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Selective iNOS inhibition reduces renal damage induced by cisplatin

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Abstract

Cisplatin is a chemotherapeutic agent used in the treatment of several cancer tumors; however, nephrotoxicity has restricted its use. Reactive oxygen species and peroxynitrite, which is formed by the reaction between superoxide anion and nitric oxide ($NO^{\bullet}$), are implicated in cisplatin-induced nephrotoxicity. In contrast, both toxic and beneficial effects of $NO^{\bullet}$ have been suggested in cisplatin-induced nephrotoxicity. Therefore, nowadays the role of $NO^{\bullet}$ in this experimental model remains controversial. The aim of the present work was to elucidate the role of $NO^{\bullet}$ in cisplatin-induced renal damage using *N*-[3-(aminomethyl)benzyl]acetamidine (1400W), a selective and irreversible inhibitor of iNOS. The mRNA levels of iNOS were increased in cisplatin-treated rats. The administration of 1400W reduced the cisplatin induced histological damage, renal dysfunction (increase in proteinuria and kidney injury molecule expression and decrease in creatinine clearance), tubulointerstitial infiltration, oxidative stress (increase in renal malondialdehyde and inmmunostaining for 4-hydroxy-2-nonenal) and nitrosative stress (immunostaining for 3-nitrotyrosine). In addition, the administration of 1400W was unable to modify systolic blood pressure in control rats. Our data demonstrate that selective iNOS inhibition reduces the cisplatin-induced nephrotoxicity and nitrosative stress which strongly suggest that in this experimental model (1) the $NO^{\bullet}$ production is toxic and (2) iNOS is the main source of $NO^{\bullet}$.

Keywords: Cisplatin; 1400W; Inducible nitric oxide synthase (iNOS); Nitric oxide ($NO^{\bullet}$)

1. Introduction

Cisplatin (Cis-diamminedichloroplatinum II) is a chemotherapeutic agent useful in the treatment of testicular, ovarian, bladder, breast, head, and neck cancers (Lebwohl and Canetta, 1998). Nephrotoxicity, however, is the dose-limiting factor for cisplatin clinical use (Daugaard and Abildgaard, 1989). Cellular and molecular mechanisms responsible for cisplatin-induced nephrotoxicity are not well understood, but there is evidence that the formation of reactive species is involved in producing renal damage. Role of oxidative and nitrosative stress is additionally supported by the protective effect of several free radical scavengers and antioxidants (Chirino et al., 2004; Masuda et al., 1994; Nishikawa et al., 2001; Tsuruya et al., 2003;

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Tsutsumishita et al., 1998) and enhanced expression of heme oxygenase-1 (Shiraishi et al., 2000) in cisplatin-induced nephrotoxicity. On the other hand, nitrosative stress involves reactive nitrogen species (RNS), such as peroxynitrite ($ONOO^-$), peroxynitrous acid, nitryl chloride (NO_2Cl) and nitrogen dioxide radical ($NO_2^\bullet$) (Coddington et al., 1999). RNS are originated from nitric oxide ($NO^\bullet$), which is synthesized by a family of nitric oxide synthase (NOS) composed of three isoforms: neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS) and all of them have been localized in the kidney (Kone, 2004). These enzymes contain heme and binding sites for flavin adenine dinucleotide, flavin mononucleotide, nicotinamide adenine dinucleotide phosphate (NADPH), tetrahydrobiopterin, and Ca^{2+}/calmodulin, essential for catalytic oxidation of L-arginine. In addition, nNOS and eNOS are constitutive enzymes, while iNOS is an inducible isoform up-regulated in response to a wide range of inductors such as lipopolysaccharides, cytokines and oxidative stress. iNOS is able to produce large amounts of $NO^\bullet$ that, under oxidative stress conditions, can react with superoxide anion ($O_2^{\bullet -}$) to form $ONOO^-$, an oxidant specie able to modify a great number of biomolecules such as amino acids, proteins, enzymes and cofactors (Szabo, 2003). A selective, irreversible and time-dependent inhibitor of iNOS, *N*-(3-(aminomethyl)benzyl) acetamidine (1400W) that is 5000-and 200-fold more potent against purified eNOS and nNOS isoforms, respectively (Garvey et al., 1997), has been helpful in investigating $NO^\bullet$ role derived from iNOS in different pathophysiological models (Kankuri et al., 2001; Mark et al., 2005; Robinson et al., 2005; Thomsen et al., 1997). 1400W has an amidine group that mimics guanidinium group of the substrate L-arginine and forms hydrogen bonds with carboxylate oxygen atoms of iNOS catalytic unit Glu-371/592 (Fedorov et al., 2003).

In contrast with non-selective NOS inhibitors such as *N*(G)-nitro-L-arginine-methyl-ester (L-NAME) (Pedraza-Chaverri et al., 1998), the administration of 1400W is unable to modify systemic blood pressure (Atkins et al., 2006; Nagareddy et al., 2005; Yaghi et al., 2004; László and Whittle, 1997) indicating the lack of effect of 1400W on eNOS activity.

We previously demonstrated that an increased $ONOO^-$ generation is involved in cisplatin-induced nephrotoxicity (Chirino et al., 2004). In addition, Deng and colleagues (2001) demonstrated an increase in iNOS mRNA levels in kidney 4 h after cisplatin administration. However, at present, the role of $NO^\bullet$ in cisplatin-induced nephrotoxicity remains controversial. In fact, it has been found that $NO^\bullet$ may be toxic (Srivastava et al., 1996; Mansour et al., 2002) or beneficial (Saad et al., 2002; Saleh and El-Demerdash, 2005). Based on the above information, we hypothesize that $NO^\bullet$ production could be toxic and that iNOS may be the source of $NO^\bullet$ in this experimental model. To test this hypothesis, a selective and irreversible iNOS inhibitor was used in the present study.

2. Materials and methods

2.1. Chemicals

Cisplatin (Cat. No. P4394, Lt 026K1291), trimethoxypropane, and 1-methyl-2-phenylindole were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 1400W (Cat No. 81520, Lt 135873) was from Cayman Chemical (Ann Arbor, MI, USA). Miniosmotic pumps Model 2001 were from Alzet (DURECT Corporation, Cupertino, CA, USA). Commercial kits for measurement of blood urea nitrogen (BUN) and creatinine concentration (Sera-pak plus creatinine and urea) were from Bayer (Tarrytown, NY, USA). Mouse monoclonal anti-4-hydroxy-2-nonenal (4-HNE) antibody (Cat. No. 24325) was purchased from Oxis International Inc. (Portland, OR, USA). Mouse monoclonal anti-3-nitrotyrosine (3-NT) antibody (Cat. No. 189542) was purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). Mouse monoclonal anti-rat macrophages/monocytes antibody (Clone ED-1, Cat. No. MAB1435) was purchased from Chemicon International Inc. (Temecula, CA, USA). Secondary antibody biotin SP-conjugated AffiniPure donkey anti-mouse IgG (Cat. No. 715-065-151) was purchased from Jackson ImmunoResearch Laboratories Inc. (West Grove, PA, USA). Declere was from Cell Marque (Hot Springs, AR, USA). ABC-kit Vectastain was from Vector Laboratories (Orton Southgate, Peterborough, UK). Diaminobenzidine substrate (Cat. No. K3466) and Mayer's Hematoxylin (Lillie's Modification) (Cat. No. S3309) were from DAKO Corporation (Carpinteria, CA, USA). Kim-1 and iNOS probes, as well as MicroAmp optical tubes and TaqMan Master Mix were obtained from Applied Biosystems (Foster City, CA, USA).

2.2. Experimental design

Experimental work followed the guidelines of Norma Oficial Mexicana Guide for the use and care of laboratory animals (NOM-062-ZOO-1999) and for the disposal of biological residues (NOM-087-ECOL-1995). 31 male Wistar rats (230–270 g) were used for this study and all animals were provided with a standard commercial rat chow diet and water. Housing room was maintained under constant conditions of temperature (18–22 °C) and lighting (12-h light/dark cycle). Animals were divided randomly in four groups: one group was used as control (Ct) and received a single intraperitoneal (ip) injection of vehicle (1.25 mL of saline solution). Second group received 5 mg/Kg/day of 1400W during three days by miniosmotic pumps (1400W group). Third group was treated with