Evaluation of the Chemoprotective Role of N-Acetylcyesteine on Cisplatin-Induced Nephrotoxicity: New Aspect of an Old Drug

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Abstract: This study aimed to evaluate the chemoprotective role of N-acetylcyesteine on experimental cisplatin-induced nephrotoxicity. Forty albino rats were divided into 4 groups (10 rats each); Group 1: normal control. Group 2: induced nephrotoxicity (cisplatin 1 mg/kg/day i.p, 7 days). Group 3: treated 1 h before cisplatin with N-acetylcyesteine orally 400 mg/kg/day, 7 days. Group 4: treated 1 h after cisplatin with N-acetylcyesteine in the same dose and duration. Cisplatin-induced nephrotoxicity (group 2) caused higher levels of serum creatinine and urea with marked DNA fragmentation, significant increase in caspase-3 reaction and significant increase in proximal convoluted tubules vacuolations when compared to the control group. N-acetylcyesteine 1 h after cisplatin offered marked protection in comparison to its administration 1 h before cisplatin, manifested as significant amelioration of all studied parameters. In conclusion, N-acetylcyesteine 1 h after cisplatin could be recommended as a well-tolerated effective chemoprotective therapy against the potential cisplatin nephrotoxicity. For the future, further researches are needed to judge the confliction about the effects of timing and different dosage regimen of N-acetylcyesteine on the antitumor efficacy of cisplatin in various types of tumors.

Key words: Cisplatin, nephrotoxicity, N-acetylcyesteine, apoptosis

INTRODUCTION

Cisplatin(cis-diaminedichloroplatinum, CDDP) is an alkylating inorganic compound that is widely used as a potent anticancer agent against solid tumors of the testes, ovaries, breasts, lungs, bladder, etc. (Greggi Antunes et al., 2000; Previti et al., 2004; Chirino et al., 2004). Cisplatin acts through activation of multiple signal transduction pathways associated with cell survival and apoptosis in various cell types in a dose-and time-dependent manner (Choi et al., 2004). However, in practice, the major dose limiting toxicity of cisplatin is the cumulative nephrotoxicity. Although reversible effects occur in 28-36% of patients treated with a single dose, renal toxicity may be cumulative and permanent with high doses or repeated cycles of treatment (MoEvoy, 2006).

The exact mechanisms of nephrotoxicity induced by cisplatin are still not fully elucidated (Previti et al., 2004; Shimeda et al., 2005; Hofmann et al., 2006). Studies in a variety of cell types have suggested that cancer chemotherapy drugs induce cell apoptosis by inducing formation of Reactive Oxygen Species (ROS) (Senturker et al., 2002) and that the generation of ROS and lipid peroxidation is responsible for the cisplatin-induced renal tubular injury (Sugihara et al., 1987; Mora et al., 2003; Xiao et al., 2003). Also, the disproportionate accumulation of cisplatin in kidney tissue may play an important role, but the therapeutic measures to prevent this proposed cause of nephrotoxicity are not available (Kroning et al., 2000).

Due to the fact that no antidote exists for cisplatin, a continuous effort has been made to protect patients from cisplatin-induced toxicities since the early 1980's (Blakley et al., 2002; Markman, 2003; Hartmann and Lipp, 2003). Hemodialysis, for example, is able to reduce only the free cisplatin in plasma, while most of cisplatin binds very quickly to plasma proteins and tissues after administration and therefore cannot be further eliminated by this procedure (Brivet et al., 1986; Lagrange et al., 1994). So, several chemoprotective agents have been tested with cisplatin-based therapies. They all have the common feature of compounds containing sulfur. Their mechanism of action is thought to be free radical scavenging, covalent binding to the agent or both, but is still not entirely understood (Hofmann et al., 2006).

N-acetylcyesteine (NAC) is a precursor of the amino acid L-cysteine and helps glutathione synthesize pathway (Tepel et al., 2000). In addition to its conventional use as
a mucolytic and as an antidoor in acetaminophen toxicity (Reynolds, 1996); many researchers reported its various therapeutic applications in renal disorders such as: prevention of kidney damage caused by injections of isoosmide, a contrast medium used in computerized tomography (CT) imaging (Tepel et al., 2000); successful treatment of pseudoporphria that complicates kidney dialysis (Vadoud-Seyedi, 2000); reduced the incidence of kidney damage in people undergoing coronary angiography (Shyu et al., 2002); protection against a number of toxic insults, including ifosfamide-induced urototoxicity (Holoye et al., 1983). Recently, thiols such as N-acetylcysteine (NAC) are increasingly used in clinical trials of platinum chemotherapy as chemoprotectants, however, the molecular mechanisms of NAC on apoptosis and cisplatin cytotoxicity remain unknown (Wu et al., 2005). So, the aim of this study is to evaluate the chemoprotective role of N-acetylcysteine on experimental cisplatin-induced nephrotoxicity.

**MATERIALS AND METHODS**

**Drugs used**

- Cisplatin (vial 50 mg/50 mL; Merck, Germany)
- N-acetylcysteine (sachet 200 mg; Sedgeco, Egypt)
- All other chemicals were supplied from Sigma-Aldrich Co.

This study was carried out on 40 albino rats weighing 100-150 g. They were handled in accordance with the guideline principles in the use of animals and provided with food and water *ad libitum* throughout the period of the work. The rats were divided into four groups (each of 10 rats) as following:

Group 1: Served as normal control group and received vehicle of isotonic saline by intraperitoneal (i.p.) injection daily for 7 days.

Group 2: Nephrotoxicity was induced by cisplatin in a dose of 1 mg kg\(^{-1}\) daily by i.p. injection for 7 days (Huang et al., 2001), (diluted in isotonic saline to a final concentration of 0.1 mg/0.5 mL).

Group 3: Treated 1 h before cisplatin with N-acetylcysteine by oral gavage in a dose of 400 mg kg\(^{-1}\) daily for 7 days (Dickey et al., 2005), (prepared as 40 mg/0.5 mL solution in distilled water).

Group 5: Treated 1 h after cisplatin with N-acetylcysteine in the same dose and duration.

Twenty-four hours from the last injection the rats were sacrificed and blood samples were obtained for measurement of serum levels of urea (mg dL\(^{-1}\)) (Fawcett and Scott, 1960) and creatinine (mg dL\(^{-1}\)) (Bartels and Böhmer, 1973) using the commercially available kits. Kidneys of each rat were dissected carefully; one kidney was processed for detection of apoptosis (DNA fragmentation) and the other kidney for immunohistochemistry of caspase-3 reaction and histopathological assessment.

**DNA extraction and apoptosis detection in kidney tissue:** Nucleic acid extraction and detection of apoptosis was done according to salting out extraction method of Aljunibi and Martinez (1997), modified by Hassab El-Nabi (2004) as following: Protein was precipitated by saturated solution of 5 M NaCl and a little bit of kidney tissue was squeezed in eppendorf tube, lysed with 600 µL lysing buffer (50 mM NaCl, 1 mM Na\(_2\)EDTA, 0.5% SDS, pH 8.3) and gently shook. The mixture was incubated overnight at 37°C then, 200 µL of saturated NaCl was added to the samples, shook gently and centrifuged at 12,000 rpm for 10 min. The supernatant was transferred to new eppendorf tubes and DNA precipitated in 600 µL cold isopropanol. The mix was inverted several times until fine fibres appear, then centrifuged for 5 min at 12,000 rpm. The supernatant is removed and the pellets were washed with 500 µL 70% ethyl alcohol, centrifuged at 12,000 rpm for 5 min, then the alcohol was tipped out and the tubes blotted on Whatman paper, until the pellets appeared to be dry, then resuspended in 50 µL of TE buffer (10 mM tris, 1 mM EDTA, pH 8) supplemented with 5% glycerol. The suspended DNA was incubated for 30-60 min with loading mix (Rnase + loading buffer) and then loaded directly into the gel.

**Immunohistochemistry of caspase-3 reaction:** Initially, tissue sections were obtained after deparaffinized by xylol overnight and hydrated in descending grades of alcohol 100, 90, 70 and 50% for 5 min each. Antigen retrieval was performed by heating slides in citrate buffer (pH 6) in microwave for 5 min and washed twice in phosphate buffer for 20 min. Then, endogenous peroxi activity was blocked by hydrogen peroxide solution for 10 min and washed twice in phosphate buffer solution (pH 7.2) for 5 min each.

The sections were treated for one hour in humidity chamber with rabbit polyclonal antibody (primary antibody), against caspase-3, labeled it as dark brown granules inside the cytoplasm of the proximal convoluted tubules. The slides were rinsed in phosphate buffered saline and biotinylated secondary antibody (Universal Multilink) was applied for 10 min and washed again in phosphate buffer for 5 min. The bound antibodies were visualized by applying streptavidine peroxidase 2 drops.
on the sections for 30 min, washed in phosphate buffer solution for 5 min and exposed for 10 min to freshly prepared Diaminobenzidine (DAB) as chromogen. After appearance of the brown color the slides were immediately washed in tap water, counterstained with hematoxylin, dehydrated and mounted. For negative control, phosphate buffer solution was used instead of the primary antibody. For statistical analysis, the frequency of the positively affected cells was scored in 100 proximal convoluted tubules from cortical tissue of each rat (Silverberg et al., 2006).

**Histological method:** The kidneys were fixed in 10% formal saline for 24 h, dehydrated with ascending grades of alcohol and paraffin embedded of 5 µm were obtained and stained with hematoxylin and eosin (Bancroft et al., 1996). For statistical analysis, the frequency of presence of intra-cytoplasmic vacuolations was scored in 100 proximal convoluted tubules from renal cortical tissue of each rat (Lortholary et al., 1993).

**Statistics:** Values of the measured parameters were presented as Mean±SD. The difference between each two groups was determined using student’s t-test. The significance was considered at p<0.05. Pearson’s correlation coefficient was applied to correlate between the parameters. The statistical analyses were processed according to the conventional procedures (Papkin, 1984), using the Statistical Program of Social Sciences (SPSS) software for windows, version 10.0. The comparison between each two proportions (frequencies) was calculated by z-test with level of significance at p<0.05 when z>1.96.

**RESULTS**

**Biochemical results:** Regarding functional parameters of the kidney (Table 1, Fig. 1, 2), it was found that cisplatin-induced nephrotoxicity (group 2) caused significant elevation in serum levels of creatinine (mg dl⁻¹) and urea (mg dl⁻¹) when compared to the control group (group 1) [1.65±0.51 vs 0.62±0.08 (p<0.001)] and 44.17±5.08 vs 29.33±3.15 (p<0.001), respectively.

Table 1: Serum levels of creatinine and urea in the different studied groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (n=10)</th>
<th>Group 2 (n=10)</th>
<th>Group 3 (n=10)</th>
<th>Group 4 (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine (mg dl⁻¹)</td>
<td>6.52±0.08</td>
<td>1.65±0.51</td>
<td>1.03±0.30</td>
<td>0.78±0.11</td>
</tr>
<tr>
<td>Serum urea (mg dl⁻¹)</td>
<td>29.33±3.15</td>
<td>44.17±5.08</td>
<td>44.17±5.08</td>
<td>38.08±3.95</td>
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NS = Non significant; n = Number; p = Cisplatin-induced nephrotoxicity (group 2) vs control (group 1); p = N-acetylcysteine 1 h before cisplatin (group 3) and N-acetylcysteine 1 h after cisplatin (group 4) vs cisplatin only (group 2); p = N-acetylcysteine 1 h after cisplatin (group 4) vs N-acetylcysteine 1 h before cisplatin (group 3).

When compared to rats with untreated cisplatin-induced nephrotoxicity (group 2), treatment with N-acetylcysteine 1 h before administration of cisplatin (group 3) resulted in significant reduction in serum creatinine [1.03±0.30 vs 1.65±0.51 (p<0.01)], with non significant reduction in serum urea [44.17±5.08 vs 44.17±5.08 (p>0.05)], while treatment with N-acetylcysteine 1 h after administration of cisplatin (group 4) resulted in significant reduction in both serum creatinine and urea [0.78±0.11 vs 1.65±0.51 (p<0.001) and 38.08±3.95 vs 44.17±5.08 (p<0.01), respectively].

**DNA fragmentation results:** There was an increase in intensity of DNA fragmentation at 200, 400, 600 and 800 bp in the kidney tissue of cisplatin treated rats (group 2) in comparison with the control group (group 1). Administration of NAC one hour before cisplatin administration (group 3) showed reduction in intensity of DNA fragmentation at 200, 400, 600 and 800 bp in
Fig. 3: The electrophoretic pattern of DNA damage in kidney tissue in rats treated with NAC one hour before as well as 1 h after cisplatin administration. There is decrease intensity of DNA fragmentation in kidney tissue at 200, 400, 600, 800 bp in NAC treated animals one hour before cisplatin administration in comparison with cisplatin treated animals. The intensity of DNA damage is less in animals treated with NAC one hour after cisplatin administration in comparison with those treated with NAC one hour before cisplatin administration (Lane 1, 2 control, lane 3, 4 cisplatin treated animals, lane 5, 6 NAC 1 h before cisplatin, Lane 7, 8 NAC 1 h after cisplatin)

comparison with group 2 (cisplatin treated animal), while NAC administration one hour after cisplatin injection (group 4) revealed more repair in DNA damage in comparison with group 3, (Fig. 3).

Results of immunohistochemistry of caspase-3 reaction:
In the control rats (group 1) most of the PCT cells mainly in subcapsular region exhibited absent or faint positive immunostaining reaction appearing as brown granules in their cytoplasm, (Fig. 4A). On the other hand group 2 and 3 showed marked increase in caspase-3 activity throughout most cells of PCT in subcapsular regions, where strong reactivity was detected (Fig. 4B-C). Group 4 showed apparent reduction in the caspase-3 activity in the cells of the PCT revealed as faint to moderate immunostaining reaction only in few proximal convoluted tubules of the subcapsular region (Fig. 4D).

As regard the statistical analysis, caspase-3 immunostaining reaction was significantly increased in cisplatin treated group (group 2) in comparison with control rats (group 1), (32.2 vs 6.7% p<0.05). Compared to group 2, group 3 showed non significant change, while group 4 showed significant decrease in caspase-3 reaction (28.4 vs 32.2% p<0.05 and 9.5 vs 32.2%, p<0.05, respectively). Group 4 showed marked decrease in caspase-3 immunostaining reaction in comparison with group 3 (9.5 vs 28.4%, p<0.05) (Table 2).

In the rats with untreated cisplatin-induced nephrotoxicity (group 2), there were significant positive correlation between the frequency of renal caspase-3 reaction and serum levels of urea (r = 0.667, p<0.05) and creatinine (r = 0.638, p<0.05), as shown in Fig. 1 and 2 respectively.

Histopathological results: Light microscopic examination of H and E stained renal sections of control rats (Group 1) revealed normal structural pattern of the proximal convoluted tubules (PCT) with distinct brush border and narrow irregular lumen lined by cubical epithelia cells with strongly eosinophilic cytoplasm with indistinct boundaries and few regularly arranged vesicular rounded central nuclei in TS section. Few tubular epithelial cells showed focal cytoplasmic vacuolations, (Fig. 5A).

Examination of renal cortex of rats received cisplatin only (group 2), (Fig. 5B) and those received N-acetylcysteine 1 h before cisplatin (group 3), (Fig. 5C) showed a conspicuous alteration in the histological structure of many PCT of the subcapsular nephrons, the affection was patchy and showed several grades of tubular lesions in between the normal ones in the form of epithelial swelling with an intraepithelial vacuolations and narrowing of their lumina; others exhibited a shorter tubular hyperchromatic epithelium with loss of striated edge and discontinuity of tubular wall; many tubules have no cellular lining and lost their nuclei while others were lined with flattened elongated cells. Most of the tubular epithelial cells were fragmented and sloughed into their lumina and some tubules contained hyaline casts. This tubular necrosis was also accompanied by scattered signs of apoptosis as evidenced by diminished cell size,
Fig. 4: A photomicrograph of the renal cortex (Avidin Biotin peroxidase for caspase-3 H.Mic. Mag. X400); (A) Control (group 1) showing faint immunostaining reaction of Caspase-3 in the cytoplasm of cells proximal convoluted tubules; (b) Cisplatin nephrotoxicity (group 2) showing strong immunostaining reaction of Caspase-3 in the cytoplasm of cells of proximal convoluted tubules; (C) NAC 1 h before cisplatin (group 3) showing strong immunostaining reaction of Caspase-3 in the cytoplasm of cells of proximal convoluted tubules mostly in the sub capsular regions; (D) NAC 1 h after cisplatin (group 4) showing moderate immunostaining reaction of Caspase-3 in cytoplasm of proximal convoluted tubules.

reduced cytoplasmic volume with condensed eosinophilic cytoplasm and nuclear chromatin.

Examination of the renal cortex of rats received N-acetylcysteine 1 h after cisplatin (group 4), revealed amelioration in the nephrotoxic effect of cisplatin manifested as an obvious recovery of many tubules. Only little pathologic changes were detected if compared with animals of group 2 and 3. The tubules showed large vesicular regularly arranged nuclei with focal tubular vacuolation, (Fig. 5D).

Statistical analysis showed significant increase in PCT vacuolations in group 2 in comparison with group 1 (48.6 vs 18.4%, p<0.05). Compared to group 2; PCT vacuolations was non significantly changed in group 3 but showed significant decrease in group 4 (43.5 vs 48.6%, p>0.05 and 26.2 vs 48.6%, p<0.05; respectively). Comparing group 4 with group 3, there was significant decrease in PCT vacuolations (26.2 vs 43.5%, p<0.05) (Table 2).

**DISCUSSION**

Cisplatin (CDDP) is currently among the most effective form of chemotherapy for a variety of malignant solid tumors (Kuhlmann et al., 1997; Blakley et al., 2002; Shmeda et al., 2005). Nevertheless, in practice, its major dose limiting toxicity is the nephrotoxicity that may be cumulative and permanent with high doses or repeated cycles of treatment (McEvoy, 2006).

The current study showed that treatment with cisplatin resulted in induction of nephrotoxicity in rats, that was manifested as significant elevation in serum urea and creatinine levels and accompanied by severe morphological changes and marked apoptosis in the proximal convoluted tubules detected by significant increase in renal caspase-3 reaction and marked DNA laddering. Moreover, there was significant positive correlation between caspase-3 with either serum urea or serum creatinine levels. The precise mechanisms involved
in nephrotoxicity induced by cisplatin have not been completely elucidated (Shimeda et al., 2005). Nevertheless, results of the present research came in concurrence with those obtained in many studies (Zhan et al., 1999; Gonzalez et al., 2001; Razzaque et al., 2002; Choi et al., 2004; Karimi et al., 2005; Wu et al., 2005; Dickey et al., 2005), demonstrating the central role of apoptosis in cisplatin-induced nephrotoxicity and highlighting a number of steps that might be targeted to minimize cisplatin-induced nephrotoxicity.

Meanwhile, two distinct pathophysiological mechanisms have been recognized as primary promoters of cellular damage, namely the inhibition of protein synthesis and glutathione (GSH) depletion. The intracellular binding of cisplatin to SH groups leads to GSH depletion, resulting in lipid peroxidation, free radical generation in the renal tubular cells and eventually mitochondrial damage; but these events were merely considered as sequelae of established cell damage (Kuhlmann et al., 1997; Shimeda et al., 2005). Leibbrandt et al. (1995) demonstrated nucleolar condensation and fragmentation in tubular cells treated by a concentration that is readily reached in patients and ultimately decrease protein synthesis in non proliferating
cells such as renal tubular epithelial cells. It is currently thought that DNA platination by cisplatin is an essential first step in its cytotoxic activity (Shellard et al., 1993; Evans et al., 1994; Johnson et al., 1994) results primarily from its ability to bind covalently to DNA preventing DNA replication and transcription (Li et al., 2001). Factors controlling DNA platination include the drug uptake, the rate of platinum adduct formation and repair and the concentration of cellular thiol. The uptake of cisplatin varies among different cells (Sadowitz et al., 2002). This was in consistence with the concept that the kidney accumulates cisplatin to a higher degree than any other organ, exceeding plasma concentrations by a factor of five and may indirectly alter renal hemodynamics where proximal convoluted tubules are expected to exhibit the most pronounced injury due to its poor vascular supply (Arany and Safirstein, 2003), with the highest intracellular concentrations are found in mitochondria, nuclei and microsomes (Kuhlmann et al., 1997).

Through these mechanisms, cisplatin triggers cellular responses involving multiple pathways, including DNA repair, transcription inhibition, cell cycle arrest and apoptosis (Siddik, 2003). Apoptosis is a programmed cell death that occurs through death receptor pathway and mitochondrial pathway (Levin et al., 1999). Although studies have revealed that cisplatin induces cell apoptosis, the mechanism in various cell types is not fully understood. Previous studies showed that apoptosis induced by cisplatin was mediated through both death receptor/caspase-8 (Fulda et al., 1998; Seki et al., 2000) and/or the caspase-9/caspase-3 pathways (Sun et al., 1999; Siddik, 2003; Choi et al., 2004; Ludwig and Oberleithner, 2004; Del Bello et al., 2004). Caspase-3 can be activated by caspase-9, which is activated by the release of cytochrome C from the mitochondria (Zhan et al., 1999; Schuler et al., 2000). Following one hour of exposure to cisplatin, platinum adducts to mitochondrial DNA were abundant and released cytochrome C within minutes of exposure (Yang et al., 2006). In fact, caspase-3 has been specifically implicated as the effector caspase responsible for DNA fragmentation by subsequent activation of DNA endonuclease that is required for formation of apoptotic DNA ladders (Liu et al., 1998; Nagata, 2000; Widlak, 2000; Basnakian et al., 2002; Wu et al., 2005). The cleavage of caspase-3 began to increase at 4 h after cisplatin, peaked at 12 h with sustained high expression levels at the 24 h time point, indicating that apoptotic signal was continuously activated by cisplatin (Wu et al., 2005).

In this context, it is of interest to understand the relationship between the dosage regimen of cisplatin and the induction of apoptosis. The cisplatin dosage regimen used in the present work was proved to induce nephrotoxicity without lethality in rats after a 7-day exposure period (Huang et al., 2001) and chosen on the basis that the multiple administrations or continuous infusion of low-dose cisplatin is an excellent regimen for cancer patients (Kurihara et al., 1995; Morazzoni et al., 1998).

It was documented that when certain dose of cisplatin was added intermittently, it would reached the same final concentration producing the ultimate cytotoxic effect, including activation of caspase-3, as if given as a single dose, although, these individual additions alone were not cytotoxic. Therefore, it was suggested that cisplatin induces apoptosis by formation of a threshold level of DNA-platinum adducts that are independent on the exposure regimen (Kishimoto et al., 2005). Ozawa et al. (1988) demonstrated that the cytotoxic ability of non-cell cycle-specific agents, such as cisplatin, depends on the concentration-time or Area Under Curve (AUC), which means that intermittent exposure low concentrations of the drug have the same effect as a single short-term exposure at a high concentration. Taeka et al. (2004) demonstrated that the amount of platination of nuclear DNA by cisplatin is proportional to the AUC.

The disproportionate accumulation of cisplatin in kidney tissue may play an important role, however, therapeutic measures to prevent this proposed cause of nephrotoxicity are not available (Kroning et al., 2000). Since even vigorous hydration (Dickey et al., 2005) or hemodialysis (Lagrange et al., 1994) could not completely eliminate toxicity, the discontinuation of cisplatin remains the only option in cases of progressive renal failure (Kuhlmann et al., 1997). Meanwhile, various thiols (SH-containing substances) such as sodium thiosulphate, amifostine, glutathione, diethylithiocarbamates and L-methionine have been tested for their efficacy to ameliorate cisplatin nephrotoxicity through prevention of GSH depletion and Reactive Oxygen Species (ROS) scavenging (Kuhlmann et al., 1997; Blakley et al., 2002; Hartmann and Lipp, 2003; Markman, 2003). But, it has been found that cisplatin could induce extensive apoptosis in the absence of any detectable oxidative stress (Senturker et al., 2002) and that GSH depletion and free radical generation in the renal tubular cells were merely considered as sequela of established cell damage (Kuhlmann et al., 1997; Shimeda et al., 2005). So, novel rationales against the prime apoptotic pathomechanisms could be emerged to protect kidneys from cisplatin toxicity.

In the current study, administration of N-acetylcyesteine one hour after cisplatin offered marked protection against nephrotoxicity when compared to the
group with untreated cisplatin nephrotoxicity. This protection was manifested as significant reduction in serum levels of urea and creatinine, amelioration of both apoptotic markers caspase-3 and DNA fragmentation as well as the histopathological changes. On the other hand, administration of N-acetylcysteine 1 h before cisplatin did not provide any significant changes in the studied parameters except the slight reduction in serum creatinine levels when compared to the group with untreated cisplatin nephrotoxicity.

N-acetylcysteine (NAC) is a thiol agent, acting as precursor of L-cysteine and glutathione pathway. Although it is currently viewed as an antioxidant scavenging free radicals (Zhang et al., 1998; Safirstein et al., 2000; Zafarullah et al., 2003), but the absence of significant correlations between GSH and chemoprotection against melphalan after 1 and 4 h from N-acetylcysteine argues against glutathione biosynthesis as a mechanism of protection (Pendyala and Creaven, 1995; Muldoon et al., 2001). Previously, Borgstrom and Kagedal (1990) estimated that the beneficial clinical effects observed after repeated dosing of NAC can not be ascribed to its accumulation in plasma.

De Flora et al. (2001) reviewed that NAC has an impressive array of mechanisms and protective effects towards DNA damage and carcinogenesis, most of which are related to its nucleophilicity, antioxidant activity and replenishment of GSH stores, inhibition of formation of adducts to mitochondrial and nuclear DNA, modulation of DNA repair, inhibition of oxidative DNA damage, regulation of cell survival and apoptosis, inhibition of induced mutations, modulation of gene expression and signal transduction pathways, inhibition of cell proliferation and progression to malignancy, inhibition of invasion and metastasis and protection towards adverse effects of other cancer chemopreventive or chemotherapeutic agents.

The mechanisms governing the effect of N-acetylcysteine on cell damage induced by anticancer agents such as cisplatin have not been fully established (Wu et al., 2005). However, there are recent growing data from in vitro and in vivo studies (Konstantinov et al., 1994; Choi et al., 2004; Wu et al., 2005; Dickey et al., 2005), suggesting that N-acetylcysteine protected against cisplatin-induced apoptosis. The N-acetylcysteine inhibition of apoptosis is presumably the result of its ability to attenuate DNA damage and other signals which ultimately trigger apoptosis (De Flora et al., 2001). Wu et al. (2005) proved that N-acetylcysteine administration reversed the cytotoxic and apoptotic effects, blocks both the death receptor and the mitochondrial apoptotic pathways induced by cisplatin, through its marked inhibition of caspase-3 signaling pathway. Another possible mechanism is attributed to the direct binding of the reactive thiol group on N-acetylcysteine to platinum (Pt) agents including cisplatin, resulting in their inactivation and formation of a complex unsuitable for tubular reabsorption (Appenroth et al., 1993; Muldoon et al., 2001; Senturker et al., 2002). Since the thiolate anion has a high affinity for Pt\(^{2+}\), Pt ions entering the cell may preferentially bind to sulfur atoms rather than the bases of DNA (Ishikawa and Ali-Osman, 1993).

In contrary, (Dizdaroglu et al., 1991; Sprong et al., 1998) demonstrated that N-acetylcysteine could induce metal-dependent H\(_2\)O\(_2\) generation and subsequently, damage to cellular and isolated DNA. They speculated that N-acetylcysteine undergoes Cu\(^{2+}\)-mediated autoxidation to generate Cu\(^{2+}\) and the thyl radical of N-acetylcysteine, binding to DNA with formation of a reactive complex causing DNA damage. However, they explained this effect on the bases that many agents possess antioxidant activity at low doses, while exhibiting prooxidant behavior at their higher doses under certain conditions of abundant metals (Cu\(^{2+}\), Fe\(^{2+}\)). Another conflicting aspect arise from a previous study proposed that cisplatin requires metabolic activation to become nephrotoxic via the metabolism of a glutathione-platinum conjugate, where preincubation of cisplatin with N-acetylcysteine results in a transient increase in the toxicity of cisplatin toward renal proximal tubular cells (Townsend et al., 2003). But this observation could be argued because in other study, the stimulation of apoptosis by N-acetylcysteine, was shown in cancer cells but not in non-cancer cells (Ziemann et al., 1999).

The impact of timing and route of N-acetylcysteine administration may provide a mechanism to reduce cisplatin side effects in vivo, without compromising therapeutic efficacy for the treatment of cancer (Wu et al., 2005). Regarding that induction of nephrotoxicity by cisplatin is assumed to be a rapid process involving reaction with proteins in the renal tubules (Heidman et al., 1985; Montine and Borch, 1990), so it is important for the protective agent to be present in renal tissue before damage occurs (Karimi et al., 2005).

The results obtained in the current work showed that only administration of N-acetylcysteine one hour after cisplatin offered marked protection against nephrotoxicity in comparison to its administration one hour before cisplatin. This protection was manifested as significant reduction in serum levels of urea and creatinine, amelioration of both apoptotic markers caspase-3 and DNA fragmentation as well as the histopathological changes.
This came in consistency with the observation that renal damage occurs within 1 h after cisplatin administration (Heidman et al., 1985; Rao and Rao, 1992). When Wu et al. (2005) explored the kinetics of N-acetylcysteine protection at 15 min, 1, 2, 4, 6 and 8 h after administration of cisplatin, they observed that N-acetylcysteine completely blocked cisplatin-induced apoptosis for all cell types when added within 1 h after cisplatin treatment. This protection was reduced when N-acetylcysteine was added 2 to 8 h after cisplatin, after which no protection was noted and these data suggest that 1 h and up to 2 h is the critical time period for rescue from cisplatin toxicity. Moreover, they estimated that there was less of such antiapoptotic effect of NAC if it was washed off the cells prior to cisplatin administration. Zunino et al. (1989) and Neuwelt et al. (2001) reported that the timing of cisplatin administration after N-acetylcysteine becomes an issue and protection against cisplatin-induced nephrotoxicity was found to be critically dependent on timing of thiol administration. However, they found that N-acetylcysteine administration 60 min prior to chemotherapy was bone marrow protective, but this result was in contrast with the rapid clearance of N-acetylcysteine, means that most N-acetylcysteine was cleared from the circulation by the time the chemotherapy was given.

From the pharmacokinetic viewpoint, it was reported that N-acetylcysteine is rapidly absorbed after oral administration with a peak plasma concentration obtained in 0.5-1 h and terminal half-life of 6.25 h (Reynolds, 1996). Moreover, N-acetylcysteine is known to have a rapid first pass metabolism in the intestine and liver after oral administration and its bioavailability is thought to be quite low (4 to 10%) (Borgstrom et al., 1986). On the other hand, cisplatin behaves in biphasic manner with initial plasma half-life of 25-49 min for the non-bound fraction and half-life of 58-73 h for total platinum (Reynolds, 1996; Dickey et al., 2005). It is known that cisplatin binds to plasma proteins very quickly after administration, where this protein-bound fraction representing more than 90% of the dose by 2 to 4 h after administration, forming an exchangeable pool that is mostly responsible for prolonged toxic effects, where it could be detected in tissue for several months afterwards (Lagrange et al., 1994; Reynolds, 1996).

Despite the potential benefits of chemoprotectants, such agents have relatively little clinical use due to concerns of impaired antitumor efficacy. The issue of potential interactions of chemoprotection with chemotherapy efficacy is of much concern, however, such interactions may be avoided by separating treatments in time (Muldoon et al., 2001; Dickey et al., 2005) and better understanding of the roles of N-acetylcysteine on the apoptotic signaling pathway in cellular and molecular prospects should enable a more rational chemoprotectant delivery regimen to maximize chemoprotective effects while minimizing any impact on antitumor efficacy in human patients (Wu et al., 2005). Several studies reported that application with N-acetylcysteine provided amelioration of the platinum induced nephrotoxicity with respect to preservation of the antitumor activity of cisplatin (Konstantinov et al., 1994; Yim et al., 1994; Muldoon et al., 2000; Neuwelt et al., 2004). Regarding cost, practicality of use, safety and efficacy, it is noteworthy that N-acetylcysteine preparations have a low cost and are of practical use and moreover, oral administration is compatible with long-term use (De Flora et al., 2001). A previous study by Hermann (1970) showed that 5.6 g/day chronic p.o. administration of N-acetylcysteine for 6 months was well tolerated, with vomiting (1 of 12) and diarrhea (4 of 12) as the only side effects. Toxicity of N-acetylcysteine is low either in human (Pendyala and Creaven, 1995) or in experimental animals (Johnston et al., 1983), although adverse effects, including anaphylactic responses, have been observed only in accidental high-dose NAC infusion in humans (Sprong et al., 1998).

In conclusion, N-acetylcysteine administration one hour after cisplatin could be recommended as a well-tolerated effective chemoprotective therapy against the potential cisplatin nephrotoxicity. For the future, further researches are needed to judge the confliction about the effects of timing and different dosage regimen of N-acetylcysteine on the antitumor efficacy of cisplatin in various types of tumors.

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REFERENCES


