Evaluation of the Effect of Acetazolamide, Mannitol and N-acetylcysteine on Cisplatin-Induced Nephrotoxicity

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List of abbreviations

Abbreviation	Meaning
5-HT3	5-hydroxytryptamine subtype 3
ACTZ	Acetazolamide
ALT	Alanine Transaminase
AST	Aspartate Transaminase
ATPASE	Adenosine triphosphatase
BSA	Body Surface Area
BUN	Blood urea nitrogen
CrCl	Creatinine clearance
Ctr1	Copper transporter
ECF	Epirubicin and fluorouracil regimen
ERK	Extracellular signal-related kinase
GC	Gemcitabine and cisplatin regimen
GFR	Glomerular filtration rate
GGT	Gamma-glutamyl transferase
GSH	Glutathione
HEK293	Human embryonic kidney cells
HER2	Human epidermal growth factor receptor 2
HER-2/neu	Gene encodes 1 of 4 trans-membrane receptor protein-tyrosine kinases that mediate cell growth, differentiation, and survival.
Hg	Hemoglobin
hOAT	Human organic anion transporter
hOAT3	human homolog organic anion transporter
hOCT	Human organic cation transporter
IL-1	Interleukin-1
MAPK	Mitogen-activated protein kinases
MDRD	Modification of Diet in Renal Disease
mEq	Milli-equivalent

Abbreviation	Meaning
MVAC	Methotrexate, vinblastine, doxorubicin and cisplatin regimen
NAC	N-acetylcysteine
OCT	Organic cation transporter
PAH	P-aminohippurate
rhuMAb HER2	Recombinant humanized anti-p185HER2/neu monoclonal antibody
ROS	Reactive oxygen species
S.Cr	Serum creatinine
TCC	Transitional Cell Carcinoma
TLC	Total Leucocyte Count
TNF	Tumor necrosis factor
WAP	Whey acidic protein

Abstract

Introduction. Cisplatin is an important anti-cancer medication used for treatment of variety of malignant tumors. The most important toxicity caused by cisplatin is nephrotoxicity. Cisplatin-induced nephrotoxicity still occurs despite intensive hydration, where reduction in glomerular filtration occurs in 28% to 36% including acute renal failure and chronic renal insufficiency. Acetazolamide (ACTZ) and N-acetylcysteine (NAC) ameliorated cisplatin-induced nephrotoxicity in rats. No study to date evaluated the protective effect of ACTZ or NAC against cisplatin nephrotoxicity in humans.

Aim of the study. To evaluate the effect of ACTZ or NAC against cisplatin nephrotoxicity in humans compared to mannitol.

Patients and methods. A prospective comparative pilot study was conducted at Nasser Institute Cancer Center (NICC), Cairo, Egypt. A total of 52 patients receiving standard hydration measures for cisplatin were allocated to three groups: 20 patients receiving mannitol, 15 patients receiving ACTZ and 17 patients receiving NAC. Patients` kidney function was monitored using serum creatinine, creatinine clearance and blood urea nitrogen, whereas kidney injury was assessed using

RIFLE criteria. Patients` liver function tests and hematological parameters were also monitored.

Results. There was a statistically significant protective effect of either ACTZ or NAC groups compared to mannitol group on kidney function. This was apparent in laboratory results as differences by time between three groups in creatinine level, creatinine clearance values and BUN levels were statistically significant with P-values 0.045, 0.012 and 0.016 respectively. However, there were no statistically significant differences between the three groups in liver function tests: aspartate transaminase (AST) and alanine transaminase (ALT) levels with P-values 0.369 and 0.182 respectively. There were no statistically significant differences in hematological parameters: hemoglobin level, total leucocytes count and platelets` count between the three groups with P-values 0.479, 0.114 and 0.256 respectively.

Conclusion. ACTZ or NAC may have protective effect against cisplatin-induced nephrotoxicity which need to be confirmed in large multicenter randomized clinical trials.

Key words. Cisplatin- nephrotoxicity- N-acetylcysteine- acetazolamide- kidney function

Introduction

Cisplatin is an important anti-cancer medication used for treatment for variety of malignant tumors. The most important toxicity caused by cisplatin is nephrotoxicity **(Yao et al., 2007, Vasaikar et al., 2018)**. Cisplatin-induced nephrotoxicity still occurs despite intensive hydration, where reduction in glomerular filtration occurs in approximately 30% of patients **(George et al., 2018).**

Cisplatin makes crosslinks with DNA that causes cytotoxic lesions in tumors and other rapidly proliferating cells **(Dasari and Tchounwou, 2014)**. Although anti-neoplastic medications targeting DNA usually have lower toxicity in non-proliferating cells, cisplatin selectively damage renal proximal tubules **(Bolisetty et al., 2016)**. Uptake of cisplatin is mainly through the organic transporter pathway. The kidney accumulates cisplatin to a greater degree than other organs and is the major route for its excretion. The cisplatin concentration in proximal tubular epithelial cells is about 5 times the serum concentration **(Yao et al., 2007).**

After accumulation of cisplatin in kidney, it interacts with sulfur containing compounds and causes an increased membrane fragility and depletion of intracellular glutathione **(Anand and Bashey, 1993)**.This effect results in increased

blood urea nitrogen (BUN) and serum creatinine. But this effect is significantly higher for creatinine than that for BUN. Elevation of serum creatinine and BUN after cisplatin treatment may be due to acute nephrotoxicity. This increase in kidney function tests may be reversible **(Arunkumar et al., 2012).**

Intensive prophylactic hydration and forced diuresis have been used for the preservation of kidney function during cisplatin treatment. Electrolyte disturbances such as decreased serum magnesium, calcium, phosphorus, potassium levels and increased serum creatinine, BUN were observed after cisplatin-based chemotherapy if no prophylactic measures used. Frequency and severity of cisplatin-induced nephrotoxicity may be reduced by supplementing intravenous electrolyte infusions and maintaining the hydration; before, during and immediately after the administration of cisplatin **(Arunkumar et al., 2012, Kimura et al., 2018).**

A large number of sulfur-containing compounds have been shown to reduce the nephrotoxicity of cisplatin without inhibiting its antitumor effect. This was shown in patients with ovarian cancer, non–small-cell lung cancer, metastatic breast cancer and metastatic colon cancer. Administration of amifostine in chemotherapeutic protocols containing cisplatin has minimized nephrotoxicity and hepatotoxicity **(Glover et al., 1989).**

Amifostine is the only FDA-approved agent for the reduction of cumulative renal toxicity in advanced ovarian and non–small-cell lung cancer patients receiving cisplatin. Amifostine, an organic thiophosphate, works by giving protective thiol group that protects against cisplatin-For induced toxicity, and this only occurs in normal cells and does not occur in malignant cells. Also amifostine reduces cisplatin-induced toxicity by binding free radicals. Moreover it binds and detoxifies platinum agents by reduction of platinum-DNA adduct formation. However, as a consequence of side effects and cost, use of amifostine in routine practice is limited **(Yao et al., 2007).** Thus we still need medication that decrease cisplatin-induced nephrotoxicity, with lowest possible side effects, has an affordable cost and does not affect cisplatin efficacy against malignant cells.

Several medications have been studied for this purpose. For example, pretreatment with the sulfur-containing compound N-acetylcysteine (NAC) has been shown to reduce cisplatin-induced nephrotoxicity in rats by prevention of BUN elevation after administration of cisplatin **(Dickey et al., 2005).** NAC significantly reduced cisplatin-induced hemodynamic, biochemical and histopathological changes. By studying the effect of giving NAC to cisplatin-treated rats, it was found that the concentration of platinum in the kidneys of cisplatin and

NAC treated rats was less than in cisplatin only treated rats by 37%. The results showed that administration of intraperitoneal NAC (500 mg /kg per day for 9 days) reversed the renal hemodynamic changes as well as the biochemical and histopathological indices of cisplatin-induced nephrotoxicity in rats **(Abdelrahman et al., 2010).** The NAC ability to protect renal cells against cisplatin-induced toxicity may involve several mechanisms including the ability to increase cellular glutathione content and its ability to complex with cisplatin **(Salahudeen et al., 1998).**

Apoptosis caused by cisplatin was completely prevented by giving NAC before cisplatin or up to one hour after cisplatin. By time, NAC ability to reduce cisplatin-induced toxicity was reduced and this ability disappeared after eight hours of cisplatin. These findings suggest that before or one to two hours after cisplatin is the best time to rescue from cisplatin-induced apoptosis **(Wu et al., 2005).**

In addition, the diuretic acetazolamide (ACTZ) was studied for its` effect on cisplatin-induced nephrotoxicity but the drug mechanism of action is still unclear. Several theories have been suggested for explaining ACTZ mechanism of action. First, ACTZ is an organic acid which may competitively decrease tubular reabsorption of cisplatin. Second, ACTZ is a sulfur containing drug and several sulfur-containing compounds