Protective effect of *N*-acetylcysteine on antituberculosis drug-induced hepatotoxicity

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Introduction **Isoniazid, rifampicin, and pyrazinamide, the first-line antituberculosis (anti-TB) drugs, are associated with hepatotoxicity.**

Aims and objectives **To study the hepatoprotective effect of *N*-acetylcysteine (NAC) on liver injury induced by anti-TB drugs.**

Methods **A randomized clinical trial was conducted on 60 new TB patients who were aged 60 years or more. Patients were randomized into two groups. In group I ($n=32$), drug regimen included daily doses of isoniazid, rifampicin, pyrazinamide, and ethambutol. Patients in group II ($n=28$) were treated with the same regimen and NAC. The patients were followed up for 2 weeks. Liver enzymes and bilirubins were measured at baseline, after 1 and 2 weeks of treatment, and whenever the patients presented with clinical symptoms of hepatotoxicity.**

Results **The mean ± SD values of aspartate aminotransferase and alanine aminotransferase were significantly higher in group I than in group II after 1 and 2 weeks of treatment. Hepatotoxicity occurred in 12 patients with (37.5%) group I and none in group II. The mean duration of treatment before the onset of hepatotoxicity was 4.67 ± 4.58 days.**

Conclusion **NAC protects against anti-TB drug-induced hepatotoxicity. *Eur J Gastroenterol Hepatol* 22:1235–1238 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Isoniazid (INH), rifampicin (RIF), and pyrazinamide (PZA), the first-line drugs used for tuberculosis (TB) chemotherapy, are associated with hepatotoxicity [1]. A high rate of hepatotoxicity has been reported in some developing countries compared with advanced countries with a similar dose schedule [2,3]. Sharifzadeh *et al.* [4] reported an incidence of 27.7% in Iran. The reasons for this higher rate of hepatotoxicity are not completely clear. Ethnic variations, advanced age, female sex, alcoholism, underlying liver disease, acetylator phenotype, hepatitis B and C virus, HIV infection, extensive pulmonary parenchymal disease, and hypoalbuminemia have been observed to be the risk factors for the development of drug-induced hepatotoxicity (DIH) because of anti-TB treatment [2].

The mechanism of DIH induced by anti-TB treatment is not yet fully understood. Sodhi *et al.* [5] proposed oxidative stress as one of the likely mechanisms for INH-RIF-induced hepatic injury. It is well established that by augmenting a cellular antioxidative defense system, especially nonprotein thiols, that is, glutathione (GSH), cells can be protected against oxidative injuries produced by various drugs and chemicals [6]. Animal studies have shown that INH-RIF-induced oxidative injury can be prevented by supporting the cellular antioxidant defense mechanism by *N*-acetylcysteine (NAC) [7,8]. However, there are no published data regarding the protective effect of NAC against hepatotoxicty induced by anti-TB drugs in humans, to our knowledge.

Therefore, we designed a clinical trial with the aim to see whether NAC could protect against anti-TB DIH.

Methods

Study population

The study was carried out in Masih Daneshvari, a tertiary-care university teaching hospital that comprised 60 newly diagnosed pulmonary TB and treatment-naive patients. Ethical permission for the study was obtained from the ethics review board of the National Research Institute of Tuberculosis and Lung Disease. All patients signed written consent before their participation in the study. The patients with two sputum specimens positive for tubercle bacilli on direct smear

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microscopy, no earlier anti-TB chemotherapy, aged 60 years and more, and agreement to participate in the study were included in this study.

The patients with alcohol consumption, viral hepatitis, hemoptysis (NAC may exacerbate the severity of hemoptysis), abnormal pretreatment liver functions level, chronic disease (asthma, liver, and kidney disease), additional hepatotoxic drug use, HIV positive, liver TB, and a moribund state were excluded from the study.

Sample size calculation

A sample size of 52 was calculated using Minitab v 15.1.1.0 statistical software (*http://www.minitab.com/support/index.html*).

Incidence of hepatitis in a similar population was 27.7% [4]. As there was no information in the literature about the size of treatment effect, a pilot study was needed to estimate the effect of NAC. Incidence of hepatitis in the NAC group by extrapolating from this study was 0.01%. Level of significance and the power of study were 0.05 and 80%, respectively.

Diagnosis of drug-induced hepatotoxicity

The presence of at least one of the following criteria was defined as DIH: (i) a rise of five times the upper limit of normal levels of serum aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT); (ii) a rise in the level of serum total bilirubin of more than 1.5 mg/dl; (iii) any increase in AST and/or ALT above the pretreatment levels together with the hepatitis symptoms [9].

Study design

The study was designed as open label clinical trial. Patients who were included in the study were randomized into two groups. In group I ($n = 32$), drug regimen included daily doses of four drugs standard therapy including INH (5 mg/kg), RIF (10 mg/kg), PZA (25 mg/kg), and ETB (15 mg/kg). Group II ($n = 28$) was treated with the same standard drug regimen and NAC (600 mg, orally, twice daily).

The patients were admitted to the hospital for the first 2 weeks of therapy according to our hospital protocol. Baseline laboratory tests including AST, ALT, and bilirubin were evaluated before anti-TB therapy. During treatment, liver enzymes and bilirubin were measured at weeks 1, 2, and whenever the patients presented with clinical features of DIH.

When DIH was detected, INH, RIF, and PZA were withdrawn. After hepatotoxicity-related symptoms had disappeared and laboratory findings had returned to normal levels, anti-TB drugs were gradually reintroduced.

Statistical analysis

Descriptive statistics were used to detail the demographics of the patients. Normally distributed variables were presented as the mean ± SD. Independent sample *t*-test and χ^2 test were used for the comparison of quantitative and qualitative data between groups I ($n = 32$) and II ($n = 28$), and in group II between patients with and without hepatitis. Pearson's relative analysis was used to evaluate the relation of liver function tests and quantitative risk factors. A P value of less than 0.05 was considered as statistically significant. Statistical analysis was performed using SPSS version 16.0 (*http://www.spss.com/tech/*).

Results

Patient demographics are shown in Table 1. Risk factors for hepatotoxicity between two groups with respect to mean age, weight, sex ratio, place of origin, diabetes mellitus, pretreatment serum AST, ALT, and bilirubin were not statistically significant. The mean ± SD values of AST and ALT were significantly higher in group I than in group II after 1 and 2 weeks of treatment. Although the mean ± SD value of serum bilirubin was significantly higher in group I than in group II after 1-week of treatment (Table 2).

Of 32 patients, 12 (37.5%) in group I developed hepatotoxicity that was induced by anti-TB drugs. All patients with hepatotoxicity exhibited hepatitis symptoms. A rise of five times the upper limit of normal levels of serum

Table 1 Patient demographics

Characteristics	Group I	Group II
Number	32	28
Mean age (years) ± SD	73.41 ± 6.72	74.46 ± 7.83
Sex ratio (male/female)	17/15	14/14
Mean weight (kg) ± SD	56.12 ± 9.97	54.16 ± 14.96
Origin		
Iranian	31	26
Non-Iranian	1	2

Table 2 Mean ± SD values of aspartate aminotransferase, alanine aminotransferase, and bilirubin levels in groups I and II

Laboratory findings	Group I ($n = 32$)	Group II ($n = 28$)
Before treatment		
AST (U/l)	27.47 ± 13.55	27.04 ± 16.24
ALT (U/l)	22.84 ± 14.35	22.50 ± 18.57
Bilirubin total (mg/dl)	0.75 ± 0.39	0.68 ± 0.32
First week after treatment		
AST (U/l)	99.44 ± 150.11*	27.68 ± 13.79
ALT (U/l)	65.78 ± 88.64*	20.96 ± 11.95
Bilirubin total (mg/dl)	1.13 ± 0.91**	0.61 ± 0.29
Second week after treatment		
AST (U/l)	57.22 ± 75.81*	27.32 ± 13.11
ALT (U/l)	58.09 ± 86.18*	21.53 ± 9.56
Bilirubin total (mg/dl)	0.73 ± 0.40	0.58 ± 0.31

*$P < 0.05$.
**$P < 0.01$.

AST and/or ALT was detected in six patients. Total billirubin level was more than 1.5 mg/dl in three patients. There was no significant relationship between age, sex, weight, and diabetes mellitus with hepatotoxicity. A significant relationship between liver function tests and quantitative risk factors was also not observed in our study. The mean duration of treatment before the onset of hepatotoxicity was 4.67 ± 4.58 days. Serum transaminases were normalized within 8.17 ± 3.76 days after stopping the treatment.

Discussion

Potentiation of the host antioxidant defense machinery could be a rational strategy to primary prevention of anti-TB DIH. NAC, a nontoxic antioxidant, is reported to prevent INH-RIF-induced oxidative liver injury in rats [7,8].

Our results, for the first time, showed that NAC protects against anti-TB drug-induced hepatic injury in humans. NAC was administered for the first 2 weeks of treatment as DIH occurs most often during this period [4,10].

The rate of hepatotoxicity induced by anti-TB drugs is high in our patients. Several factors have been implicated in the development of DIH because of anti-TB treatment. American Thoracic Society states that based on the variables (anti-TB regimens, study populations, definitions of hepatotoxicity, monitoring, and reporting practices) a wide range of hepatotoxicity from 5% to as high as 33% can be expected [11]. Huang *et al.* [12] reported that *N*-acetyltransferase 2 slow acetylator status and age were the only independent risk factors for DIH because of anti-TB treatment. In addition, it was also observed that slow acetylators were prone to develop more severe hepatotoxicity than rapid acetylators. The acetylator status distribution in Iranians shows a considerable prevalence of slow acetylators over rapid acetylators [13,14]. In contrast, an inclusion criterion for our study was age of 60 years and above. As DIH is significantly higher in the patients above 65 years of age [10], this criterion was defined to include more patients with hepatotoxicity in the study. Twelve patients (37.5%) with anti-TB treatment did experience DIH, whereas there was no DIH in patients with anti-TB treatment and NAC.

Recently, Venketaraman *et al.* [15] indicated that GSH levels are compromised in TB patients, and this decrease correlates with protective immunity. They concluded that administration of NAC to TB patients might improve GSH contents and the host-immune responses. In our study, hepatoprotection by NAC may be by acting as a precursor of GSH synthesis and by triggering various antioxidant mechanisms.

A limitation of our study is the lack of using placebo. As NAC was not manufactured in our country at the time of study design and approval, we could not provide a placebo intervention. Therefore, the study was carried out as a randomized open-label clinical trial. This study was the first clinical trial conducted to assess the protective effect of NAC against anti-TB DIH. A selected group of TB patients (susceptible patients to DIH) for a relatively short-term administration period (probable period of development of DIH) were included in this study. The apparent protective effects of NAC were marked in this group. For generalizing these results to clinical practice, future studies in a wider patient sample over a longer follow-up period are required.

Conclusion

The results indicate that NAC protects against DIH induced by INH, RIF, and PZA combination treatment in an elderly population. Given the limitations of the trial, that is, short follow-up period and the open-label design, the results have to be interpreted with caution and require future studies.

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Conflicts of interest none declared.

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