Annexin A5 as a New Potential Biomarker for Cisplatin-Induced Toxicity in Human Kidney Epithelial Cells

Yeo-Jung Kwon¹, Jin-Joo Jung¹, Na-Hee Park¹, Dong-Jin Ye¹, Donghak Kim², Aree Moon³ and Young-Jin Chun¹*

¹College of Pharmacy, Chung-Ang University, Seoul 156-756, ²Department of Biological Sciences, Konkuk University, Seoul 143-701, ³College of Pharmacy, Duksung Women’s University, Seoul 132-714, Republic of Korea

Abstract
Cisplatin is a member of platinum-containing anti-cancer drugs that causes cross-linking of DNA and ultimately cancer cell apoptosis. The therapeutic function of cisplatin on various types of cancers has been widely reported but the side effects have been discovered together and nephrotoxicity has been regarded as major side effect of cisplatin. To select candidates for new sensitive nephrotoxicity biomarker, we performed proteomic analysis using 2-DE/MALDI-TOF-MS followed by cisplatin treatment in human kidney cell line, HK-2 cells, and compared the results to the gene profile from microarray composed of genes changed in expression by cisplatin from formerly reported article. Annexin A5 has been selected to be the most potential candidate and it has been identified using Western blot, RT-PCR and cell viability assay whether annexin A5 is available to be a sensitive nephrotoxic biomarker. Treatment with cisplatin on HK-2 cells caused the increase of annexin A5 expression in protein and mRNA levels. Over-expression of annexin A5 blocked HK-2 cell proliferation, indicating correlation between annexin A5 and renal cell toxicity. Taken together, these results suggest the possibility of annexin A5 as a new biomarker for cisplatin-mediated nephrotoxicity.

Key Words: Cisplatin, Annexin A5, Nephrotoxicity, Biomarker, Proteomic analysis

INTRODUCTION
Cisplatin ((cis-diammine-dichloro-platinumII) is the first member of metal-containing chemotherapeutic drugs and its first clinical treatment was performed for testicular cancer in 1979 (Prestayko et al., 1979). Since the first clinical trial, cisplatin has been widely used as an intravenously administered anti-cancer agent that is available to be applied to various types of cancers such as carcinomas of testis, ovary, neck and head. This practical anti-cancer drug binds and interacts with DNA to form adducts, mainly causing intrastrand cross-links (Sherman and Lippard, 1987; Siddik, 2003). DNA adducts formed by cisplatin activate several signaling pathways that ultimately trigger apoptosis such as those of ATR, p53 and MAPK (Wang et al., 2000; Damia et al., 2001), which makes cisplatin possible to be used as an anti-cancer drug.

In spite of various abilities of cisplatin as a chemotherapeutic drug, limits in clinical use still exist due to serious side effects such as oto-, myelo-, neuro- and nephrotoxicity (Laurell and Jungnelius, 1990; Harmers et al., 1991; Treskes and van der Vijgh, 1993). Among them, nephrotoxicity is known as representative dose-limiting side effect of cisplatin that induces promotion of apoptosis and necrosis of renal epithelial cells (Hanigan and Devarajan, 2003) and considerable efforts have been contributed to overcome it. To establish effective way that makes reduction of side effects possible, studies to identify the level or mechanism of nephrotoxicity occurred by cisplatin have been widely investigated.

It has been significantly emphasized that the biomarkers sensitive for nephrotoxicity are essential to detect the level of toxicity and reduce it. For this reason, various types of nephrotoxic biomarkers such as NGAL (neutrophil gelatinase-associated lipocalin) and NAG (N-acetyl-β-D-glucosaminidase) have been discovered and some of them are in clinical use (Devarajan, 2007; Franke et al., 2010). Despite serious necessity and much effort, widely accepted and sensitive biomarker for nephrotoxicity still has not been discovered yet and it needs to be investigated further.
Annexin A5 has specific properties that it requires higher calcium concentration than other types of annexin proteins for phospholipid binding and it forms two-dimensional networks on lipid bi-layers through binding with phosphatidylserine to activate its function (Raynal and Pollard, 1994; Reviakine et al., 2000). Recently, it also has been identified that this networks are available to open a new portal for cellular entry, which is able to suggest the possibility for contribution of annexin A5 to apoptosis (Kenis et al., 2004). These properties of annexin A5 make it possible to suggest that annexin A5 may be involved in cisplatin-induced toxicity and could be used as a new biomarker for side effect of cisplatin.

In present study, we performed experiments to find new sensitive biomarker for nephrotoxicity. Screening for selection of candidates, proteomic analysis was performed. Because annexin A5 was selected as one of the most potential candidates, validation of sensitivity of annexin A5 for cisplatin-induced nephrotoxicity has also been implemented.

MATERIALS AND METHODS

Reagents
Cisplatin was purchased from Sigma-Aldrich (St. Louis, MO, USA). DMEM medium, penicillin or streptomycin was obtained from Welgene (Daegu, Korea). Fetal bovine serum (FBS) or en-...