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ORIGINAL INVESTIGATION

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Prevention of lithium nephrotoxicity in a novel one-hour model in rats

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Abstract It is well established that lithium can cause morphologically visible damage to the kidneys of humans and animals. Although the clinical significance of its nephrotoxicity is debatable, it would be desirable to find a method to prevent lithium's effect on the kidneys. Toward this end, we have developed a novel method for producing nephrotoxicity that will be useful for research on prevention. A single, large, toxic dose of lithium chloride (LiCl) caused necrosis of the distal convoluted tubules, which was visible by light microscopy in 30 min, had fully developed in 1 h, and had disappeared by the next day. The lesions were seen after IP or IV injections of fasted rats of three different strains. Equivalent doses of NaCl, KCl, $MgCl_2$ and combinations thereof had no such effect, nor did they inhibit nephrotoxicity when incorporated into the LiCl solution. However, relatively small doses of LiCl injected by any route 3 or 24 h beforehand prevented the nephrotoxicity. The mechanism of prevention is not known, but it does not involve reduction of lithium levels in the kidneys.

Key words Lithium · Nephrotoxicity · Rat

Introduction

Significant nephrotoxicity from lithium treatment of bipolar disorders is rare (Schou 1988), but lesser aberrations of renal function are more common (Chan et al. 1981; Walker 1993). In addition, kidney biopsy studies have shown morphologic lesions (Hestbech et al. 1977; Kincaid-Smith et al. 1979; Aurell et al. 1981; Walker 1993). Animal studies have uniformly shown morphologic damage, but multiple doses, usually over several weeks, have been administered (Table 1 and Myers et al. 1980). The experiments described heretofore have been somewhat cumbersome for research aimed at prevention of morphologic damage to kidney, and few such studies have been reported (Olesen et al. 1980). We have resorted to the use of single, large, toxic doses of lithium and found morphologic evidence of nephrotoxicity in 30 or 60 min. The site of injury, distal convoluted tubule (DCT), in this immediate nephrotoxicity is the same as the site in the much more protracted experiments reported previously. Although large, toxic doses are, of themselves, not clinically significant, we suggest that our model is eminently suited for experiments on prevention of nephrotoxicity. Further work will be needed to determine if prevention of toxicity in our acute model can be applied to subacute and chronic toxicity.

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Materials and methods

Inbred Lewis and outbred Sprague-Dawley (SD) rats were bred in this Institute and maintained on Laboratory Rodent Diet 5001 (PMI Feeds, St Louis, Mo., USA) with tap water freely available except as indicated below. Female Long-Evans rats were purchased from Harlan Sprague-Dawley Co., Indianapolis, Ind., USA. Institutional principles of laboratory animal care were followed. Lithium chloride (LiCl) dissolved in distilled water, either 0.5 M (hypertonic) or 0.15 M (isotonic), was injected intraperitoneally (IP), intravenously (IV), subcutaneously (SC) or orally by gavage tube (PO). Exactly 1 h later, blood for analysis was obtained from the vena cava and the rats were exsanguinated under CO_2 anesthesia. Both kidneys were fixed in Bouin's fluid. A cross-section through the medullary pyramid was embedded in paraffin, sectioned and stained with hematoxylin-eosin and periodic acid-Schiff-hematoxylin (PAS). Damage to the distal convoluted tubules was scored on randomized slides without knowledge of the treatment, as follows: 2+, necrotic tubules in many areas of the subcapsular renal cortex, seen at ×100 magnification; 1+, only a few necrotic cells or tubules, scattered, requiring a short search and sometimes requiring higher magnification. Serum lithium was determined by atomic absorption spectroscopy, as described (Levine et al. 1993). In some experiments,

Table 1 Morphologic literature on nephrotoxicity produced by lithium in animals[a]

Authors and year	Li Rx	Duration	Modalities[b]	Site[c]	Notes
Radomski et al. (1950)	Oral	3–21 weeks	LM	DC	Dogs
Schou (1958)	IP	10–14 days	LM	P	
Evan and Ollerich (1972)	IP	12–60 days	EM	DC	
Evan (1973)	IP	12–60 days	EM	HDC	
Nielsen (1975)	?	1 month	EM	HDC	Dogs
Hestbech et al. (1978)	Food	3 or 9 weeks	LM	DCI	
Lim et al. (1979)	Oral	5–15 days	EM	GPDC	
Olesen et al. (1980)	Food	3–12 weeks	LM	DCI	
Easley (1981)	IP	3 days	LM,EM	DC	Dogs
Christensen et al. (1981)	Food	8 weeks	LM	DC	
Plenge et al. (1981)	Food or IP	5 months	LM	DC	
Jacobsen et al. (1982)	Food	3–21 days	LM,HC	DC	
Christensen et al. (1982)	Nursing[d]	8 or 16 weeks	LM	GPDI	Newborn
Nagi et al. (1982)	IP	8 weeks	LM,EM	DC	
McAuliffe and Olesen (1983)	Food	7 weeks	LM,EM	GPD	
Kling et al. (1984)	Food	3–18 weeks	LM, ^{3}H	DC	
Solez et al. (1984)	Food	16 weeks	LM, SC	DCI	
Ottosen et al. (1987)	Food	1–4 weeks	EM,Morph	C	
Ottosen et al. (1988)	Food	3 weeks	LM,HC,Morph	DC	
Dorup et al. (1988)	Nursing[d]	8 weeks	LM,EM,Morph	DC	Newborn
Marcussen et al. (1989,1990,1991)	Nursing[d]	8 or 16 weeks	LM,EM,Morph	GPDI	Newborn
Christensen et al. (1997)	Nursing[d]	16 weeks	LM,Morph	GPDCI	Newborn
Levine et al. this paper	IP,IV	1 h	LM	DC	

[a]Rats, except as specified
[b]*LM*, light microscopy; *EM*, electron microscopy; *HC*, histochemistry; *Morph*, morphometry; ^3H, tritiated thymidine uptake; *SC*, scanning electron microscopy
[c]Site of lesion or site of principal study: *G*, glomeruli; *P*, proximal tubule; *H*, loop of Henle; *D*, distal convoluted tubule; *C*, collecting tubule; *I*, interstitial tissue
[d]Lithium in food after weaning

one kidney in entirety, but decapsulated, was homogenized in 0.1 N HCl, centrifuged, and the clear supernate was analyzed for lithium by the same method.

Results

Female SD rats fasted for 2 days and then injected IP with 1.0 mEq/100 g LiCl appeared slightly sedated. One hour later, serum Li levels were 10–15 mEq/l, as reported previously (Ho et al. 1970). Kidneys were often tan in color. Microscopically, the outer cortex had normal glomeruli and proximal convoluted tubules with intact brush borders. In contrast, many of the DCT were partly or completely lined by necrotic cells (Figs 1,2). Many of the necrotic cells had lost cytoplasm into the lumen, and remaining cytoplasm appeared fuzzy and irregular at the lumenal surface. Nuclei were pyknotic and many had been shed into the lumen. Frequently, necrotic cells alternated with intact cells in the same tubule. No necrotic cells were seen in the inner cortex, where the pars recta of the proximal tubules predominate, or in the inner medulla, where the thin limbs of Henle's loop and the collecting tubules predominate, and there were only a few scattered pyknotic cells in the outer medulla, where the thick limbs of Henle's loop predominate. The PAS stain did not add any further information. Rats studied only 30 min after LiCl injection had fewer DCT with lesions and fewer necrotic cells in damaged tubules. DCT lesions 2 h after LiCl were similar or less than those described after 1 h. They were much less after 5 h and were no longer present after 24 h.

Important differences in occurrence of nephrotoxicity were found depending on the duration of prior fasting (Table 2). Abundant lesions were found 1 h after LiCl in both Sprague-Dawley and Lewis rats after 2 days of fasting (2+ scores). After a 1-day fast, only the SD females had abundant lesions. Without any prior fast, neither strain had abundant lesions. Both IV and IP routes for injecting LiCl were effective.

Female Long-Evans rats had the same response as SD rats to various doses of LiCl after fasting for 2 days in both strains. Tonicity of the inoculum did not determine the results. One mEq/100 g LiCl produced severe necrosis of DCT, whether injected IP as a hypertonic solution (0.5 M, 2 ml/100 g body weight) or an isotonic solution (0.15 M, 6.5 ml/100 g). Half of that dose produced milder necrosis of DCT, whether injected as a hypertonic or isotonic solution. One quarter of that dose produced only minimal lesions of DCT (four rats each group).

The specificity of the nephrotoxic reaction to LiCl was tested by study of rats (fasted for 2 days) 1 h after IP injections of other electrolytes. No renal lesions were produced by 1 mEq/100 g NaCl or KCl or by the same

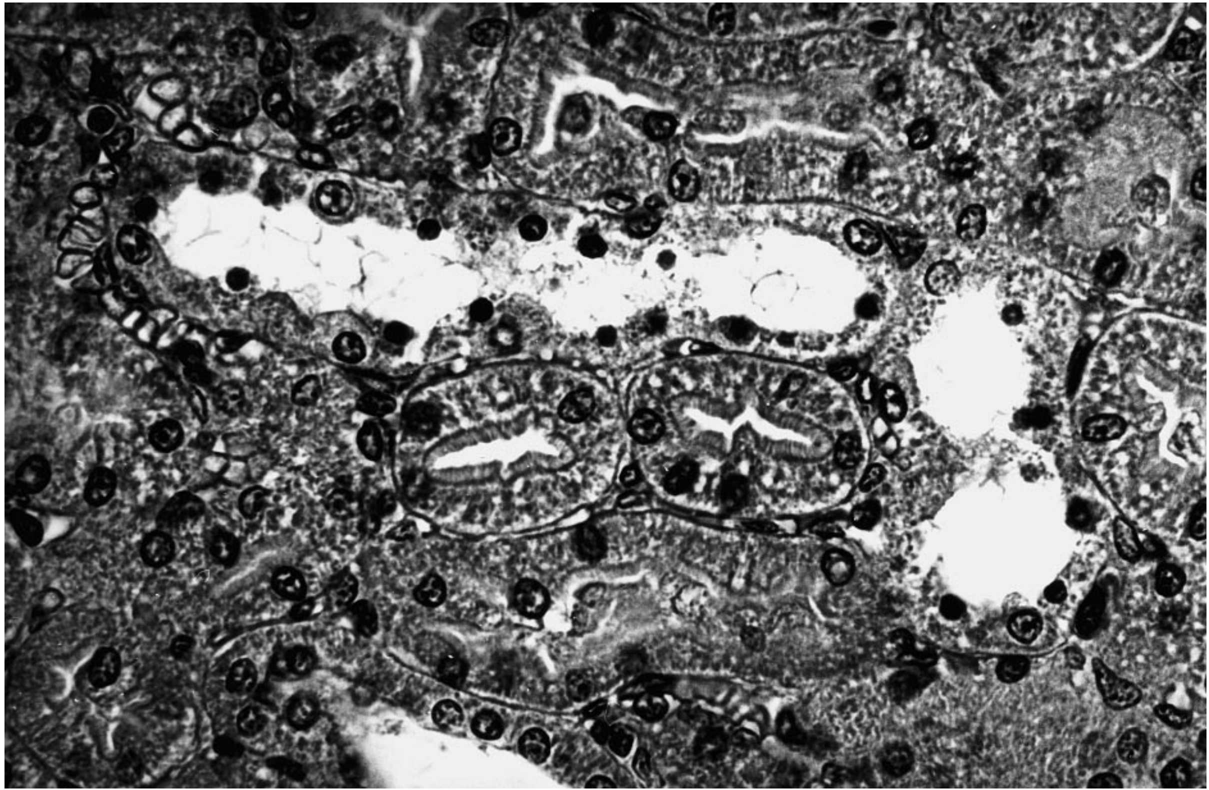

Fig. 1 The distal convoluted tubule (crossing the field just above center and turning downward on the right) exhibits severe pyknosis and shrinkage of most nuclei, pallor and disintegration of cytoplasm, and desquamation of both nuclei and cytoplasmic fragments into the lumen. A few viable cells remain. Most of the other tubules are from the proximal convolution; nuclei and cytoplasm are intact and brush borders are easily identified, especially in the two tubules in the center. One hour after LiCl injection. Hematoxylin and eosin, ×400

amounts of NaCl and KCl combined in the same volume of inoculum. Nor did NaCl, KCl or the combination of chlorides inhibit LiCl nephrotoxicity when administered together with lithium in the same doses and volume. $MgCl_2$, 1 mEq/100 g, was tested by the SC route with the same lack of effect. Urea and sucrose, examples of non-electrolytes, were tested at 1 mEq/100 g by the IV route and no renal damage was found at 1 h.

Prevention of acute DCT necrosis

Acute DCT necrosis produced in 1 h by a single large dose of LiCl was prevented by pretreatment with the same chemical. A relatively small preliminary dose (0.25 mEq/100 g) was effective when administered 3 or 24 h before the nephrotoxic dose, but doses at shorter or longer intervals were not effective (Table 3).

The dose and route needed for prevention when injected 1 day before the nephrotoxic treatment was explored in detail (Table 4). LiCl prevented nephrotoxicity regardless of the route, and the minimal effective dose only varied from 0.08 to 0.30 mEq/100 g. The preventive dose did not have to be given by the same route as the nephrotoxic dose.

In the experiments on prevention of DCT necrosis, the left kidney was assayed for lithium content. There was no correlation between DCT necrosis and lithium concentration which varied from 14.8 to 17.8 mEq/kg renal tissue (Table 3). Therefore, the decrease of DCT necrosis by LiCl pretreatment had not been accomplished by reducing the lithium content of the kidney after challenge. Also, serum was assayed for lithium in all the prevention experiments of Table 4 and there was no correlation between serum levels of lithium and DCT necrosis.

Discussion

Despite many years of intensive research on lithium and its effect on renal function and structure (Table 1), the immediate, severe necrosis of DCT has not been described previously. Previous investigators have injected low doses of lithium repeatedly in order to mimic the clinical situation. In contrast, we have used a single large, toxic dose in order to have a simple model with an obvious endpoint that would identify the target and facilitate research on prevention. Furthermore, we studied the effects of lithium early, before

Fig. 2 The distal tubule in the center has ten viable nuclei and only one pyknotic, necrotic nucleus. In contrast, the other distal tubules (*left*, *right*, and *right upper*) have far more pyknotic than viable nuclei. A proximal tubule and part of a glomerulus (*middle of bottom*) are intact. Hematoxylin and eosin, ×400

Table 2 Effects of fasting on nephrotoxicity of LiCl

Rat	Fast[a]	Necrosis score[b]
Sprague-Dawley female	2 days	2,2,2,2
	1 day	2,2,2,2
	None	0,0,0,0
Lewis female	2 days	2,2,2,2
	1 day	0,0,0,0
	None	0,0,0,0
Sprague-Dawley male	2 days	2,2,2,2,1
	1 day	1,1,1,1
	None	1,1
Lewis male	2 days	2,2,1,1
	1 day	0,0
	None	0,0

[a]Water freely available
[b]LiCl challenge, 1.0 mEq/100 g. Kidneys removed 1 h later. Histologic scores of necrosis in distal convoluted tubules recorded individually for each rat

Table 3 Timing of LiCl pretreatment to prevent nephrotoxicity

LiCl pretreatment IP		LiCl challenge IP	
Time	Dose mEq/100 g	DCT necrosis[a]	Lithium in kidney mEq/kg
−3 min	Sham	2,2	16.2,15.2
−3 min	0.25	2,2	16.3,15.4
−30 min	0.25	2,2	17.8,17.1
−1 h	0.25	2,2	16.7,16.4
−3 h	0.25	1,1	16.4,15.0
−24 h	0.25	1,0	15.6,14.8
−5 days	Sham	2,2	Not done
−5 days	1.0	2,2	
−8 days	Sham	2,2	Not done
−8 days	0.5	2,2	
−8 days	1.0	2,2	

[a]LiCl challenge, 1.0 mEq/100 g IP in female Sprague-Dawley rats. Kidneys removed 1 hour later. Histologic scores of necrosis in distal convoluted tubules (DCT) recorded individually for pairs of rats. Rats fasted 2 days before challenge

debridement and regeneration could conceal the stigma of nephrotoxicity. Amdisen (1979) has noted that the glomerular ultrafiltrate becomes increasingly concentrated as it passes down the nephron because 99% of the water is reabsorbed compared to only 80% of the lithium, leading to a distal intratubular concentration of 60 mmol/l or more. Such a high concentration of a foreign cation is likely to be toxic, especially if some reabsorption occurs in the DCT and causes high intracellular levels of lithium (Thomsen 1978; Thomsen et al. 1993). Toxic doses of lithium reduced the glomerular filtration rate (Thomsen 1978), which might augment nephrotoxicity by local and systemic mechanisms. In addition, it has been suggested that kidney

Table 4 Dose and route of LiCl pretreatment to prevent nephrotoxicity

LiCl pretreatment[a]		LiCl challenge IP[b]
Route	Dose mEq/100 g	DCT necrosis
IP	1.00	0,0
	0.30	0,0
	0.23	0,0
	0.15	2,1
	0.08	2,2
	Controls	2,2,2,2
IV	0.3	0,0
	0.23	0,0
	0.15	0,0
	0.08	1,1
	Controls	2,2
SC	0.60	0,0
	0.45	0,0
	0.30	1,0
	0.15	2,1
	Controls	2,2,2,1
PO	1.00	0,0
	0.90	0,0
	0.75	0,0
	0.60	0,0
	0.45	0,0
	0.30	1,1
	0.15	2,2
	Controls	2,2,2,2

[a]LiCl pretreatment 24 h before challenge. Controls given saline or sham inoculation
[b]LiCl challenge, 1.0 mEq/100 g IP in Sprague-Dawley female rats. Kidneys removed 1 h later. Rats fasted 2 days before challenge
Histologic scores of necrosis in distal convoluted tubules (DCT) recorded individually for pairs of rats

cells may be intrinsically more susceptible to lithium's toxicity than other cells (Matthopoulos et al. 1995). The rapid disappearance of the lesions is explained by desquamation of the necrotic cells into the urinary stream, and restoration of the tubular lining by the remaining cells. The completeness of this restoration will have to await ultrastructural studies that have been started. The absence of visible light microscopic changes cannot always be used as an indicator of normal kidney function.

Necrotic lesions of DCT were favored by fasting prior to lithium administration (Tables 2 and 3). Fasting or a diet low in Na and/or K reduces sodium excretion in the urine of rats (Boim and Schor 1992; Thomsen et al. 1993). Inasmuch as Li and Na compete for reabsorption in the renal proximal tubules, the decrease of Na caused by fasting allows more Li to be reabsorbed, thereby maintaining high serum levels and contributing to nephrotoxicity.

Tubular necrosis occurring in a mere 1 h after injection of a nephrotoxic agent has been reported in other systems (Dunn and Polson 1926; Preuss et al. 1975). It is of some interest that one of these reports, although entitled "Experimental uric acid nephritis" (Dunn and Polson 1926), actually involved the injection of lithium urate, and the damage was in the distal portions of the nephron. A role for lithium was not discussed but, in a later study on lithium urate, a nephrotoxic role for the lithium component was considered and rejected (Smith and Lee 1957). In light of our findings, this rejection should be re-investigated. By coincidence, it was lithium urate that Cade used to make his seminal discovery of the effect of lithium on manic patients (Copolov 1994).

The mechanism by which pretreatment with lithium prevents nephrotoxicity from a subsequent dose of the same reagent is currently under investigation. The lack of correlation between DCT necrosis and lithium levels in the kidney shows that prevention is not caused by altering the absorption of the challenge dose of lithium or its transport to the kidney. However, alternations of lithium levels in the DCT itself could not be detected by our assays of whole kidneys. A few hours are required for the resistant state to develop, and within a few days the susceptible state has returned. One possibility is the transient synthesis of a protective material like heat-shock protein or metallothionein in the DCT cells themselves. However, extrarenal factors (humoral, neural, vascular) could be responsible.

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