropic pharmacological effects of NOV-002 are attributable to the glutathione disulfide component of the drug and modulation of cellular redox balance is a feature central to NOV-002s mechanism of action [3]. Interference with the formation of glutathione-cisplatin conjugates may reduce toxicity to proximal tubule cells [5] suggesting that this conjugate is involved in nephrotoxicity. Cisplatin conjugation to glutathione may occur in multiple organs and indeed, short-term treatment of rodents with cisplatin shows that multiple metabolites can be formed [11]. Since the glutathione-platinum conjugate is catalyzed by GGT and glutathione disulfide is an endogenous substrate of GGT [3], mechanistically, the protective effect of NOV-002 in the kidney may be attributed to its ability to act as a competitive substrate for the enzyme. In addition, it would seem that the chemoprotective effects of NOV-002 extend beyond the bone marrow and immune system reported previously [2,12] and confirmed in our present study, and may define a more systemic property for the drug.

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