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A study on free radical-induced renal toxicity due to cyclophosphamide and its amelioration with N-acetyl cysteine

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Abstract

A study was conducted to evaluate the protective effect of N-acetyl cysteine (NAC) against experimentally-induced renal toxicity due to cyclophosphamide (CYP) in rats. Three groups (2, 3 and 4) of female W*istar Kyoto* rats with 6 animals in each group were administered with CYP @ 40 mg/kg bwt on 1st, 3rd, 5th and 7th day by intraperitoneal route. Group 2 remained as toxic control. Group 3 received NAC from 1st to 14th day @ 0.2% of feed and group 4 was treated with NAC from 8th to 14th day. Group 1 was maintained on basal diet for 14 days. The study revealed a significant ($P < 0.05$) increase in the concentration of serum creatinine, blood urea nitrogen, thiobarbituric acid reacting substances (TBARS) and protein carbonyls, and a decrease in the reduced glutathione (GSH) concentration of kidney. These results are suggestive of CYP-induced oxidative stress resulting in renal damage. Feeding of NAC could not offer protection against CYP-induced renal damage in this study.

Keywords: Cyclophosphamide (CYP), N-acetyl cysteine (NAC), Oxidative stress.

Introduction

Cyclophosphamide (CYP), the derivative of nitrogen mustard, had been widely used as anticancerous agent and immunosuppressant. It acts as a bifunctional alkylating agent (Moore, 1991). It undergoes metabolic activation by hepatic microsomes and forms two active meatabolites - phosphoramide mustard and acrolein (Georgia *et al*., 2005). Acrolein is responsible for the toxic effects of CYP. One of the side effects of CYP is renal damage, which was thought to be due to induction of free radical formation and further tissue damage by its metabolite (Senthilkumar *et al*., 2006).

N-acetyl cysteine (NAC) is the acetylated derivative of cysteine, an aminoacid. It enters into glutathione synthesis inside the cell (Flora *et al*., 2001; Zhang *et al.,* 2008). NAC also neutralizes the free radicals produced in the body (Dekhuijzen, 2004) and therefore it has been used successfully to treat GSH deficiency in a wide range of infections, genetic defects, metabolic disorders, HIV infection, chronic obstructive pulmonary disease (Kondala *et al*., 2007) and iopromide-induced kidney damage (Tepel *et al*., 2000). As NAC has been very effective in conditions of oxidative stress, the present study was taken up to investigate CYP-induced oxidative stress and its protection by NAC on kidney.

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Materials and Methods

24 female albino rats of *Wistar Kyoto* strain weighing about 200-250 g were procured from National Institute of Nutrition, Hyderabad. Animals were placed on commercial standard mash feed for rats and provided water *ad libitum*. Animal were randomly divided into 4 groups with 6 animals in each group and were maintained as per the following treatment schedule for 14 days.

Group 1 : Basal diet control (1-14 days).

Group 2 : Cyclophosphamide toxic control (CYP @ 40 mg/kg bwt on 1st, 3rd, 5th and 7th day by intraperitoneal route.

Group 3 : CYP @ 40 mg/kg bwt on 1st, 3rd, 5th and 7th day by intraperitoneal route and N-acetyl cysteine given orally @ 0.2% in feed from 1st to 14th day.

Group 4 : CYP @ 40 mg/kg bwt on 1st, 3rd, 5th and 7th day by intraperitoneal route and N-acetyl cysteine given orally @ 0.2% in feed from 8th to 14th day.

Blood samples were collected at the end of 1st and 2nd week by retro-orbital puncture under ether anesthesia. Sera samples were separated to estimate serum creatinine and blood urea nitrogen (BUN) by commercially available diagnostic kits. At the end of 2nd wk, animals were sacrificed and kidneys were collected for estimation of reduced glutathione (GSH; Moron *et al.*, 1979), thiobarbituric acid reacting substances (TBARS; Balasubramanian *et al.*, 1988) and protein carbonyls (Levine *et al.*, 1990). The experimental protocol was accepted by Institutional Animal Ethics Committee. The data were subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) 15th version. Differences between means were tested using Duncan's multiple comparison test and significance was set at $P<0.05$.

Results and Discussion

In the present study, the markers of renal damage, blood urea nitrogen (BUN) and serum creatinine, which would rise in serum only when the kidney functioning ability is below 30%, were found increased significantly (Table 1-2) in all the three groups administered with CYP (groups 3, 4 and 5) at the end of 1st and 2nd week as compared to basal diet control group (1). This might be due to the severe damage of kidney caused by CYP. Similar results were also observed by Henry *et al.* (1990), Senthilkumar *et al.* (2006) and Sudharsan *et al.* (2006). There is also a significant decrease in the levels of GSH (Table 3) and a rise in the concentration of TBARS and protein carbonyls in kidney (Table 4-5). TBARS are the lipid peroxidation products formed by the action of free radicals on membrane lipids of cell and protein carbonyls are the oxidation products of proteins. Generation of free radicals might be due to the attack of nucleophilic centers of the cellular components by the electrophilic metabolites of CYP.

The levels of serum creatinine and BUN in NAC treated groups at the end of 1st and 2nd week were not significantly different from the CYP toxic group. Also the cellular GSH level, which was expected to augment with NAC showed no increase in groups 3 and 4. The concentration of TBARS and protein carbonyls revealed similar results. N-acetyl cysteine, a chelator of free radicals known to be useful in paracetamol poisoning and in

Table 1: Blood urea nitrogen (mg/dl) in different groups of rats.

Group	Week	
	1st	2nd
1. Basal Diet Control (1-14 d)	9.28 ± 0.85^{aA}	9.65 ± 0.82^{aA}
2. CYP Control(1,3,5,7 d)	21.44 ± 1.30^{bA}	23.19 ± 2.49^{bA}
3. CYP (1,3,5,7 d) + NAC (1-14 d)	20.94 ± 3.36^{bA}	19.30 ± 2.90^{bA}
4. CYP (1,3,5,7 d) + NAC (1-14 d)	21.50 ± 3.94^{bA}	20.08 ± 2.61^{bA}

Values are Mean + SE (n = 6); One way ANOVA (SPSS); Means with different alphabets as superscripts differ significantly ($P<0.05$); Capital alphabets (Horizontal comparison); Small Alphabets (Vertical Comparison)

Table 2: Serum creatinine (mg/dl) in different groups of rats.

Group	Week	
	1st	2nd
1. Basal Diet Control (1-14 d)	1.05 ± 0.12^{aA}	1.07 ± 0.12^{aA}
2. CYP Control (1,3,5,7 d)	2.26 ± 0.16^{bA}	2.29 ± 0.34^{bA}
3. CYP (1,3,5,7 d) + NAC (1-14 d)	1.97 ± 0.97^{bA}	2.00 ± 0.47^{bA}
4. CYP (1,3,5,7 d) + NAC (1-14 d)	2.20 ± 0.19^{bA}	2.23 ± 0.18^{bA}

Values are Mean + SE (n = 6) One way ANOVA (SPSS); Means with different alphabets as superscripts differ significantly ($P<0.05$); Capital alphabets (Horizontal comparison); Small Alphabets (Vertical Comparison)

Table 3: GSH concentration (μM /mg protein) in kidney in different groups of rats

	Group	2nd Week
1.	Basal Diet Control (1-14 d)	23.16 ± 1.20^{b}
2.	CYP Control (1,3,5,7 d)	10.58 ± 1.41^{a}
3.	CYP (1,3,5,7 d) + NAC (1-14 d)	14.53 ± 1.50^{a}
4.	CYP (1,3,5,7 d) + NAC (1-14 d)	13.75 ± 2.52^{a}

Values are Mean + SE (n = 6); One way ANOVA (SPSS); Means with different alphabets as superscripts differ significantly ($P < 0.05$)

Table 4: TBARS concentration (μM MDA /mg protein) in kidney in different groups of rats

	Group	2nd Week
1.	Basal Diet Control (1-14 d)	0.07 ± 0.01^{a}
2.	CYP Control (1,3,5,7 d)	0.17 ± 0.01^{b}
3.	CYP (1,3,5,7 d) + NAC (1-14 d)	0.17 ± 0.02^{b}
4.	CYP (1,3,5,7 d) + NAC (1-14 d)	0.16 ± 0.03^{b}

Values are Mean + SE (n = 6); One way ANOVA (SPSS); Means with different alphabets as superscripts differ significantly ($P < 0.05$)

Table 5: Protein carbonyls concentration (nM/ 100 mg protein) in kidney in different groups of rats

	Group	2nd Week
1.	Basal Diet Control (1-14 d)	0.01 ± 0.06^{a}
2.	CYP Control (1,3,5,7 d)	2.67 ± 0.25^{b}
3.	CYP (1,3,5,7 d) + NAC (1-14 d)	2.63 ± 0.20^{b}
4.	CYP (1,3,5,7 d) + NAC (1-14 d)	2.62 ± 0.45^{b}

Values are Mean + SE (n = 6) One way ANOVA (SPSS); Means with different alphabets as superscripts differ significantly ($P < 0.05$)

conditions of GSH deficiencies for replenishing GSH, was found to be ineffective in providing protection against CYP-induced oxidative damage of kidney at the dosage schedule used in this study.

References

Balasubramanian, KA, Manohar, M and Mathan, VI (1988). An unidentified inhibitor of lipid peroxidation in intestinal mucosa. *Biochim Biophysic Acta*, **962**: 51-58.

Dekhuijzen, PNR (2004). Antioxidant properties of *N*-acetylcysteine: their relevance in relation to chronic obstructive pulmonary disease. *European Respiratory J.*, **23**: 629-636.

Flora De S, Izzotti, A, D' Agostini, F and Balansky (2001). Mechanisms of N-acetylcysteine in the prevention of DNA damage and cancer, with special reference to smoking – related end – points. *Carcinogenisis*, **22**: 999 – 1013.

Georgia, JP, Dianne, C, Michael, B, Sally, L, Eric, W, Brian, H, Colin, JH and Roland, CW (2005). Role of hepatic cytochrome P450s in the pharmacokinetics and toxicity of cyclophosphamide: Studies with the hepatic cytochrome P450 reductase Null mouse. *Cancer Res.*, **65**: 4211-4217.

Henry, SF, Colvin, OM, Aisaka, K, Jim, P, Bossen, EH, Reimer, KA, Powell, JB, John H, Gross, SS, Roberto, L, Bigner, DD and Griffith, OW (1990). Glutathione protects cardiac and skeletal muscle from cyclophosphamide-induced toxicity. *Cancer Res.*, **50**: 2455-2462.

Kondala, RA, John, J, Leonard, AH and Leonore, AH (2007). N-Acetylcysteine - a safe antidote for cysteine/glutathione deficiency. *Current Opinion in Pharmacol.*, **7**: 355-359.

Levine, RL, Garland, D, Oliver, CN, Amici, A, Climent, I, Lenz, AG, Ahn, BW, Shaltiel, S and Stadtman, ER (1990). Determination of carbonyl content in oxidatively modified proteins. Methods in Enzymol., 186: 464-478.

Moore, MJ (1991). Clinical Pharmacokinetics of cyclophosphamide. *Clinical Pharmacokinetics*, **20**: 194-208.

Moron, MS, Depierre, JW and Mannervik, B (1979). Levels of glutathione, glutathione reductase and glutathione S transferase in rat lung and liver. *Biochim Biophysic Acta*, **582**: 67-68.

Senthilkumar, S, Devaki, T, Manohar, BM and Babu, MS (2006). Effect of squalene on cyclophosphamide-induced toxicity. *Clinica Chimica Acta*, **364**: 335-342.

Sudharsan, PT, Mythili, Y, Selvakumar and Varalakshmi, P (2006): Lupeol and its ester inhibit alteration of myocardial permeability in cyclophosphamide administered rats. *Mol. Cell Biochem.*, **292**: 39-44.

Tepel, M, van der Giet, M and Schwarzfeld, C (2000). Prevention of radiographic-contrast-agent-induced reductions in renal function by acetylcysteine. *New Engl J, Med.*, **343**: 180-184.

Zhang, S, Chai, FY, Yan, HGY and Harding, JJ (2008). Effects of N-acetylcysteine and glutathione ethyl ester drops on streptozotocin-induced diabetic cataract in rats. *Mol Vision*, **14**: 862-870.

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