N-Acetylcysteine ameliorates lithium-induced renal failure in rats

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Abstract

Background. Prolonged lithium treatment may induce progressive deterioration of renal function in humans and experimental animals. N-Acetylcysteine (NAC) has been shown to be effective in the prevention of hypoperfusion and toxin-induced renal failure, but its effect on lithium nephrotoxicity has not been evaluated yet. The purpose of this study was to examine a possible renoprotective effect of NAC against lithium-induced renal failure in a rat model.

Methods. Moderate renal failure was induced in 40 Sprague–Dawley rats using a 5 week protocol including 3 weeks of lithium chloride administration in the drinking water. The animals were divided randomly into two equal groups receiving either 10 mg/kg NAC or saline by two daily intraperitoneal injections. In week 6, the glomerular filtration rate (GFR) was assessed by 99mTechnetium diethylene triaminepentacetic acid, and serum creatinine, blood urea nitrogen (BUN) and 24 h urinary protein and osmolarity were measured. Kidneys were excised for pathological evaluation.

Results. At the end of the lithium protocol, the GFR was significantly higher in the NAC-treated group compared with the control group, 0.92 ± 0.35 vs 0.56 ± 0.25 ml/min/100 g, respectively, P = 0.002. Serum creatinine and BUN were also significantly lower in the NAC-treated group 1.009 ± 0.107 vs 1.143 ± 0.118 mg/dl, P = 0.001, and 83.9 ± 6.8 vs 88.95 ± 7.1 mg/dl, P = 0.28, respectively. The percentages of tubular necrosis and tubular lumen obstruction, evaluated by light microscopy, were significantly lower in the NAC-treated group, P = 0.002 and P = 0.007, respectively.

Conclusions. NAC treatment has a renoprotective effect against lithium-induced renal failure in a rat model.

Keywords: N-acetylcysteine; lithium; rats; renal failure

Introduction

Prolonged lithium therapy has been associated with several forms of renal injury. Nephrogenic diabetes insipidus is the most common; however, renal tubular acidosis, nephrotic syndrome and interstitial nephritis with renal failure are not infrequent [1].

A slowly progressive decline in glomerular filtration rate (GFR) may develop in 15–20% of lithium-treated patients [2,3]. Presne et al. have found that the prevalence of lithium-related end-stage renal disease (ESRD) in France is 2 per 1000 dialysis patients and that the average latency between onset of lithium therapy and ESRD is 20 years [4]. Moreover, even after withdrawal of lithium therapy, renal function may continue to deteriorate [5].

N-Acetylcysteine (NAC) has been shown to be effective in the prevention of contrast media-induced nephrotoxicity and in hypoperfusion and toxin-induced renal failure in human and animal models [6–8]. Lithium therapy may cause severe damage to the mitochondria and to the endoplasmic reticulum in patients with lithium nephrotoxicity, implicating ischaemia as a pathogenetic mechanism [3]. Thus far, the role of NAC in mitigating lithium-induced renal failure has not been investigated. The purpose of the present study was to examine a possible protective effect of NAC against lithium-induced renal failure in a rat model.

Materials and methods

Animals

The experiments were conducted according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. All the animals included in the study...
were born and bred in significant pathogen-free conditions in the animal facilities of Assaf Harofeh Medical Center. The maintenance was according to the guidelines of the local ethics committee for animal experimentation, and the experimental protocol received their approval. Rats were fed a regular chow routinely purchased by our animal facilities via Harlan Laboratories.

Induction of renal failure

The study included 40, 1-month-old, Sprague–Dawley male rats (mean weight 200 ± 50 mg). Renal failure was induced by lithium chloride according to the following protocol (the solution given orally as a drinking substance): (i) 15% LiCl solution for 1 week; (ii) regular tap water for 1 week; (iii) 7% LiCl solution for 2 weeks; and (ii) regular tap water for 1 week.

In a preliminary experiment, this regimen induced moderate renal failure (~60% reduction of GFR), that was consistent at least for 8 weeks after the lithium administration was terminated. The GFR was 1.59 ± 0.3 ml/min/100 g at baseline, 0.5 ± 0.21 ml/min/100 g at the end of the lithium protocol and 0.4 ± 0.19 ml/min/100 g 8 weeks following the end of the lithium protocol.

N-Acetylcysteine treatment protocol

Rats were divided randomly into two groups (20 rats each). Starting the first day of the LiCl protocol, the treatment group received intraperitoneal (i.p.) NAC (Flumil Antidoto 20%, Zambon S.A, Spain) 10 mg/kg twice daily (at 6 a.m. and 6 p.m.), and the control group received i.p saline for 5 weeks. The dose of NAC was relatively close to the daily dose used by us in a previous study performed on humans [(17 mg of NAC)/(kg of human weight)] [8].

Biochemical studies

At baseline and 1 week after ending NAC treatment, in week 6 of the study protocol, animals were placed individually in metabolic cages for 24 h urine collection. Total urinary protein was determined using a Cobas-Mira autoanalyser. Urine osmolarity was measured using a Fiske Osmometer. Blood samples were procured for measurements of creatinine and blood urea nitrogen (BUN) from the caudal vein. Creatinine measurements were performed using the Rate-Blanked creatinine/Jaffé method on a Roche/Hitachi autoanalyzer. BUN measurements were performed using the kinetic UV assay for urea nitrogen on a Roche/Hitachi autoanalyzer.

Glomerular filtration rate assessment

GFR was assessed at baseline and in week 6 of the study by 99mTc Technetium diethylene triaminepentaacetic acid (99mTc-DTPA) injection. At 60 min after i.p injection of 0.1 ml of 1 μCi/ml 99mTc-DTPA, one 50 μl blood sample was procured from the caudal vein and diluted in 2 ml of phosphate-buffered saline (pH 7.4). Standard solution was prepared from 0.1 ml of 1 μCi/ml 99mTc-DTPA stock solution. Radioactivity of the samples was measured in a γ counter (LKB, USA) and GFR values were calculated using the following formula [9]:

\[
\text{GFR} = \frac{V \times \ln(CPM_0/CPM_t)}{t}
\]

where V is the volume of distribution (0.3 × weight in mg); CPM0 is the radioactivity counts per min of standard solution; CPMt is the radioactivity counts per min 60 min after 99mTc-DTPA injection; and \(t\) is the time (min) between the 99mTc-DTPA injection and the blood sample procurement.

In a preliminary study, we evaluated the accuracy of the 99mTc-DTPA method in 20 1-month-old rats. The mean GFR was 1.59 ± 0.3 ml/min/100 g. The reproducibility of the method was evaluated by repeated GFR measurements after a 3 day interval, in the same rats, under the same diet and physical conditions; the coefficient of variation was 8 ± 3%. Six weeks later, in the same rats under the same conditions, the mean GFR was 1.51 ± 0.4 ml/min/100 g.

Pathological evaluation

After GFR evaluation, 10 rats (five from each group), randomly selected, were sacrificed by halothane overdose, their kidneys removed, preserved in formalin and subsequently embedded in paraffin. Large sections (1 μm) were cut perpendicularly to the renal capsule, in order to ensure that both cortex and medulla would be present in each section. Samples were stained with haematoxylin–eosin dye and 20 fields (×400) were selected randomly for light microscope evaluation. Stratification of the kidney damage was made using the percentage of necrotizing cell in damaged tubules per total count of the tubules, and tubular lumen obstruction was established by counting the percentage of granular casts or cellular fragments out of total tubules counted.

Statistical analysis

The statistical analysis was performed using SPSS version 11 software. Parametric data are expressed as means ± SD. The statistical differences between the groups were evaluated by unpaired t-test and the differences within each group by paired t-test. P-values <0.05 were considered statistically significant.

Results

All 40 rats participating in the study survived and the results obtained were included in the final analysis. There was no statistically significant weight difference between the groups before (145 ± 24 vs 152 ± 21 mg), treatment and control groups, respectively) and after the lithium treatment (257 ± 32 vs 243 ± 37 mg, treatment and control groups, respectively).

Table 1 depicts data on serum creatinine, BUN, GFR, 24 h urine volumes, urinary protein and urinary osmolarity obtained at baseline and at the end of the lithium protocol (week 6). At the end of the lithium protocol, there was a significant decrease in the mean GFR compared with baseline for both groups (P < 0.01 for both groups). The mean GFR in the NAC-treated group at the end of the lithium protocol...
Table 1. Biochemical and histological parameters at baseline and at the end of the lithium induced renal failure protocol, in the N-acetylcysteine (NAC)-treated (n = 20) and control (n = 20) groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Week 6</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAC</td>
<td>Control</td>
<td></td>
<td>vs.</td>
</tr>
<tr>
<td>Plasma creatinine (mg/dl)</td>
<td>0.46±0.01</td>
<td>0.47±0.012</td>
<td>0.96</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dl)</td>
<td>25.4±1.6</td>
<td>25.1±1.7</td>
<td>0.98</td>
</tr>
<tr>
<td>GFR (ml/min/100 g)</td>
<td>1.54±0.3</td>
<td>1.61±0.4</td>
<td>0.98</td>
</tr>
<tr>
<td>Urine volume (ml/24 h/100 g)</td>
<td>6.1±1.4</td>
<td>6.0±1.3</td>
<td>0.96</td>
</tr>
<tr>
<td>Urine osmolarity (mmol/l)</td>
<td>989±104</td>
<td>985±107</td>
<td>0.94</td>
</tr>
<tr>
<td>Urine protein (g/24 h)</td>
<td>0.25±0.3</td>
<td>0.26±0.27</td>
<td>0.93</td>
</tr>
<tr>
<td>Tubular necrosis (%)</td>
<td>44.2±9.5</td>
<td>58.9±21.0</td>
<td>0.007</td>
</tr>
<tr>
<td>Tubular lumen obstruction (%)</td>
<td>36.5±9.5</td>
<td>58.9±21.0</td>
<td>0.007</td>
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</table>

#Discussion

Lithium is the first line of treatment in patients with bipolar affective disorder and is the only drug that has clearly demonstrated antisuicide effects [10]. Unfortunately, prolonged lithium administration is often detrimental to renal tissue [1–5]. The wide use of lithium therapy, taken together with the fact that renal function may continue to deteriorate even after discontinuing lithium treatment [5], emphasizes the need for a protective strategy against its toxicity. The results of the present study demonstrated that concomitant administration of NAC has a protective effect against lithium-induced moderate renal failure in a rat model. NAC-treated rats had significantly higher GFRs and significantly less distal tubular necrosis and obstruction compared with the untreated group.

Lithium-induced renal failure is a slowly progressive disease and its rate of progression is related to both duration and cumulative dose of the drug [4,5]. In this study, we used a rat model of 5 weeks gradual exposure to lithium (3 weeks treatment during a 5 week protocol), resulting in ~60% reduction in the mean GFR. Three weeks of exposure to lithium, which is 5–10% of the life expectancy in Sprague–Dawley rats, may resemble 4–8 years of lithium therapy in humans. The wide use of lithium has clearly demonstrated antisuicide effects [10]. Unfortunately, prolonged lithium administration is often detrimental to renal tissue [1–5]. The wide use of lithium therapy, taken together with the fact that renal function may continue to deteriorate even after discontinuing lithium treatment [5], emphasizes the need for a protective strategy against its toxicity. The results of the present study demonstrated that concomitant administration of NAC has a protective effect against lithium-induced moderate renal failure in a rat model. NAC-treated rats had significantly higher GFRs and significantly less distal tubular necrosis and obstruction compared with the untreated group.

Light microscopy evaluation of kidney tissue samples from the control as well as from the NAC-treated groups at the end of the lithium treatment protocol revealed glomeruli with normal appearance of the basement membrane and without signs of cellular proliferation (Figure 2a and b). However, differences in glomerular diameter were detected in the control group. Some of them were shorter while others were prolonged. These differences were not observed in the NAC-treated group.

Tubular damage was demonstrated in both groups. In the control group, tubules were dilated and patchy swelling of tubular cells was evident. In some of the cells, karyolysis was present and cells were vacuolated. More prominent damage was demonstrated in the distal than in the proximal tubules. Tubular lumen obstruction by broad granular casts and cell fragments was present in 58.9 ± 21.0% of counted tubules (Figure 2a, Table 1). In the NAC-treated group, there were significantly fewer tubular dilatations, as well as swelling of tubular cells (Figure 2b). Obstruction of the tubular lumen of the NAC group was noted in 36.5 ± 9.5%, significantly less than in the control group, P = 0.007 (Table 1). Similarly, the percentage of necrotizing tubular cells was significantly lower in the NAC-treated group. Thus, in the control group, 71.4 ± 20.5% of tubular cells were damaged, while in the NAC-treated group only 44.2 ± 10.3% revealed a similar injury, P = 0.002 (Table 1).
lithium protocol and 0.4 ± 0.19 ml/min/100 g 8 weeks after the end of the lithium protocol). However, according to the histopathological evaluation performed in week 6 of the experiment, 1 week after ending lithium exposure, no visible evidence for chronic renal damage was found (the observed lesions represented tubular necrosis and obstruction, rather than interstitial fibrosis), implicating acute rather than chronic renal failure as the primary mechanism.

Although the predominant form of renal disease associated with lithium therapy is tubulointerstitial nephropathy, electron microscope examinations have demonstrated severe lesions in the mitochondria and endoplasmic reticulum in renal tissue of patients with

Fig. 2. Light microscopy after exposure to lithium in the control (a) and in the N-acetylcysteine-treated groups (b). Light microscopy reveals glomeruli with normal appearing basement membrane and no signs of cellular proliferation in both groups. (a) Diffuse tubular necrosis and several dilated tubuli with intraluminal tubular cells fragments. (b) Normal tubular cells and tubules.
lithium nephrotoxicity, implicating ischaemia as one of the pathogenetic mechanisms [3,5]. Under such circumstances, any other co-morbid conditions such as diabetes or a decrease in renal blood flow may result in a further reduction in tissue oxygenation and augment lithium-mediated toxicity.

Local nitric oxide (NO) production plays a major role in the maintenance of adequate blood supply to the renal medulla. Previous animal [11] and human [12,13] studies have demonstrated that NAC administration can increase NO-mediated vasodilation. In agreement with this concept of NAC as a renal vasodilator, recent studies on the protective effects of NAC in the context of contrast media toxicity have demonstrated a transient increase in GFR, paralleled by a decrease in serum creatinine in NAC-pre-treated patients [8]. The possible underlying mechanisms may be an increase in NO production by NAC [8], and amplification of NO’s vasodilatory effect by stimulation of calcitonin gene-related peptide release, the principal transmitter in capsaicin-sensitive sensory nerves that is widely distributed in vascular tissues [14]. Another hallmark of renal vasoconstriction is increased oxidative stress, being both the cause and the result of ischaemic insults. Increased local production of superoxide results in a decrease of NO bioavailability (from the reaction of NO with superoxide) and the formation of peroxynitrite, a cytotoxic oxidant on its own, causing further worsening of ischaemia [15]. By interfering with peroxynitrite-related pathways, NAC has been shown to play a protective role in models of gentamicin- and cisplatin-mediated nephropathies as well as in ischaemia/ reperfusion renal injuries [6,7]. Recently, Heyman et al. have demonstrated that NAC can also ameliorate renal vasoconstriction by mechanisms other than prostaglandins or NO [16]. In addition to NAC’s ability to preserve the microcirculation, exposure to NAC can improve mitochondrial function by lowering the concentration of reactive oxygen species [17]. The ability of NAC to improve medullary ischaemia and mitochondrial function may be responsible for its protective effect in the rat model of lithium-induced renal failure.

Nephrogenic diabetes insipidus, the most common form of lithium-induced renal injury, results from intracellular lithium accumulation [18,19]. The potassium-sparing diuretic, amiloride, can improve and possibly prevent lithium-induced nephrogenic diabetes insipidus [20]. Amiloride blocks some of the sodium channels in the collecting duct cells and therefore minimizes further local lithium accumulation in patients with prolonged lithium therapy. In this study, lithium induced a significant decrease in urine osmolarity in both groups. However, at the end of the lithium treatment protocol, urine osmolarity in the NAC-treated group did not differ from that of the control group, apparently indicating that NAC does not exert its renoprotective effect via interference with lithium accumulation in collecting ducts.

In conclusion, co-administration of NAC during lithium chloride ‘therapy’ has a significant renoprotective effect in a rat model of lithium-induced renal failure. If relevant in humans, this finding may be of a major importance, introducing for the first time a safe, inexpensive and feasible method for attenuation of lithium-induced nephrotoxicity.

Conflict of interest statement. None declared.

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Received for publication: 16.6.04
Accepted in revised form: 6.10.04