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Age-related differences in kidney injury biomarkers induced by cisplatin

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ABSTRACT

Acute kidney injury (AKI) occurs in a half of cisplatin (CDDP)-treated patients. Traditional biomarkers including blood urea nitrogen (BUN) and serum creatinine (SCr) are still used for detection of CDDP-induced AKI, but these biomarkers are not specific or sensitive. The aim of this study was to identify the specific and sensitive biomarkers against CDDP-induced renal injury between young (3-week-old) and old (20-week-old) rats. All animals were intraperitoneally injected once with CDDP (6 mg/kg). After 3 days, all animals were sacrificed and serum, urine, and kidney tissues were collected. Urinary and serum biomarkers as well as histological changes were measured. CDDP-induced proximal tubular damage was apparent from histopathological examination, being more severe in 3-week-old rats accompanied by increased number of TUNEL-positive apoptotic cells. This was associated with elevated urinary kidney injury molecule-1 (KIM-1), glutathione-S-transferase alpha (GST-α), vascular endothelial growth factor (VEGF), and tissue inhibitor of metalloproteinases-1 (TIMP-1). In contrast, the levels of neutrophil gelatinase-associated lipocalin (NGAL) and osteopontin were significantly increased in 20-week-old rats after CDDP treatment. These results indicate that the use of age-specific urinary biomarkers is necessary to diagnosis of CDDP-induced AKI. Especially, urinary KIM-1, GST-α, TIMP-1, and VEGF levels may help in the early diagnosis of young patients with CDDP-induced AKI.

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1. Introduction

Cisplatin (CDDP) is one of the most effective chemotherapeutic agents that are widely used for the treatment of malignant cancer (Sahni et al., 2009; Yao et al., 2007; Wang and Lippard, 2005). However, despite its effectiveness, one of the limiting uses of CDDP is nephrotoxicity. Although CDDP-induced nephrotoxicity is manifested in a number of ways, the most prominent outcome is acute kidney injury (AKI). Previous studies have been shown that approximately one-third of patients experience AKI after CDDP chemotherapy as evidenced by reduced glomerular filtration rate (GFR), increased blood urea nitrogen (BUN), and increased serum creatinine (SCr) (Hanigan and Devarajan, 2003; Meyer and Madias, 1994). Pathologically, CDDP-induced renal toxicity is characterized by tubular degeneration and necrosis in the S3 segment of the proximal tubules with loss of microvilli, alterations in the number and size of lysosomes, and mitochondrial vacuolation (dos Santos et al., 2012; Stewart and Bolt, 2012).

The nephrotoxicity of CDDP has been documented in adults and pediatric patients, but the side effects of CDDP-induced AKI in children differ from adults (Goren et al., 1986). Thus age is a critical factor that needs to be considered in chemotherapy (Aleksa et al., 2001; Skinner et al., 1998). In general, the responsiveness to drugs may vary between children and adults due to differences in drug absorption, distribution, metabolism and excretion (Faustman et al., 2000; Kacew, 2001). The effect of aging on CDDP-induced renal injury is still controversial in clinical trials; a few animal studies have been shown that young rats are less susceptible to cisplatin-induced nephrotoxicity than adult rats. The severity of kidney histopathological alterations and biomarker changes were greater in 80-day-old rats than in 10-, 25- and 40-day old rats, indicating that older animals were more susceptible to CDDP-induced side effects (Espandiari et al., 2010). Similarly, CDDP (6 mg/kg) produced nephrotoxicity as evidenced by physiological, biochemical and histological parameters changes, which were more severe in older than younger animals (Ali et al., 2008). In addition, CDDP-induced nephrotoxicity was less prominent in young (3-week-old) as compared to adult (12-week-old) rats due to the relative accumulation of CDDP in the kidney tissue of young rats (Jongejan et al., 1986). Although these findings may appear contradictory to the postulation that older animals are more susceptible to adverse consequences following CDDP treatment, it needs to be emphasized that renal functions in young animals are not fully developed. Therefore, the role of age in CDDP-induced kidney injury remains controversial and warrants further study.

For evaluation of kidney toxicity, BUN and SCr are considered as reliable biomarkers for detecting nephrotoxicity. Furthermore, several urinary enzymes including r-glutamyl transferase, alkaline phosphatase, lactate dehydrogenase (LDH), and N-acetyl-β-D-glucosaminidase (NAG) are used as biomarkers of nephrotoxicity (Stonard et al., 1987). However, the changes of these enzymes are not early or specific stage of nephrotoxicity, thus specific diagnostic parameters for the detection of AKI has been already indicated in previous studies (Wang et al., 2013; Hoffmann et al., 2010; Ferguson et al., 2008). Newly identified sensitive biomarkers from serum or urine may be utilized for early diagnosis of potential risk of drug-induced nephrotoxicity. Han et al. (2002) reported that kidney injury molecule-1 (KIM-1) was detected in the urine of patients with acute tubular necrosis and may be used as an effective biomarker for renal proximal tubule injury. The KIM-1 protein was also accepted as a marker of proximal tubular injury in animal models and humans (Vaidya et al., 2010; Prozialeck et al., 2009; van Timmeren et al., 2006). Urinary neutrophil gelatinase-associated lipocalin (NGAL) may serve as an early sensitive marker of AKI following cardiac surgery (Bennett et al., 2008). Increases in urinary cystatin-C, KIM-1, glutathione-S-transferase alpha (GST-α), and epidermal growth factor (EGF) in CDDP-induced AKI in rats have been recently reported (Togashi et al., 2012; Tonomura et al., 2010).

There are some limitations in trying to compare sensitive biomarkers for CDDP-induced nephrotoxicity in different age groups. Therefore, it was interest to identify reliable and sensitive biomarkers for nephrotoxicity using the multiplex panel of biomarkers and to determine whether age was a factor. The aim of this study was to determine whether AKI following treatment with CDDP is more severe in younger than older patients. We examined the sensitive and reliable age-specific biomarkers associated with CDDP-induced AKI in the urine of different aged rats.

2. Materials and methods

2.1. Chemicals

CDDP (cis-diamminedichloroplatinum(II) dichloride, purity ≥99.9%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). NGAL, clusterin and β-actin primary antibodies and horseradish peroxidase (HRP)-conjugated secondary antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The primary antibodies for vascular endothelial growth factor (VEGF), calbindin, β_2-microglobulin (β-MG), osteopontin (OPN), Netrin-1, and high mobility group protein B1 (HMGB1) were purchased from Abcam (Cambridge, MA, USA). KIM-1 was purchased from R&D Systems (Minneapolis, MN, USA). Antibodies for detection of apoptosis-related proteins were purchased from Abcam (Cambridge, MA, USA).

2.2. Experimental design

Sprague-Dawley male rats were obtained from Charles River Laboratory Animals Resources (Seoul, Korea) and were maintained in a specific pathogen free (SPF) room under a 12 h light/dark cycle. The ambient air temperature and relative humidity were set at 23 ± 2 °C and 55%, respectively. Prior to experimentation, all animals were checked for any overt signs of illness and only healthy animals were selected. Tap water and rodent chow were provided ad libitum. Male rats (6 per group) were treated at 3-weeks-of-age or 20-weeks-of-age with a single intraperitoneal (i.p.) injection of CDDP 6 mg/kg b.w. dissolved in saline. Rats in the control group received a single i.p. injection of saline (1 ml/kg b.w.). The experimental protocol was approved by Pusan National University Laboratory Animal Care Service (Busan, Korea) in accordance with the Korea Food