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ORIGINAL ARTICLE

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Relationship between serum lithium concentration and kidney damage in a preclinical model

Georgina P. Ossani[1,2,3] | Ana M. Uceda[1,2] | Néstor R. Lago[1] | Diego J. Martino[3,4]

[1]Centre of Experimental and Applied Pathology, Department of Pathology, School of Medicine, University of Buenos Aires, Buenos Aires, Argentina

[2]Laboratory of Experimental Medicine, Hospital Alemán, Buenos Aires, Argentina

[3]National Council of Scientific and Technical Research (CONICET), Buenos Aires, Argentina

[4]Institute of Cognitive and Translational Neuroscience (INCyT), INECO Foundation, Favaloro University, Buenos Aires, Argentina

Correspondence
Georgina P. Ossani, J.E. Uriburu 950, 5th floor, CABA, Argentina.
Email: georginaossani@gmail.com

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Abstract

Objectives: The aim of the present study was to assess whether there is a relationship between serum lithium concentrations and the magnitude of kidney damage in a preclinical model.

Methods: Thirty Wistar male rats were randomized into three groups: control group fed ad libitum powered standard diet for 3 months; and experimental groups fed ad libitum the same diet supplemented with 30 or 60 mmol/kg diet for 3 months (LowLi and HighLi groups respectively). Laboratory parameters were assessed at months 1 and 3 and histopathological changes were evaluated after 3 months.

Results: Serum lithium levels in experimental rats were within therapeutic range used in humans throughout the entire experiment. After 3 months of treatment, lithium levels were statistically higher in HighLi group. Rats of the LowLi group showed dilation of cortical tubules although with similar clearance of creatinine. Rats from the HighLi group had greater histopathological damage in addition to lower creatinine clearance than the other two groups.

Conclusions: Our study suggests that during long-term treatments, even with serum lithium levels within the therapeutic range used in humans, the risk of kidney damage could increase proportionally to the serum lithium concentration.

KEYWORDS
chronic kidney disease, kidney damage, lithium, side effect

1 | INTRODUCTION

Despite several decades of use, lithium remained the "gold standard" in the long-term prophylactic treatment of bipolar disorder (BD).[1] One disturbing adverse effect associated with the prolonged use of lithium is the development of chronic kidney disease (CKD), which may be less infrequent than was commonly thought. In fact, stage 3 of CKD (defined as a decrease in the estimated glomerular filtration rate—eGFR—below 60 mL/min/1.73 m^2) could be observed in up to 20%-40% of patients under long-term treatment with lithium, a percentage that could be higher even among older adults.[2-4] Although the development of CKD does not always imply serious clinical concerns, between 1% and 2% of patients could progress to an end stage renal disease (or CKD stage 5) requiring dialysis or kidney transplantation.[5,6]

In this context, knowledge of the risk factors for the development of CKD associated with long-term lithium treatment is challenging, and could contribute to a more rational use of this therapeutic agent. There is some convergent evidence that the risk of developing CKD could be associated with the duration of treatment (often more than a decade), old age, prior episodes of lithium toxicity, medical comorbidities (ie, diabetes or hypertension), and the concomitant use of other medications with nephrotoxic potential (such as angiotensin converting enzyme inhibitors or non-steroidal anti-inflammatory agents).[7] On the contrary, the risk associated with serum lithium concentrations—when they are within the therapeutic

range—is controversial. Some studies suggested that serum lithium levels were not associated with decline in eGFR,[8,9] consistent with a report that showed that lithium treated patients with and without CKD did not significantly differ in their lithium levels (0.58 vs 0.59 mmol/L).[10] In contrast, in a retrospective analysis of laboratory data, median serum lithium concentrations higher than the overall median (0.60 mmol/L) were associated with an eGFR < 60 mL/min/1.73 m^2.[11] Similarly, a recent large multisite study found that a higher serum lithium concentration was an independent predictor of CKD stage 3.[12] In addition, some preclinical models seem to show that kidney damage (measured as an increase in creatinine or histopathological changes) is dose-dependent.[13-15] However, these studies did not have serial measurements of serum levels, so the results could be confused by episodes of lithium intoxication. To date no studies have been conducted in animal models specifically designed to evaluate the relationship between serum lithium levels and the risk of kidney damage. Then, the aim of the present study was to assess whether there is a relationship between serum lithium concentrations and the magnitude of kidney damage in a preclinical model.

2 | MATERIALS AND METHODS

2.1 | Animals

Thirty *Wistar* male rats (180-220 g) from the Laboratory Animal Facility, Faculty of Pharmacy and Biochemistry, University of Buenos Aires were randomized into three groups: control group fed ad libitum powered standard diet (GEPSA) for 3 months; and two experimental groups fed ad libitum the same diet supplemented with 30 (LowLi group) or 60 (HighLi group) mmol of lithium/kg diet for 3 months. Based on literature, 3 months of treatment with a drug in rats is a period of chronic exposure.[16,17] All groups had free access to tap water and the experimental groups received additionally physiological solution to maintain sodium balance and to avoid lithium intoxication. Animals were individually housed in suspended wire-bottomed cages with a photoperiod of 12 hours light-12 hours dark (lights on from 07:00AM to 07:00PM) and a temperature of 20 ± 2°C. Rats were introduced into metabolic cages and urine was collected for 24 hours after 1 and 3 months of experiment. Blood samples were collected from caudal artery after 1 month and from abdominal aorta at the end of the experiment. The animals were anesthetized with ketamine/xilazine (50/10 mg/kg body weight). The kidneys were removed for histopathological analysis. Authors have adhered to appropriate NIH Guide for the Care and Use of Laboratory Animals. Housing, handling, and experimental procedures were approved by the Laboratory Animal Use and Care Committee of the School of Medicine of the University of Buenos Aires (Protocol number: 2366/2017).

2.2 | Serum and urine analysis

Creatinine concentrations in urine and serum were measured by the Jaffe´s kinetic method. Lithium concentration in serum was determined by direct potentiometry. Creatinine clearance was calculated by the standard formula. All determinations were assessed after 1 and 3 months of experiment.

2.3 | Histopathology and immunohistochemistry

Left kidney was fixed in buffered-formalin and embedded in paraffin. In order to analyze histopathological alterations 3 μm sections were cut and stained with hematoxylin and eosin and PAS, using conventional protocols. Regions of interest were examined on a Nikon microscope and photographed with a digital camera. Conventional protocols of immunohistochemistry were used for labeling Aquaporine-2 (Santa Cruz, sc-28629, Lot#K2408, rabbit polyclonal).

2.4 | Statistics

The normality of the variables was studied by graphic (Q-Q Plot, Box-Plot) and analytic methods (Kolmogorov-Smirnov). Since variables had not normal distribution, they were compared by the Kruskal-Wallis and Mann-Whitney tests. Values of $P < .05$ were considered statistically significant. Despite the asymmetric distribution of variables, results are also expressed as mean and standard deviation to improve understanding.

3 | RESULTS

3.1 | Serum and urine analysis

Serum lithium levels were within therapeutic range used in humans in experimental rats throughout the entire experiment. After 1 month, there was no statistical difference between both experimental groups but after 3 months of treatment, lithium levels were statistically higher in HighLi group (Table 1). Creatinine levels were higher in the HighLi group compared to controls after 3 months. Creatinine clearance was lower in HighLi group compared to control and LowLi groups reaching significance after 3 months of experiment (Table 1 and Figure 1).

3.2 | Histopathology and immunohistochemistry

Both experimental groups showed damage of the cortical collecting tubules, evidenced as dilatation, hypertrophy, and hyperplasia of the lining epithelium compared to controls. However, the magnitude of the histopathological damage was greater in the HighLi group compared to the LowLi group, with more evident cellular changes and dilatation (Figure 2A-C). AQP2 immunostaining allowed confirming that the injured tubules were cortical collecting tubules (Figure 2D).

4 | DISCUSSION

The main objective of this study was to evaluate the role of serum lithium concentration in the risk of developing kidney damage. Unlike previous preclinical studies,[13-15] we took special care that serum lithium levels of both experimental groups were within the

TABLE 1 Laboratory determinations

	Control (1) Mean(SD)/median(range)	LowLi group(2) Mean(SD)/median(Range)	HighLi group(3) Mean(SD)/median(range)	Kruskal-Wallis	Mann-Whitney
Creatinine (mg/dL)					
1 month	0.34(0.04)/0.33(0.15)	0.39(0.05)/0.38(0.18)	0.39(0.12)/0.35(0.44)	$\chi^2 = 4.642$; $P = .098$	
3 months	0.41(0.16)/0.37(0.54)	0.42(0.06)/0.41(0.16)	0.49(0.12)/0.50(0.40)	$\chi^2 = 6.416$; $P = .04$	1 vs 2 Z=−1.82; $P = .068$ 1 vs 3 Z=−1.970; $P = .049$ 2 vs 3 Z=−1.783; $P = .075$
Lithium (mmol/L)					
1 month		0.50(0.05)/0.50(0.20)	0.69(0.30)/0.55(0.80)	$\chi^2 = 21.835$; $P < .001$	1 vs 2 Z=−4.192; $P < .001$ 1 vs 3 Z=−4.052; $P < .001$ 2 vs 3 Z=−1.245; $P = .213$
3 months		0.39(0.01)/0.40(0.05)	0.71(0.13)/0.70(0.40)	$\chi^2 = 27.632$; $P < .001$	1 vs 2 Z=−4.264; $P < .001$ 1 vs 3 Z=−4.059; $P < .001$ 2 vs 3 Z=−3.982; $P < .001$
Creatinine clearence					
1 month	2.23(0.64)/2.28(2.22)	2.33(0.51)/2.29(1.40)	1.90(0.66)/1.85(1.92)	$\chi^2 = 2.023$; $P = .364$	
3 months	2.77(0.49)/2.77(1.53)	2.69(0.45)/2.51(1.28)	1.64(0.68)/1.58(2.55)	$\chi^2 = 13.692$; $P = .001$	1 vs 2 Z=−0.227; $P = .82$ 1 vs 3 Z=−3.180; $P = .001$ 2 vs 3 Z=−3.175; $P = .001$

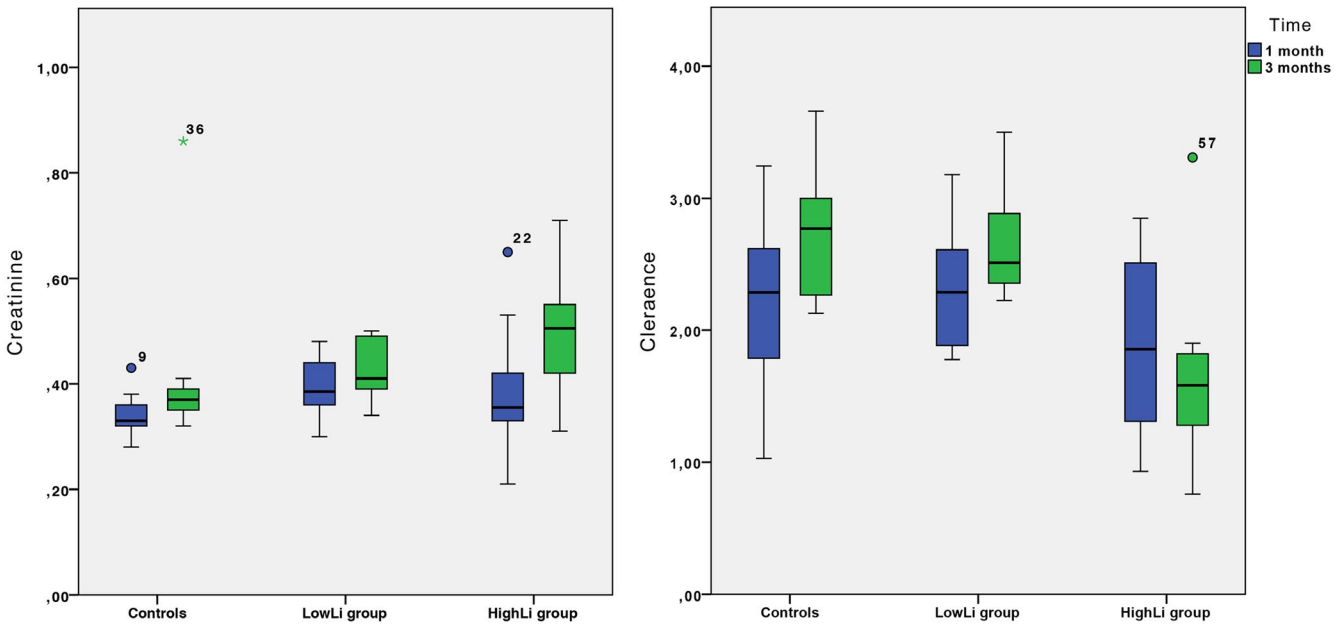

FIGURE 1 Change over time between the three experimental groups (controls, low, and high lithium levels) in serum creatinine (mg/dL) and clearance of creatinine (mL/min)

therapeutic range used in humans to avoid the potential confounding effect of episodes of lithium intoxication.

LowLi group did not show differences regarding controls on serum creatinine levels or creatinine clearance; however, this experimental group showed dilatation of the cortical collecting tubules with minimal changes in the tubular cells. These findings highlight that some histopathological damage could occur even in the absence of alterations in the analytical measures of habitual use for the monitoring of lithium treatment, standing out the importance of developing early biomarkers of renal injury.[18]

In contrast, although compared to controls, HighLi group did not show differences in laboratory data during the first month of treatment, they had higher values of serum creatinine and decreased creatinine clearance at 3 months. This finding suggests a time-dependent effect of lithium treatment on the development of renal damage, which agrees with previous data from preclinical and clinical

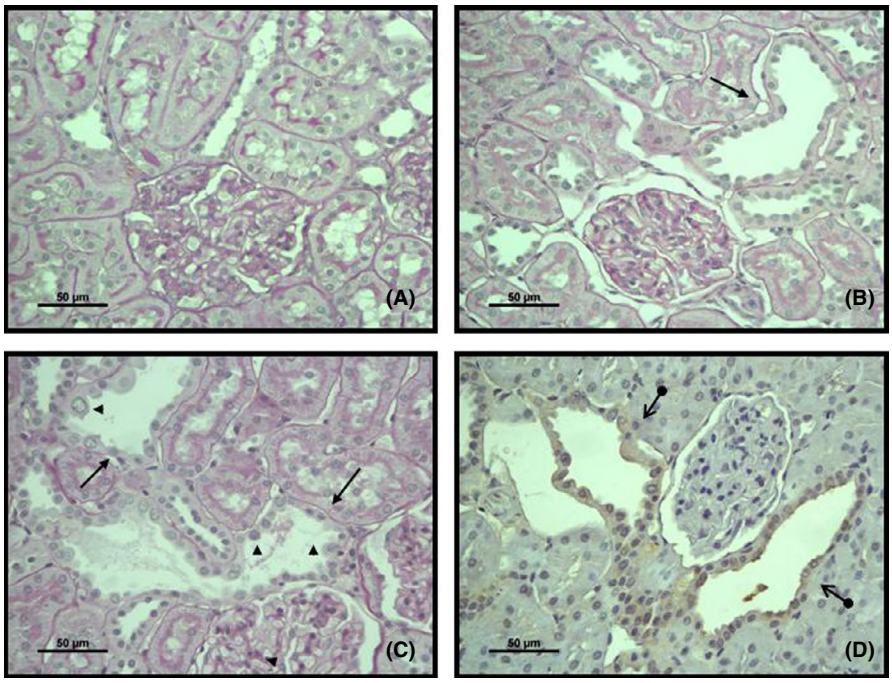

FIGURE 2 **A**, Control, PAS (400×); (**B**) LowLi group 3 months, PAS (400×), arrow indicates dilatation of the cortical collecting tubules; (**C**) HighLi group 3 months, PAS (400×), arrows indicate dilatation of the cortical collecting tubules and arrowheads show cellular changes of hypertrophy and hyperplasia; (**D**) HighLi group 3 months, AQP-2 (400×), arrows indicate positive staining in cortical collecting tubules

studies.[7,19] In addition, compared with the LowLi group, the HighLi group had a lower creatinine clearance and more histopathological damage characterized by greater dilatation of the cortical collecting tubules, hyperplasia, and hypertrophy with the presence of binucleated cells and macronuclei and focal tubular atrophy.

To the best of our knowledge this is the first study to show both analytically and histopathologically the fact that, even within the therapeutic ranges usually used in humans, the risk of kidney damage might increase proportionally to the serum lithium concentration. These findings agree with those of some recent clinical studies that reported that higher serum lithium concentrations were associated with an eGFR < 60 mL/min/1.73 m^2.[11,12] Taken together, these confluent results from preclinical and clinical studies could suggest that patients under long-term lithium therapy could reduce the risk of CKD as long as the lowest possible therapeutic serum concentrations are used. Future studies could use more stratified samples in order to assess whether the increased risk of developing CKD occurs throughout the entire therapeutic range of serum lithium concentration. In any case, periodic monitoring of serum lithium levels and renal function through eGFR are mandatory during lithium maintenance treatment.[20] A recent review from the ISBD/IGSLI Task Force on treatment with lithium recommended for adults that the standard lithium serum level should be 0.60-0.80 mmol/L with the option to reduce it to 0.40-0.60 mmol/L in case of good response but poor tolerance.[21] If the CKD associated with chronic lithium treatment can be stopped or even reversed by lowering serum levels is an issue to be elucidated in future studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ORCID

Georgina P. Ossani https://orcid.org/0000-0001-6478-9539
Diego J. Martino https://orcid.org/0000-0002-8608-9346

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