A RANDOMIZED CONTROLLED TRIAL OF INTRAVENOUS N-ACETYLCYSTEINE IN THE MANAGEMENT OF ANTI-TUBERCULOSIS DRUG-INDUCED LIVER INJURY

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SUMMARY: N-Acetylcysteine did not shorten time to alanine transaminase below 100 U/L in a randomized placebo-controlled trial of hospitalized adults with anti-tuberculosis drug-induced liver injury. However, participants who received N-Acetylcysteine had shorter hospital stays. Mortality did not differ by study arm.

ABSTRACT

Background: Liver injury is a common complication of first-line anti-tuberculosis therapy. N-acetylcysteine (NAC) is widely used in patients with paracetamol toxicity with limited evidence of benefit in liver injury due to other causes.

Methods: We conducted a randomized, double-blind, placebo-controlled trial to assess whether intravenous NAC hastens liver recovery in hospitalized adult patients with anti-tuberculosis drug induced liver injury (AT-DILI). The primary endpoint was the time for serum alanine aminotransferase (ALT) to fall below 100 U/L. Secondary endpoints included length of hospital stay, in-hospital mortality and adverse events.

Results: Fifty-three participants were randomized to NAC and 49 to placebo. Mean age was 38 (SD±10) years, 58 (57%) were female and 89 (87%) were HIV-positive. Median serum ALT and total bilirubin at presentation were 462 U/L (IQR 266-790) and 56 μmol/L (IQR 25-100) respectively. Median time to ALT<100 U/L was 7.5 days (IQR 6 -11) in the NAC arm and 8 days (IQR 5 -13) in the placebo arm. Median time to hospital discharge was shorter in the NAC arm (9 days; IQR 6-15) than in the placebo arm (18 days; IQR 10-25), hazard ratio 1.73 (95% CI 1.13-2.65). Mortality was 14% overall and did not differ by study arm. The study infusion was stopped early due to an adverse reaction in 5 participants receiving NAC [nausea and vomiting (3), anaphylaxis (1), pain at drip site (1)].

Conclusion: NAC did not shorten time to ALT<100 U/L in participants with AT-DILI, but significantly reduced length of hospital stay. NAC should be considered in management of AT-DILI.

KEYWORDS: Tuberculosis, Anti-tuberculosis therapy, N-Acetylcysteine, Drug induced liver injury

INTRODUCTION

Liver injury is the most common severe adverse drug reaction caused by first-line anti-tuberculosis therapy (ATT) with an estimated incidence of 2-28% depending on the definition of drug-induced liver injury (DILI) used and the population studied [1]. Liver injury due to first-line anti-tuberculosis therapy (AT-DILI) may cause prolonged hospitalization [2], and is associated with increased mortality [3, 4]. There is currently no specific therapy for AT-DILI. The management of suspected AT-DILI includes the cessation of all potentially hepatotoxic anti-tuberculosis drugs (rifampicin, isoniazid, pyrazinamide), possible introduction of alternative ATT, monitoring of liver function tests, and supportive care while awaiting liver recovery. When the serum alanine aminotransferase (ALT) falls below 100 U/L, ATT rechallenge may be considered [5].

N-acetylcysteine (NAC) is widely used as treatment for paracetamol liver toxicity [6] and may provide benefit for other causes of hepatitis [7, 8]. A prospective cohort study of 155 participants with non-paracetamol acute liver failure, found improvement in transplant-free survival in those treated with NAC compared with historical controls; DILI was the cause of liver failure in 38% [9]. A randomized controlled trial of NAC in 173 participants with non-paracetamol acute liver failure found improved transplant free survival overall, but the study was under-powered to assess efficacy in the small DILI subgroup (26% of participants) [10]. A systematic review concluded that there was insufficient evidence to support the use of NAC in non-paracetamol induced liver injury [11].

There is evidence that NAC may prevent AT-DILI. NAC improved liver histology in rats exposed to high dose isoniazid and rifampicin intraperitoneally [12]. NAC reduced cellular and mitochondrial membrane damage, and apoptosis in an in vitro study using human hepatocellular carcinoma cells exposed to toxic doses of isoniazid, rifampicin and pyrazinamide in various combinations [13]. Oral

NAC administered to participants during the first two weeks of ATT prevented increases in ALT in a small open label randomized controlled trial [14].

We hypothesised that NAC would shorten the duration of AT-DILI and conducted a randomized, double-blind placebo-controlled trial of intravenous NAC in adults with suspected AT-DILI.

METHODS

Study Participants

We recruited adult patients with a diagnosis of AT-DILI at 3 public sector hospitals in Cape Town, South Africa: Groote Schuur Hospital (tertiary level academic hospital), New Somerset Hospital (secondary level hospital) and Khayelitsha District Hospital. To be eligible for recruitment, a patient had to meet the American Thoracic Society criteria for AT-DILI requiring cessation of ATT [5], by either having an ALT of more than three times the upper limit of normal if symptoms of hepatitis were present, or an ALT of more than 5 times the upper limit of normal without symptoms of hepatitis. Other inclusion criteria were age 18 years or older, taking first-line ATT for the treatment of active TB and liver injury that was attributed to ATT. We included both participants who were admitted to hospital because of AT-DILI and those who developed AT-DILI while in hospital. Patients with acute liver failure, which we defined as fulminant hepatitis resulting in coagulopathy (INR>1.5) and an altered mental status [15], were eligible for inclusion.

We excluded patients with asthma, because of risk of NAC-induced bronchospasm, pregnant patients, and patients known to have viral hepatitis at the time of screening.

The primary endpoint was the time to ALT falling below 100 U/L. Secondary endpoints included time to hospital discharge, in-hospital mortality and study infusion related adverse events.

Study Procedures:

This study was a pragmatic randomized controlled trial, nested within routine clinical care. Participants were investigated and managed by hospital clinicians, except for administration of the study infusion and monitoring for adverse reactions by a member of the study team. Hospital clinicians, who were blinded to treatment allocation, made the decision to discharge participants from hospital, in line with their clinical judgement.

We collected baseline demographic, clinical, pharmacological and biochemical data on all study participants at the time of randomization. We graded hepatic encephalopathy using the West Haven score [16] (Supplementary Table 1). As part of routine clinical work-up, participants were tested for acute viral hepatitis A (anti-hepatitis A IgM), acute viral hepatitis B (hepatitis B surface antigen and anti-core IgM) and viral hepatitis C (anti-hepatitis C total antibodies and polymerase chain reaction).

Study participants were randomized 1:1 to receive intravenous NAC or placebo. Randomization was stratified by site and performed in blocks of 10 using a computer-generated randomization schedule. Study pharmacists at each site had restricted access to the randomization schedule and prepared the study infusion according to treatment allocation. Investigators and study participants were blinded to treatment allocation.

NAC was dosed and administered according to the regimen for paracetamol overdose as per manufacturer provided guidelines: 150 mg/kg over 1 hour, 50 mg/kg over 4 hours and 100 mg/kg over 16 hours (see Supplementary Table 2 for the weight-based dosing schedule). We used 0.9% saline as diluent for the NAC and placebo infusions, except for participants with acute liver failure or hypoglycaemia (serum glucose < 3.5 mmol/l), for whom 5% dextrose was used. Intravenous NAC is colourless and indistinguishable from placebo when mixed with either saline or dextrose solution.

Study participants were closely monitored for adverse events during the first hour of the study infusion and at the beginning and end of each infusion bag. A study investigator reviewed participants clinically at least twice weekly during hospital admission. We graded severity of adverse events using DAIDS categories [17]. All participant deaths were reviewed by an independent physician, to assess whether the study drug was implicated in the death. The serum ALT was monitored by the clinical care team at least twice weekly until it fell below 100 U/L as per standard clinical practice.

Analysis

We powered the study to detect a 33% reduction in time for ALT to fall below 100 U/L with 80% power and an alpha value of 0.05. We assumed a mean time for ALT to fall below 100 U/L in the placebo arm of 18 days (SD±10), based on the ALT normalization time after cessation of ATT reported in a trial of ATT rechallenge regimens [18] . We calculated that we would require 88 participants (44 in each arm); we inflated the sample size to 100 participants to allow for deaths and loss to follow-up.

Analysis was by modified intention to treat. We included all randomized participants in whom we commenced the study infusion in the analysis. Continuous variables were described using means and standard deviations if parametrically distributed or medians and ranges if non-parametrically distributed. Categorical variables were described using counts and percentages. Time to ALT <100 U/L and time to discharge from hospital were described using Kaplan-Meier analyses. For calculation of time to hospital discharge we used the interval from study consent to discharge home or to a chronic care facility. The analysis of time to hospital discharge included both patients presenting with AT-DILI before admission to hospital and those who developed AT-DILI during hospital admission. We performed a log rank test to compare survival curves. We performed a univariable Cox regression to calculate a hazard ratio (HR) for ALT falling below 100U/L, and a HR for

hospital discharge, with a 95% confidence interval (CI). Data were analysed using Stata (Version SE/15.0 Statacorp Texas USA).

Ethics

This study was conducted according to the guidelines of the Helsinki Declaration of 2013 and the ICH principles of Good Clinical Practice [19, 20]. The protocol was approved by the Western Cape Department of Health, University of Cape Town Human Research Ethics Committee (HREC 087/2012) and the South African Health Products Regulatory Authority. Participants provided written informed consent. Our research ethics committee granted us permission to include patients with hepatic encephalopathy who were too sick to consent at presentation, and to seek their consent after they had recovered from encephalopathy. We were also granted permission to include the data of participants who did not recover from encephalopathy, in the analysis. The trial was registered with the South African National Clinical Trials Registry (SANCTR: DOH-27-0414-4719)

RESULTS

We screened 125 patients with suspected AT-DILI, 20 of whom were excluded (Figure 1). We enrolled 105 participants of which 54 were randomized to the NAC arm and 51 were randomized to the placebo arm. Three randomized individuals (1 in the NAC arm and 2 in the placebo arm) were not administered the study infusion and were not included in the analyses: 1 participant withdrew consent, 1 participant disclosed that they were asthmatic (an exclusion criterion), and 1 participant could not be administered the study infusion as there was no study team member available to commence and monitor the infusion (Figure 1).

The baseline characteristics of the 102 participants who started the study infusion were similar in the NAC and placebo arms (Table 1). Sixty participants (59%) had pulmonary TB, 37 (36%) had extra-

pulmonary TB and in 5 participants (5%) the site of TB was not specified. Twenty-two participants (22%) had previously received a course of ATT before this treatment episode. One hundred participants (98%) were taking intensive phase ATT (rifampicin, isoniazid, pyrazinamide and ethambutol) and 2 participants (2%) were taking continuation phase ATT (rifampicin and isoniazid) at the time of presentation with a liver injury. Eighty-nine participants (87%) were HIV positive, of whom 31 were taking an efavirenz-containing ART regimen, 9 were taking a lopinavir-ritonavir-containing ART regimen and 23 were taking cotrimoxazole at the time of presentation with a liver injury. Four participants (1 in the NAC arm and 3 in the placebo arm) reported an alcohol consumption greater than 14 units/week. Twelve participants in the NAC arm and 9 participants in the placebo arm developed the AT-DILI during a hospital admission.

Seventy-four participants (73%) reported symptoms of AT-DILI at screening. The most commonly reported symptoms and signs were jaundice (47%), vomiting (43%), nausea (33%) and abdominal pain (25%). Eleven participants presented with hepatic encephalopathy, of whom 9 had mild encephalopathy (West Haven coma score of 1-2) and 2 had moderate encephalopathy (West Haven coma score of 3). Eighty-two participants had a prolonged INR (>1.1) and 11 had a serum sodium of less than 125 mmol/L.

None of the participants had evidence of viral hepatitis based on serology at the time of enrolment. However, during the course of the trial three participants were found to have serological evidence of chronic viral hepatitis B. In addition, in two hepatitis B surface antigen-positive participants, anti-core IgM quantification was not requested by the clinical care team, so acute hepatitis B could not be excluded. One of these participants was also hepatitis A total antibody positive but hepatitis A IgM was not performed to exclude acute hepatitis A.

Five participants (1 in NAC arm and 4 in placebo arm) were assessed by study investigators as having other diseases that could have caused hepatitis (leptospirosis, disseminated Emergo mycosis, hepatocellular carcinoma, sepsis, and TB immune reconstitution inflammatory syndrome). Eight

participants (5 in NAC arm and 3 in placebo arm) were assessed by study investigators as possibly having another drug implicated in the liver injury: efavirenz in 3, lopinavir-ritonavir in 2, cotrimoxazole in 2 and fluconazole in 1.

The time to ALT <100 U/L was similar in the treatment arms (Figure 2A), with a median of 7.5 days (IQR 5.5 -11) and 8 days (IQR 5 -13) in the NAC and placebo arms respectively. The HR for ALT falling below 100U/L was 1.03 (95% CI 0.68 to 1.57).

Time to discharge from hospital was shorter in the NAC arm than in the placebo arm (Figure 2B), with a median of 9 days (IQR 6-15) in the NAC arm, and 18 days (IQR 10-25) in the placebo arm. The HR for hospital discharge was 1.73 (95% CI 1.13 to 2.65).

The overall mortality was 14% and did not differ by treatment arm. The causes of death were ATT-induced liver failure in 9, chronic lung disease in 2, sepsis in 1, *Pneumocystis jirovecii* pneumonia in 1 and post liver biopsy haemorrhage in 1. Study drug was not implicated in any of the deaths.

There were 16 adverse events (AEs) during the study infusion, 13 in the NAC arm and 3 in the placebo arm. The study infusion was stopped early due to an AE in 5 participants, all of whom were receiving NAC (Table 2).

Serious AEs that occurred during study follow-up (after study drug administration) are described in Supplementary Table 3. None of these serious AEs were assessed by the investigators as being caused by the study drug; 19 serious AEs were assessed as possibly caused by other drugs.

DISCUSSION

We found no significant difference in our primary outcome (time to ALT <100 U/L) between participants with AT-DILI who received NAC or placebo. However, the median time to hospital discharge was 9 days shorter in the NAC arm. NAC was generally well tolerated, but all 5 AEs that resulted in discontinuation of the study infusion occurred in the NAC arm.

NAC is widely used to treat patients with paracetamol overdose; however, the quality of evidence for its efficacy in this setting is limited [21]. In contrast to paracetamol, which has a direct dose dependant hepatotoxic effect via intermediary metabolites, the mechanism of AT-DILI is thought to be an idiosyncratic, immune response to the covalent binding of drug metabolites to liver proteins [22]. Furthermore, the duration of liver injury due to ATT is typically much longer than that due to paracetamol. Despite these differences in the pathogenesis of AT-DILI and paracetamol induced liver injury, NAC prevented increases in ALT in rats and humans exposed to ATT [12, 14] ; and intravenous NAC improved transplant-free survival in a randomized placebo-controlled trial in participants with non-paracetamol induced liver failure, some of whom had DILI [10] .

In our trial there was no difference in our primary efficacy endpoint of time to ALT <100 U/L between treatment arms. However, ALT is not a good biomarker of liver injury: ALT concentrations may be normal during the early stages of hepatocyte injury or apoptosis; ALT may be elevated due to mechanisms other than hepatocyte injury (e.g. membrane blebbing, increased hepatic expression, macroenzymes) [23] ; and ALT may be released from tissues other than liver such as skeletal muscle, kidney and heart [24] . Novel biomarkers of liver injury such as microRNA, are more specific for liver

injury than ALT and increase earlier than ALT in patients with paracetamol induced liver injury [25-27].

In our trial, participants with AT-DILI who received NAC had significantly shorter hospital stays, which was a prespecified secondary endpoint of the trial. Our study was blinded, therefore the clinical decision to discharge participants from hospital could not have been biased by knowledge of treatment allocation. Our finding therefore suggests that NAC hastened clinical recovery, which is consistent with findings of shorter hospital stay in the NAC arm of a randomized placebo-controlled trial of NAC in participants with non-paracetamol induced liver failure. [10].

There are several potential explanations for the shorter hospital stay in the NAC arm of our study, despite similar time to ALT resolution. First, improved recovery from AT-DILI due to NAC may not have resulted in a difference in ALT trajectory between groups, but might have been detected had we used a better biomarker of liver injury than ALT. Second, the anti-oxidant effects of NAC may be beneficial in people living with HIV (87% of our participants), who often have glutathione depletion [28] . A randomized placebo controlled trial of NAC in HIV positive participants with low glutathione concentrations reported reduced mortality in participants who received NAC, but open label NAC was offered to all participants after a short (8 week) randomized phase; a further limitation of the study was that it was conducted in the era before effective combination ART. Third, it is possible that NAC could have improved tuberculosis as there is some evidence that NAC inhibits *Mycobacterium tuberculosis* growth and augments the mycobactericidal activity of first-line ATT in vitro [29, 30]. Fourth, the shorter hospital stay in the NAC arm could have been due to chance.

Our study had limitations. First, we included patients who were taking drugs other than ATT, which have also been associated with DILI (e.g. efavirenz and cotrimoxazole). Second, we did not exclude viral hepatitis A and B in 2 participants in the NAC arm; and we did not test for viral hepatitis E in our cohort as it is not part of local routine clinical care. Third, HIV prevalence was high in our cohort and

therefore our findings may not be generalizable to other populations. Fourth, our primary outcome was a reduction in ALT, which has limitations as a biomarker of DILI.

Further studies are needed to determine NAC clinical benefits in AT-DILI in populations with low HIV prevalence. Future trials of NAC for AT-DILI should have a clinical primary outcome and use more appropriate markers of DILI than ALT.

CONCLUSION

Our randomized controlled trial did not demonstrate any effect of NAC on reducing the time to ALT <100 U/L in patients with AT-DILI. However, length of hospital stay was significantly shorter in the NAC arm. NAC, which is widely available, should be considered in the management of AT-DILI.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Table 1. Baseline Characteristics of Participants by Study Arm

Baseline Characteristics	NAC (n=53)	Placebo (n=49)
Age years, mean (±SD)	37 (±10)	38 (±9)
Female, n (%)	34 (64)	24 (49)
Weight kg, median (IQR)	55 (47-67)	53 (45-63)
First time on TB treatment, n (%)	41 (77)	39 (80)
Duration of TB treatment, days, median (IQR)	18 (10-31)	25 (15-40)
HIV positive, n (%)	44 (83)	45 (92)
ART, n (%) Efavirenz, n (%) Lopinavir-ritonavir, n (%)	23 (43) 18 (34) 5 (9)	17 (35) 13 (27) 4 (8)
Cotrimoxazole, n (%)	8 (15)	15 (31)
Symptoms of DILI, n (%)	39 (74)	35 (71)
Encephalopathy, n (%)	6 (11)	5 (10)
ALT U/L, median (IQR)	448 (286-685)	384 (266-566)
Total bilirubin, µmol/L, median (IQR)	55 (19-93)	65 (30-117)
ALP U/L, median (IQR)	170 (101-248)	175 (113-253)
INR, median (IQR)	1.5 (1.2-2.2)	1.3 (1.1-2.2)
Albumin g/L, median (IQR)	26 (19-30)	23 (20-29)
Sodium mmol/L, mean (±SD)	131±5	129±5
CD4 count cells/mm^3, median (IQR) (HIV positive participants)	89 (40 -285)	75 (12-144)

Abbreviations: ALT, alanine transferase; ALP, alkaline phosphatase; ART, antiretroviral therapy; HIV, human immunodeficiency virus; INR, international normalised ratio; IQR, interquartile range; SD, standard deviation; TB, tuberculosis

Table 2. Adverse Events during Study Drug Infusion

Adverse Event	NAC (n=53)	Placebo (n=49)
Nausea and/or vomiting	9 (infusion discontinued in 3)	2
Rash	1	0
Pruritis	1	0
Pain at drip site	1 (infusion discontinued in 1)	0
Hypotension	0	1
Anaphylactoid reaction	1 (infusion discontinued in 1)	0
Total	13	3

Figure legends

Figure 1. Screening, Randomization and Follow-up

Abbreviations: ALT alanine transaminase, ATT antituberculosis therapy, DILI drug induced liver injury, LFTs liver function tests, NAC N-acetylcysteine

Figure 2. Cumulative estimates of time to ALT<100 U/L and time to hospital discharge in participants with anti-tuberculosis drug-induced liver injury randomized to N-acetylcysteine or placebo

Abbreviations: ALT alanine transaminase, NAC N-acetylcysteine