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Abstract

Lithium induces nephrogenic diabetes insipidus (NDI) and microcystic chronic kidney disease (CKD). As previous clinical studies suggest that NDI is dose-dependent and CKD is time-dependent, we investigated the effect of low exposition to lithium in a long-term experimental rat model. Rats were fed with a normal diet (control group), or with the addition of lithium (Li^+ group), or with lithium and amiloride (Li^+/Ami group) during 6 months, allowing obtaining low plasma lithium concentrations (0.25±0.06 and 0.43±0.16 mmol/l respectively). Exposition to low concentrations of plasma lithium levels prevented NDI but not microcystic dilations of kidney tubules, that were identified as collecting ducts (CD) on immunofluorescent staining. Both hypertrophy, characterized by an increase in the ratio of nuclei per tubular area, and microcystic dilations were observed. The ratio between principal cells and intercalated cells was higher in ~~dilated~~ microcystic than in hypertrophied tubules. There was no correlation between AQP2 mRNA levels and cellular remodeling of the CD. Additional amiloride treatment in the Li^+/Ami group did not allow consistent morphometric and cellular composition changes compared to the Li^+ group. Low exposition to lithium prevented overt NDI but not microcystic dilations of the CD, with differential cellular composition in hypertrophied and ~~dilated~~ microcystic CDs, suggesting different underlying cellular mechanisms.

Introduction

Lithium (Li^+) is the first line treatment for bipolar disorder (1–3), but its high efficacy is counterbalanced by long-term adverse effects (4). Among them, renal adverse effects are the most significant and are divided into 2 entities, the nephrogenic diabetes insipidus (NDI) and the microcystic chronic kidney disease (CKD) (5,6).

Regarding Li-induced microcystic CKD, previous experimental studies have reported tubular dilations and microcysts, developing as soon as 1 month after Li initiation (7–9). Interstitial fibrosis and uremia have also been induced in long-term exposition models up to 6 months (7,10). Based on morphological analyses and lectin staining, distal tubules and/or collecting ducts have been indirectly identified as the potential origin of dilations and microcysts (7,11). However, no specific staining has been reported, and the exact cellular composition of these cysts is not established. In this respect, experimental data have shown collecting duct remodeling characterized by cellular proliferative signals and an increase in the ratio between type A intercalated cells and principal cells (12–14).

Li-induced NDI is characterized by early polyuria and polydipsia due to renal resistance to vasopressin secondary to a decrease in AQP2 apical membrane addressing and expression (15). Whether a mechanistic relationship exists between Li-induced NDI and CKD remains to be determined. Moreover, the relationship between cellular composition, microcystic tubular dilations and resistance to the action of vasopressin is yet to be demonstrated.

As a matter of fact, NDI develops shortly after Li initiation in humans as well as in experimental models (16), while CKD is inconstant and very slowly progressive (17). Recent clinical studies suggest differential determinants of Li-induced NDI and CKD. While Li daily dose is the main determinant of the former (18), Li treatment duration is reported to be independently associated with the latter (19).

Amiloride is a therapeutic strategy in Li-induced NDI, based on case series of patients treated with lithium (20,21). Amiloride is a specific blocker of the Epithelial Sodium Channel (ENaC), hence of Li entry in principal cells (22–24). Regarding Li-induced microcystic CKD, two studies

have shown a reduction in kidney fibrosis and tubular dilations in rats treated with Li during 6 months at therapeutic doses when amiloride was concomitantly administered (25,26).

Based on these findings, the objective of this study was to evaluate in a rat model the effect of Li under-exposition on NDI and microcystic tubular dilations, the potential relationship between them and the efficacy of amiloride to prevent both.

Results

Under-exposition to lithium resulted in no overt NDI but in microcystic dilations of the collecting ducts

Plasma lithium concentrations in rats treated with Li alone (Li^+ group) were 0.25 ± 0.06 mmol/l. The Li^+ group displayed non-significantly higher urine output and lower urine osmolality than control rats (**figure 1**). Urine concentration challenging tests (hydric restriction and desmopressin injection) did not reveal a significantly altered urine concentration ability in the Li^+ group at 3 and 6 months of treatment. After 6 months of treatment, plasma creatinine was not statistically different between the 2 groups, and no overt interstitial fibrosis was observed on histopathological assessment (**figure 2**). However, consistent and marked tubular morphological changes were observed in the Li^+ group, characterized by cortical and outer medullary microcystic tubular dilations, sometimes associated with intratubular casts. Semi-automated cortical tubular area measurement showed significantly higher tubular areas and minimal diameters in the Li^+ group compared to controls (**figure 2**).

A differential cellular composition of collecting ducts was observed in ~~dilated~~ microcystic and non-~~dilated~~ microcystic collecting ducts

~~Dilated~~ Microcystic tubules stained for markers of principal (PC) and type A intercalated (AIC) cells, demonstrating that the tubular segment involved in these ~~dilations~~ microcystic dilations were collecting ducts (CD) (**figure 3**). Morphometric analysis of these collecting duct showed an increase in the size of cells and in the number of nuclei per tubular area in Li-treated rats compared to control rats, suggestive of epithelial hypertrophy. This was equally observed in the cortex, the outer medulla and the inner medulla (**figure 4**). Hypertrophy was not associated with positive Ki67 staining of CD cells at 6 months (**figure 3**).

The analysis of the composition of CDs revealed a decrease in the ratio between PCs and AICs in the Li group in the cortex, the outer medulla and the inner medulla (**figure 4**). However, the cellular composition of ~~dilated~~ microcystic CDs was different, lined with a majority of PCs, conversely to hypertrophied CDs (**figure 4**).

Long-term exposure to lithium has been demonstrated to induce remodeling of the collecting ducts, particularly in the inner medulla, characterized by the presence of pendrin-positive cells (27). Therefore, we assessed pendrin expression across various renal segments to determine whether low-dose exposure altered its expression pattern. Consistent with prior findings, we observed that pendrin expression was markedly higher in the cortex compared to the deepest segments, by at least a five-fold increase (28), with no significant differences observed within each segment between control and lithium-treated rats (figure 4)

Despite no overt NDI, AQP2 expression decreased in Li-treated rats, with ~~weak~~ minor correlation with cellular remodeling

On qualitative assessment of immunofluorescent staining, no difference in the expression pattern of AQP2 was observed between the experimental groups (figure 3). AQP2 mRNA levels were analyzed in the cortex, the outer stripe of the outer medulla (OSOM), the inner stripe of the outer medulla (ISOM) and the inner medulla, showing that mRNA levels were significantly lower in Li-treated rats compared to controls in all these segments, despite the absence of significantly higher urine output, with a decreased AQP2 expression gradient in the Li^+ group (**figure 5**). There was no statistical correlation between cellular and morphometric quantifications of CDs and the level of AQP2 expression except a correlation between AQP2 expression and the PC/AIC ratio that was found only in the outer medulla (rho=0.61, p=0.03).

Amiloride treatment had no effect on microcystic dilations but decreased the type A intercalated/principal cells ratio within the ~~non~~ hypertrophied~~dilated~~ portions of the collecting ducts

Plasma lithium levels were 0.43 ± 0.16 mmol/l in rats treated with lithium and amiloride (Li^+/Ami group, p=0.01 compared with rats in the Li^+ group). Rats treated with Li and amiloride did not differ from the Li^+ group in terms of urine output, urine osmolality and plasma creatinine, and urine concentration ability after challenging tests, but with a lower weight gain than the two other groups over the study period (**figure 1**). Tubular area quantifications were also similar

between the Li^+ and the Li^+/Ami group (**figure 2**). Increased number of nuclei per external area was prevented exclusively in the renal cortex of the Li^+/Ami group, with no statistical difference in the outer or the inner medulla (**figure 3**). This observation was associated with a less extended decrease in the PC/AIC ratio compared to the Li^+ group (**figure 3**), and no difference in this ratio between ~~dilated~~ microcystic et ~~non-dilated~~ hypertrophied CDs (**figure 4**). Finally, the reduction in AQP2 mRNA expression ~~decrease~~ was more ~~important~~pronounced, though not reaching statistical significance throughout all the kidney segments in the Li^+/Ami group compared to the Li+ group (**figure 5**). Overall, amiloride did not prevent AQP2 transcription decrease after 6 months of lithium exposure.

Discussion

Our study showed in a rat model of Li exposition that lower plasma lithium concentrations resulted in the no overt nephrogenic diabetes insipidus, but did not prevent morphological changes and cellular remodeling consisting in microcystic dilations and hypertrophy of the collecting ducts.

The usual standard target plasma lithium levels are 0.6-1 mmol/l. However, several clinical trials suggest clinical efficacy of lower doses in bipolar disorder, with lithium concentrations of 0.4 mmol/l (29). Our previous study evaluating vasopressin resistance and NDI in Li-treated patients showed that the main determinant of urine output was Li daily dose, directly affecting plasma lithium concentrations, independently of treatment duration (18). Our experimental findings in a model with low plasma concentrations are consistent with the clinical findings as, even though a significant decrease in AQP2 expression was observed, no significant change in urinary output, urine osmolality, or urine concentration ability was evidenced. Immunofluorescent staining of AQP2 showed no apparent decrease in fluorescence intensity between the groups allowing to identify PCs with this staining. This suggests a resistance to the action of vasopressin to a certain degree, that might be obvious during hydric restriction challenge.

Despite the absence of polyuria or major AQP2 expression decrease, morphological changes were observed. These morphological changes involved the CDs, as suggested by previous studies with no direct evidence to date (11,16). Two features coexisted in all the analyzed kidneys, namely CD hypertrophy, characterized by an increase in the number of cells per tubular area, and microcystic dilations, characterized by CD enlargement and cellular stretching. Interestingly, cellular composition was different in ~~dilated~~ microcystic and hypertrophic CDs, with a majority of PCs in the former and a higher proportion of AICs in the latter. While Bissler *et al.* reported that cysts developed in a murine model of tuberous sclerosis complex originated from AICs (30,31), cysts in experimental models of autosomal polycystic kidney disease (ADPKD) consisted both of PCs and ICs, with a major role of PCs in cyst development (32,33). Of note, primary cilia dysfunction represents an essential promoter of

renal cysts in ADPKD as well as in various cystic kidney diseases (generically referred to as ciliopathies), and are only present in PCs within the CDs (34).

Cellular remodeling has been previously reported during lithium treatment as well as during acute kidney injury (12,35). In our study, no proliferation was seen after 6 months of Li+ treatment as assessed by Ki67 staining. Cells also presumably remained differentiated, with no double positive cells found on immunofluorescent analysis ($AQP2^+/AE1^+$). In this regard, Trepiccione *et al.* demonstrated the presence of double-positive cells bearing markers of both PCs and of AICs after Li discontinuation in a murine model (36). These cells were particularly concentrated at the end of long-row distribution of AICs, suggestive of a trans-differentiation and/or recovery process inducing PCs following Li suspension. The existence, time course and mechanisms of a potential CD cell proliferation during Li therapy are still a matter of debate. While Christensen *et al.* have found proliferation among PCs (37), De Groot *et al.* reported a G2 arrest of the cell cycle in these cells and a shift towards a higher proportion of AIC, in accordance with our findings (13). This shift might be due to trans-differentiation of PC into AIC rather than proliferation, a mechanism that is likely mediated by Notch signaling inactivation (38). This trans-differentiation is not associated with cellular proliferation in physiological conditions as well as during acute kidney damage (35). Early events remain to be evaluated in our model in order to determine a potential trans-differentiation with or without proliferation signals. Interestingly, the remodeling phenomena were barely correlated with the decrease in AQP2 expression, whereas one could have hypothesized such a correlation based on the decrease in the proportion of PCs. Our findings rather suggest that Li effect on urine concentration and on cellular remodeling are uncoupled. Accordingly, our previous reports evaluating patients treated with Li for bipolar disorder showed differential determinants of Li-associated NDI and Li-associated kidney texture changes using kidney MRI radiomic analyses (18,39).

We did not evidence elevated plasma creatinine levels or renal interstitial fibrosis suggestive of a decrease in renal function after 6 months of treatment, whereas interstitial fibrosis was reported by Walker et al. (7) after the same duration of treatment with higher exposition to

lithium. It remains to be determined whether cellular remodeling is deleterious in the long term, but our results, put in the perspective of previous literature, suggest that lowering kidney exposition to Li might be nephroprotective in a certain extent.

Finally, we studied the effect of amiloride treatment in this experimental under-exposed model. We found no difference between the group without and the group with amiloride regarding urine concentration. AQP2 mRNA expression was even lower, although not significantly, in the Li^+/Ami group. However, this should be interpreted with caution as plasma lithium concentrations were higher, though still at infra-therapeutic levels, than in the Li^+ group. This is likely attributable to angiotensin II-mediated stimulation of proximal tubular sodium (and lithium) reabsorption, which occurs secondary to the natriuretic effect of Amiloride (40). The lower weight gain observed in the Amiloride treated rats may be indicative of a relative reduction in extracellular volume due to natriuresis. These findings also align with those reported in a prior study by Bedford et al (41). As a monovalent cation Li enters tubular cells using the same transporters as sodium. As a specific ENaC blocker, amiloride has been proven to inhibit Li entry in PCs *in vitro* and *in vivo* (22). Conversely to our findings, Bedford *et al.* demonstrated improved urine concentration and increased AQP2 expression when Li-exposed rats were treated with amiloride (41). Small clinical trials have shown an increase in urine osmolality and urinary AQP2 excretion after treating patients with amiloride (20,21). In a long term (5 months) murine model of Li nephrotoxicity, Kalita-De Croft *et al.* also showed that amiloride prevented renal fibrosis (25), although altered tubular morphology and microcystic dilations were not prevented, consistently with our study. The disparity in Li exposition among studies could account for the differences in our findings. In our study, a mild effect of amiloride was observed on cellular remodeling, in particular in the renal cortex, with a lesser decrease in the PC/AIC ratio. This effect is in line with recent literature showing an important crosstalk between PCs and AICs, especially during acidosis (23,42–44).

Our study displays limitations. First, due to the long-term treatment study design and the animal facility space limitations, we lacked an experimental group with plasma lithium concentrations at the therapeutic ranges, that might further evaluate the potential effect of amiloride on

hypertrophy and microcysts development. We thus based our protocol on previous literature showing NDI, kidney fibrosis and uremia, and microcystic kidney disease in the long-term setting (7,8,25). Second, our study also lacked early timepoint assessments in order to evaluate the early biochemical and morphological changes induced by exposition to lower plasma lithium levels. In particular, the evaluation of cell cycle disruption by Ki67 staining at 6 months was insufficient, and assessments at earlier time points are necessary to contextualize our model within previous literature. Li triggers the activation of cell cycle pathways at very early stages following initiation (45). Further studies at therapeutic levels of lithium exposition with early timepoints of assessments would thus help better evaluate CD remodeling in this setting. Finally, our transcript analyses could not be complemented by protein analyses due to the unavailability of kidney tissue.

In conclusion, under-exposition of kidneys to lithium resulted in the prevention of overt nephrogenic diabetes insipidus although AQP2 expression was decreased, but did not prevent collecting duct hypertrophy and microcysts, with only minor effect of amiloride on cellular remodeling. Future studies are needed to better decipher the differential role of signaling pathways leading to hypertrophy and intercalated cell number increase on the one hand, and microcysts generated from principal cells on the other hand.

Experimental

Animals

All procedures were performed in accordance with the French animal care legislation, and were approved by the Ethics committee of the Université Paris Cité and by the French Ministry of Research (approval # 26674-2020020714451509). Wild-type male Sprague-Dawley rats (mean weight of 100-125 g, Janvier Labs) were fed a normal rat diet with free access to water. Rats were randomly allocated into 3 groups: (1) control group, (2) lithium treatment group, (3) amiloride and lithium treatment group (n=8 for each group) for a duration of 6 months. Lithium carbonate (Sigma) was added to the dry food at 0.1% (0.05% of wet food, corresponding to 2,7 mmol/kg of body weight of lithium). Amiloride was added to the diet at a concentration of 200 mmol/kg of dry food, as previously described (22). Both Lithium and Amiloride were blended with wet ground food, totally solubilizing in the water used to wet the food. Food was subsequently dried to form dense kibbles. Control food underwent a similar processing method.

Sample processing

Urine and plasma were collected at 4, 5 and 6 months during metabolic cage housing. Sublingual blood collection was performed under gas anesthesia, followed by centrifugation of the 500 µl blood sample with 10% heparinization at 5000 rpm. The supernatant plasma was then collected and stored at -20˚C until further processing for biochemical analyses. Creatinine measurement was performed using a Konelab device (ThermoFischer Scientific). Urine osmolality was measured using the Advanced 3250 osmometer (Advances Instruments). Plasma lithium was measured by mass spectrometry (Inductively Couple Plasma Mass Spectrometry, ICP-MS, NexION 2000 spectrometer, Perkin Elmer). At the end of the protocol (6 months), after anesthesia, the left kidney was rapidly excised, partly embedded into Optimal Cutting Temperature embedding medium (OCT, Fisher Healthcare) and frozen for fluorescent immunostainings, and partly frozen and conserved at -80°C for subsequent RNA analyses. Aorta was subsequently cannulated in order to perfuse the right kidney with isotonic saline

first, and 4% paraformaldehyde (PFA) second. The right kidney was removed, fixed in 4% PFA solution, embedded in paraffin and further processed for Masson trichrome staining (3µm sections).

Urine concentration tests

Rats were monitored in metabolic cages for a duration of 24 hours each session. Water deprivation and desmopressin challenging tests were performed separately twice, after 3 and 6 months of treatment in metabolic cages. For the final timepoint (6 months), metabolic cage assessments were conducted 2 days prior to sacrifice. This timeframe was consistent for all rats. Although the assessments were spread over two days due to technical constraints, the sacrifices were scheduled accordingly, also spread over two days to ensure uniformity in the duration between the experiment and sacrifice. They respectively consisted in a 6-hour hydric restriction with urine collection at 2, 4 and 6 hours and in an intramuscular injection of desmopressin (DDAVP) (0.75 µM/kg) after 2 hours of hydric restriction, with subsequent urine collection at 2 and 4 hours after injection.

Immunofluorescent assay

Snap-frozen kidney samples were processed after cryosection (4µm sections) for immunofluorescence microscopy using a two-step staining, the first step with primary antibodies (goat anti-AQP2 SC-9882, 1/500, Santa Cruz, rabbit anti-AE1 20112, 1/800, Cell Signaling), and secondary antibodies (donkey anti-goat AF488, SC-362255, Santa Cruz, donkey anti-rabbit AF555 A-31572, 1/500, ThermoFischer Scientific), the second step with direct FITC-labeled Ki67 antibody specific for Ki67 (1/500; Abcam ab281847).

Morphometric analyses

Whole sagittal sections of kidneys were scanned using Axioscan Z1 slide scanner (Zeiss) after Masson trichrome staining or immunofluorescent labeling. Tubular area was semi-automatically measured using the QuPath software (46). Briefly, approximately one sixth of

the cortex was selected on Masson-stained tissue slices, and vessels (arteries and veins) were manually excluded. A pixel classification method was trained from manual annotations, in order to differentiate tubular lumen and parenchyma. A cut-off of 1500 μm^2 was then applied on 'lumen' objects in order to exclude glomerulus urinary spaces and peritubular capillaries. An average of 436 ± 223 tubules were analyzed in each kidney (including all the tubules present within the renal cortex).

The ratio between principal cells (stained with AQP2) and type A intercalated cells (stained with AE1, type 1 anion-exchanger), and between the number of cells and the tubular area was assessed in ~~dilated~~ microcystic and ~~non-dilated~~hypertrophied collecting ducts. Only cells with visible nucleus were counted, and all the tubules displayed closed lumen.

RT-PCR

Kidney samples were separated immediately after excision to obtain specific samples from the cortex, the outer stripe of the outer medulla, the inner stripe of the outer medulla, and the inner medulla (including the papilla). Total RNA was extracted from these samples using the RNeasy Micro-Kit according to the manufacturer's protocol (Qiagen). Reverse transcription was performed using first-strand cDNA synthesis kit for RT-PCR (Roche Diagnostics). qPCR was then performed on a LightCycler (Roche Diagnostics) using a SYBR green kit (LightCycler 480 SYBR Green Master, Roche Diagnostics) for AQP2 (forward primer: 5'- ACC TGG CTG TCA ATG CTC TC - 3' and reverse primer: 3'- GG ACG GGA GAG GTA ACC AAA - 5'), and Pendrin (forward primer: 5'- ACT GGA ACT CTG AAC TCC CG - 3' and reverse primer: 3'- CAAAGAACCCACACTGCTCC - 5') normalizing to the ribosomal protein S23 (RPS23) trancript levels (forward primer: CTC ACG CAA AGG GAA TTG T~~...~~, reverse primer: CAA TGA AGT TCA AGC AAC CG~~...~~).

Statistical analyses

All data are presented as percentages or mean ± SEM values. As the parameters did not display normal distribution, n~~N~~on-parametric Mann-Whitney or Kruskal-Wallis tests were

performed to test differences between groups. Non-parametric Spearman test was used to test correlations between AQP2 expression and the cellular quantifications. The significance level of a statistical hypothesis test was set at 0.05. All statistical analyses and graphs were performed using Prism GraphPad.

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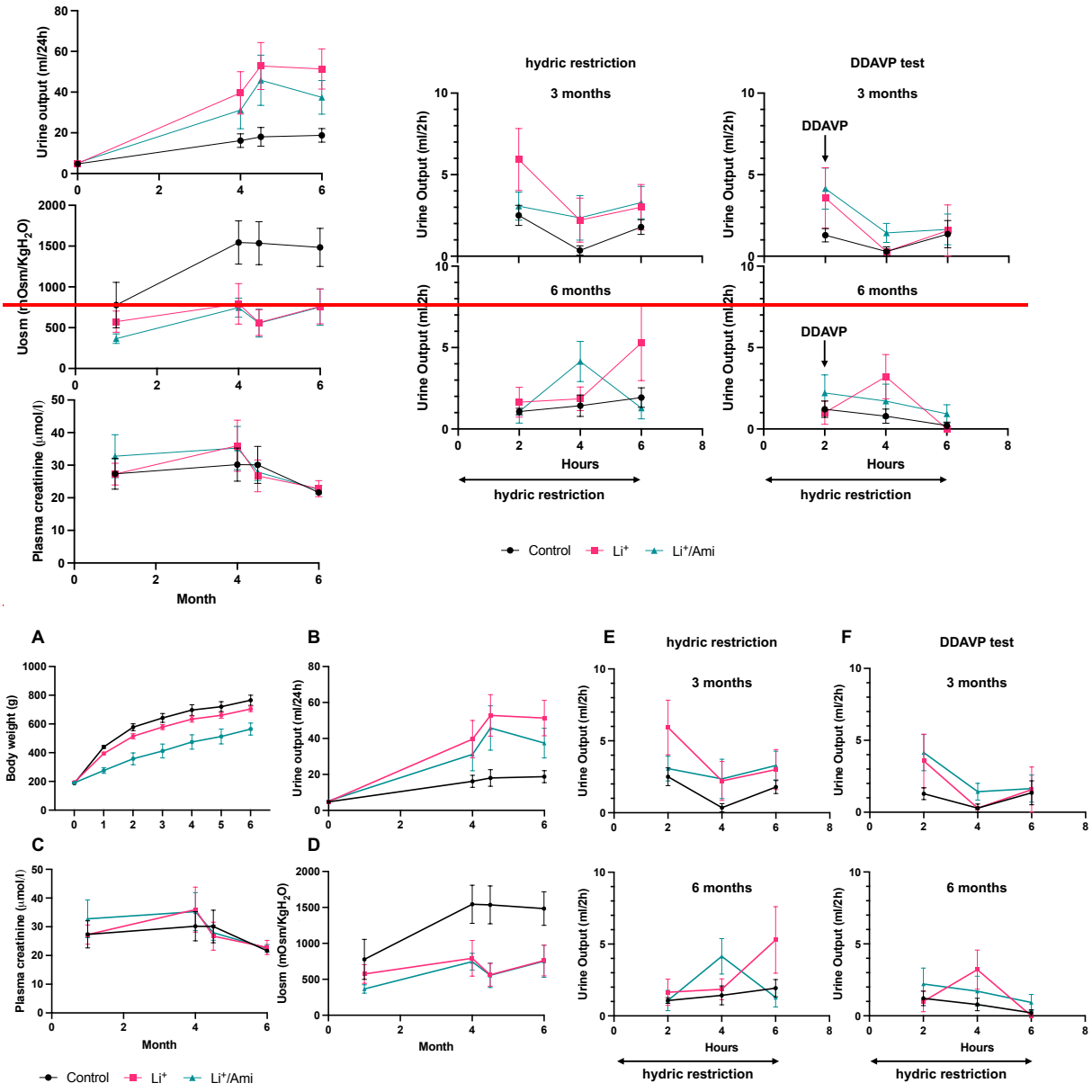
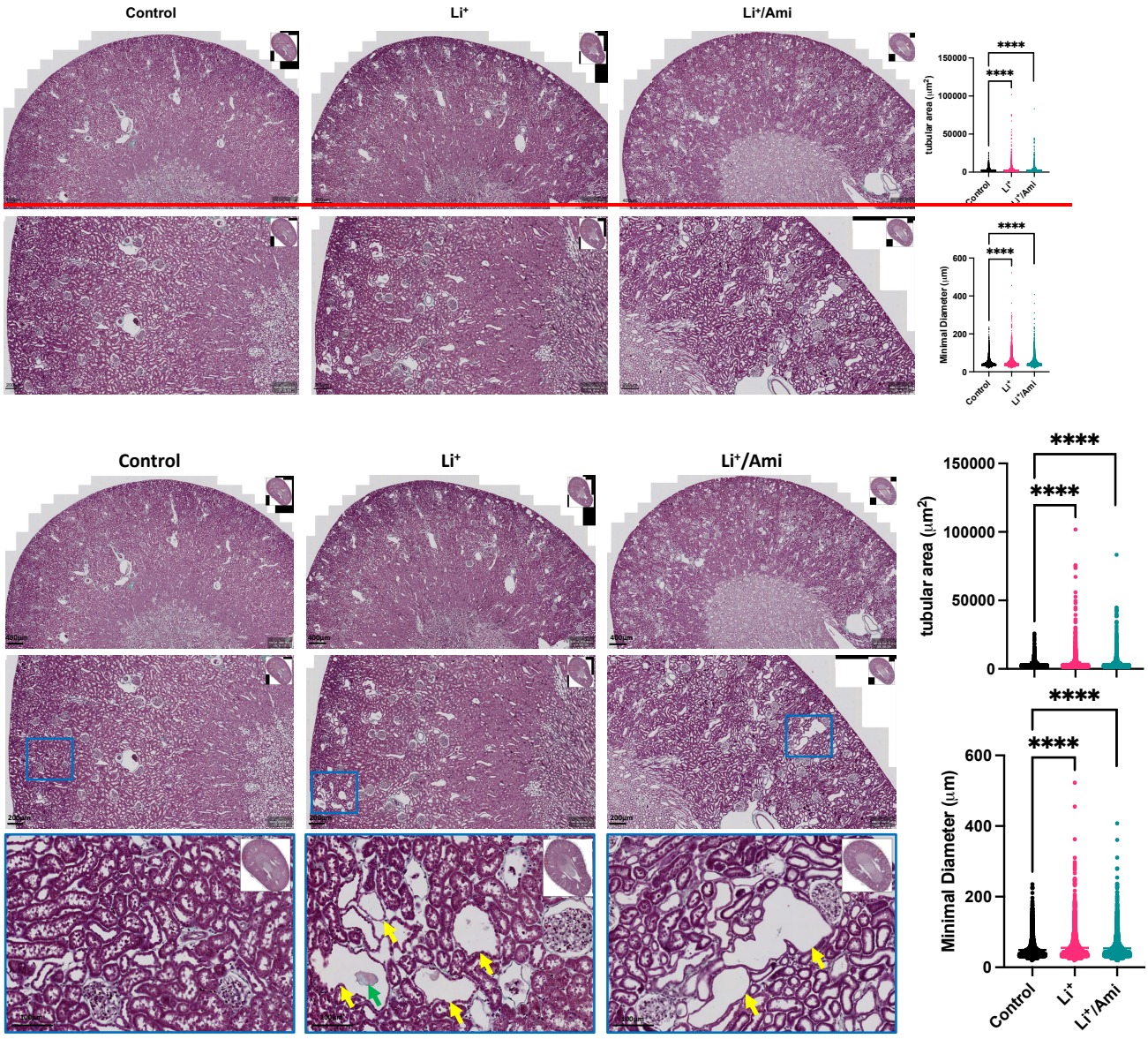
Figures

Figure 1. Weight, uUrine concentration and plasma creatinine levels.



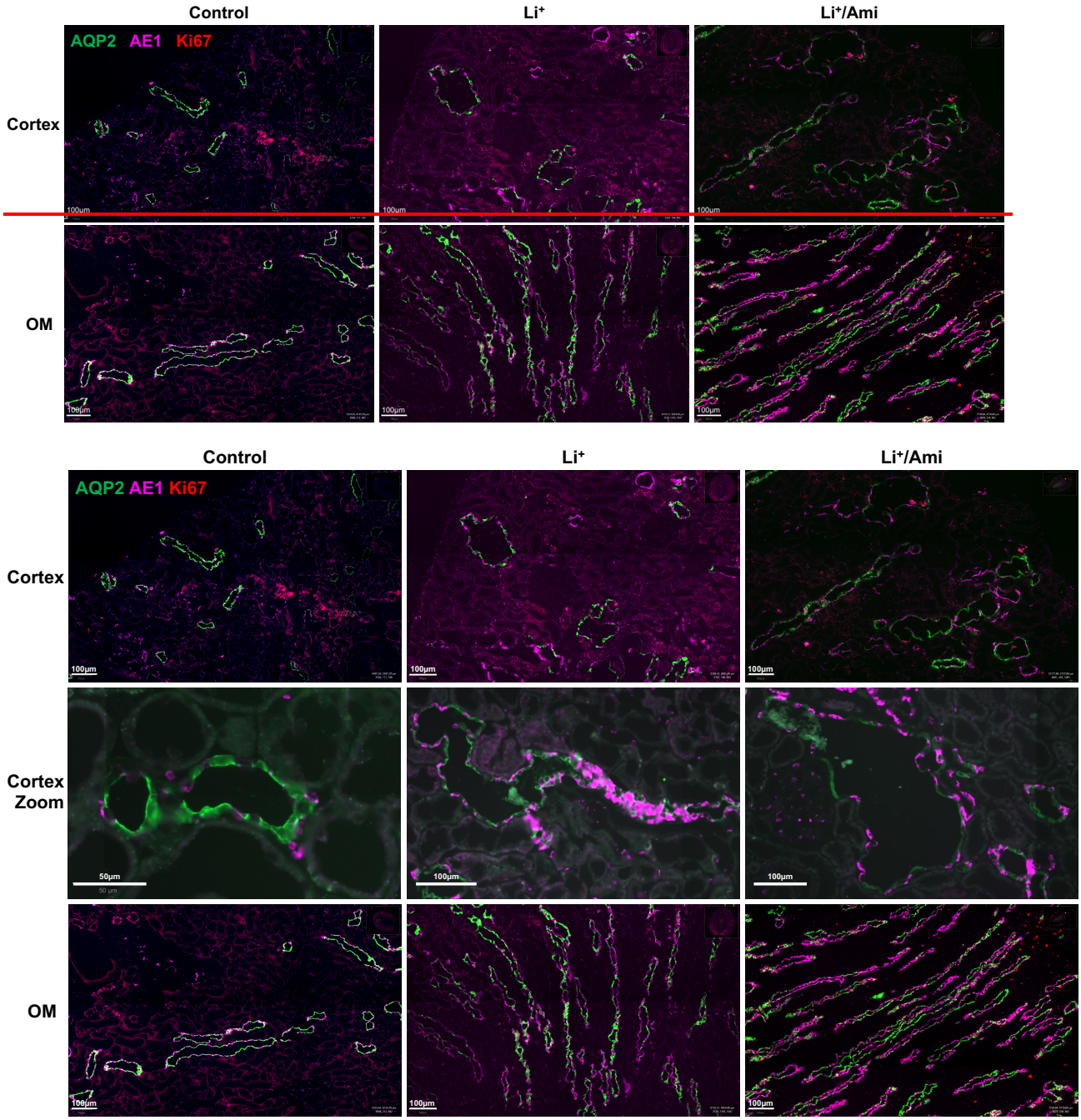
Data are expressed in mean and SEM. Uosm: urine osmolality, Li^+: lithium, Li^+/Ami: lithium and amiloride treatment. Weight gain was significantly lower in the Li^+/Ami group. There were no other statistically significant difference between the groups in all the analyses.

Figure 2. Masson's trichrome staining of kidneys and quantification of tubular areas and minimal tubular diameters.



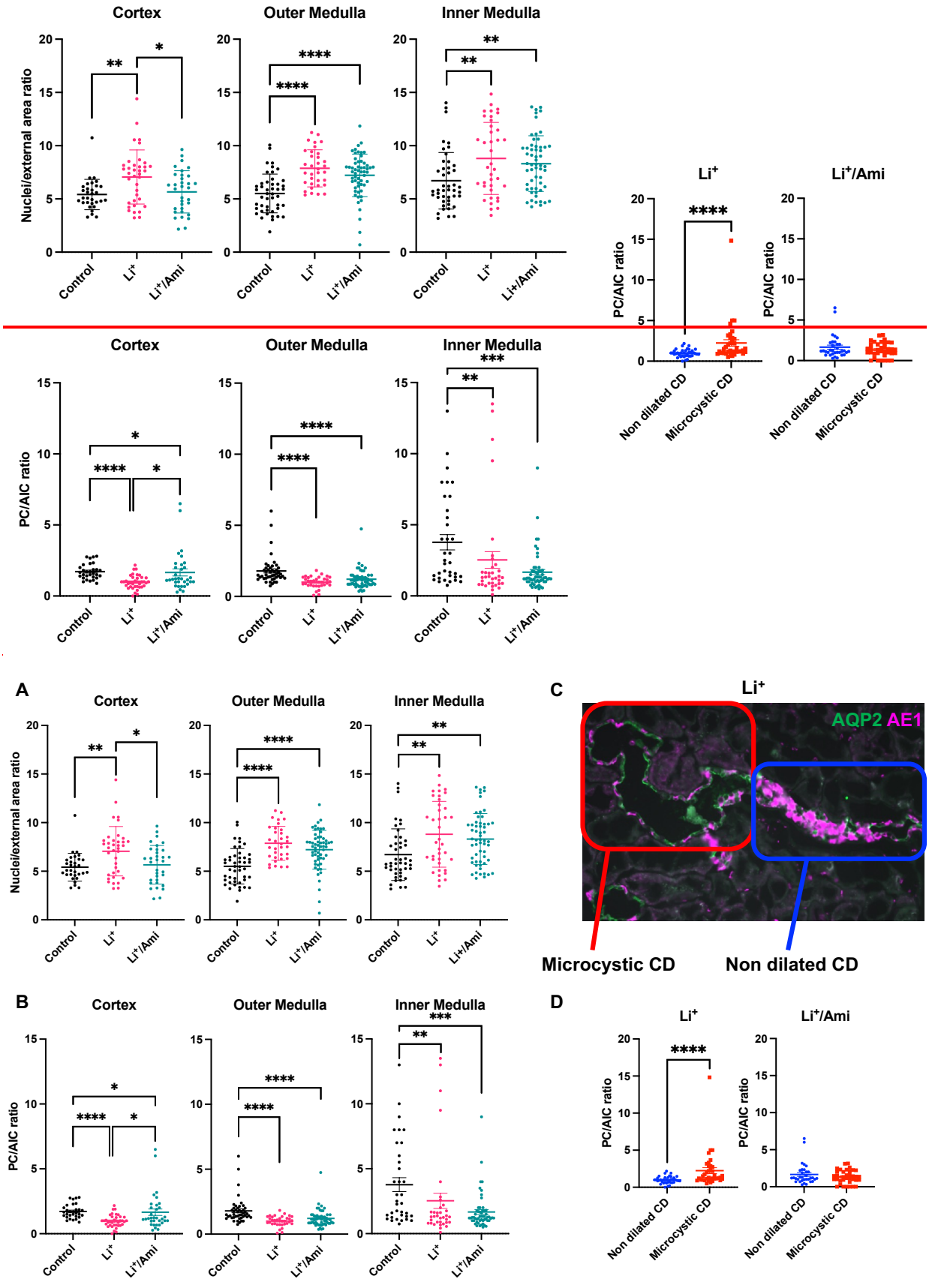
Representative images of trichrome staining (left) with zoomed-in images showing microcystic tubular structures (yellow arrows) in the Li^+ and the Li^+/Ami group, sometimes enclosing tubular casts (green arrow). A~~,~~ ~~a~~utomated tubular areas and minimal diameters in each group (right). Only statistically significant differences are represented. **** $p<0.0001$. Uosm: urine osmolality, Li^+: lithium, Li^+/Ami: lithium and amiloride treatment.

Figure 3. Immunofluorescent staining of collecting duct cells



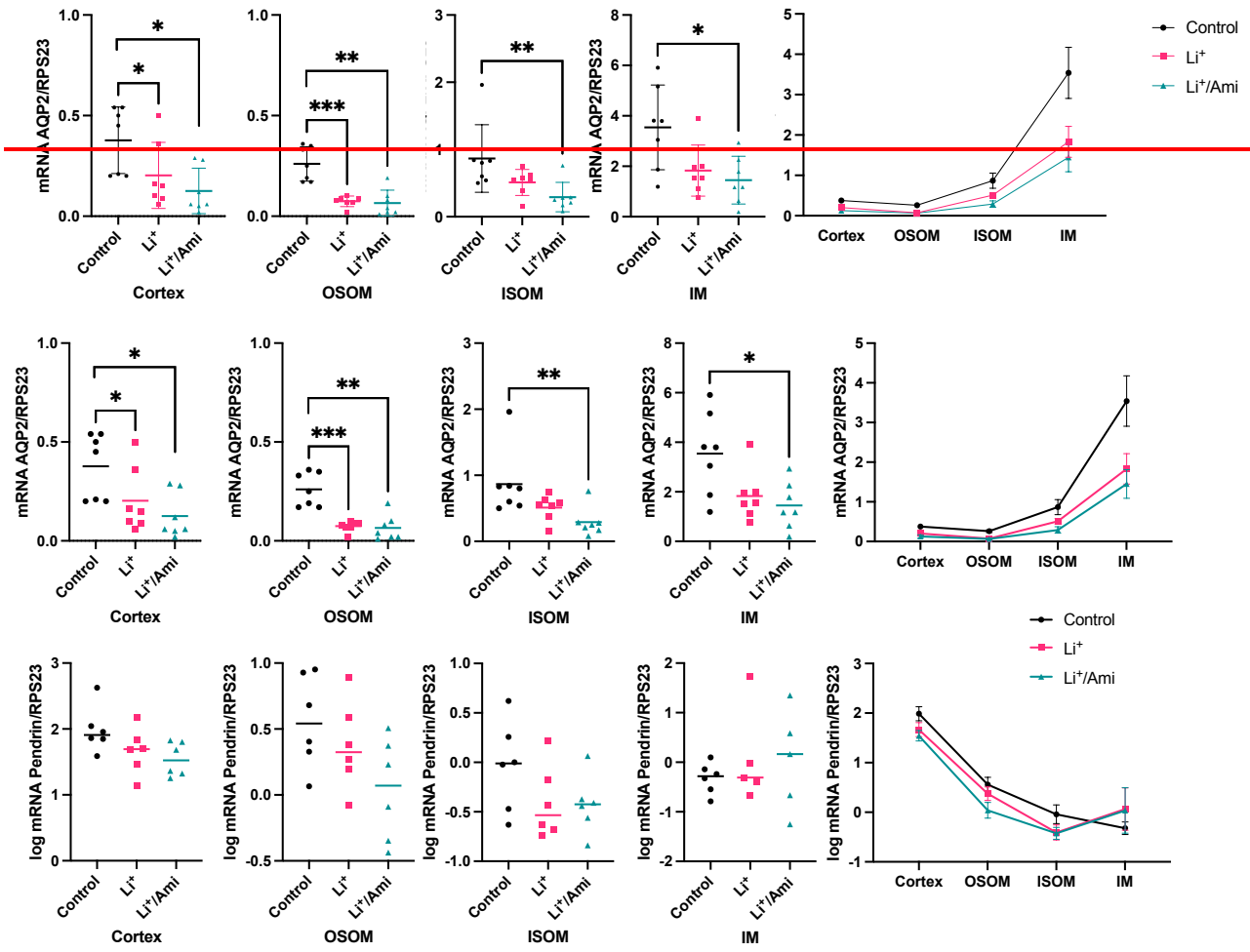
Representative images of the cortex and the outer medulla (OM) of immunofluorescent staining of type 2 aquaporin (AQP2), type 1 anion exchanger (AE1) and Ki67 of kidney slices of control rats, rats treated with lithium (Li^+) and rats treated with lithium and amiloride (Li^+/Ami). No collecting duct cells stained positive for Ki67.

Figure 4. Morphometric analyses and cellular composition of collecting ducts



~~Upper panel~~A, ratio between the numbers of DAPI-stained nuclei and external surface measurement of the collecting ducts in the cortex, the outer medulla and the inner medulla of control rats, rats treated with lithium (Li^+), and with lithium and amiloride (Li^+/Ami). ~~Lower panel~~B, ratio between principal cells (PC) and type A intercalated cells (AIC) in the cortex, the outer medulla and the inner medulla of control rats, rats treated with lithium (Li^+) and rats treated with lithium and amiloride (Li^+/Ami). ~~Right panel~~C, representative image of a longitudinal section of a collecting duct in the Li^+ group, showing a microcystic portion delineated by a majority of AQP2+ cells, and a consecutive portion with minimal lumen (non-dilated) delineated by a majority of AE1+ cells. This aspect was observed only in the Li^+ and the Li^+/Ami groups. D, ratio between principal cells (PC) and type A intercalated cells (AIC) in the cortex of rats treated with lithium (Li^+) and rats treated with lithium and amiloride (Li^+/Ami) in non-dilated (hypertrophied) and in dilated (microcystic) collecting ducts (CD). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

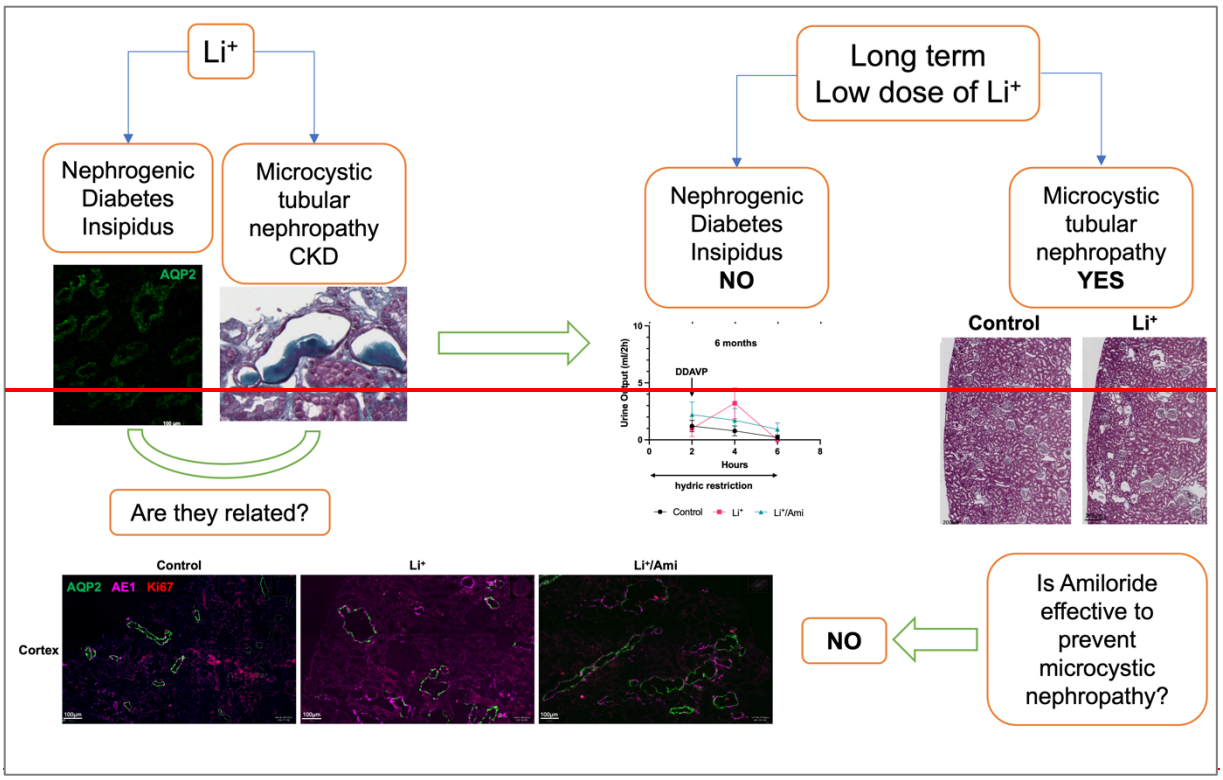
Figure 5. AQP2 expression in the different segments of the kidney

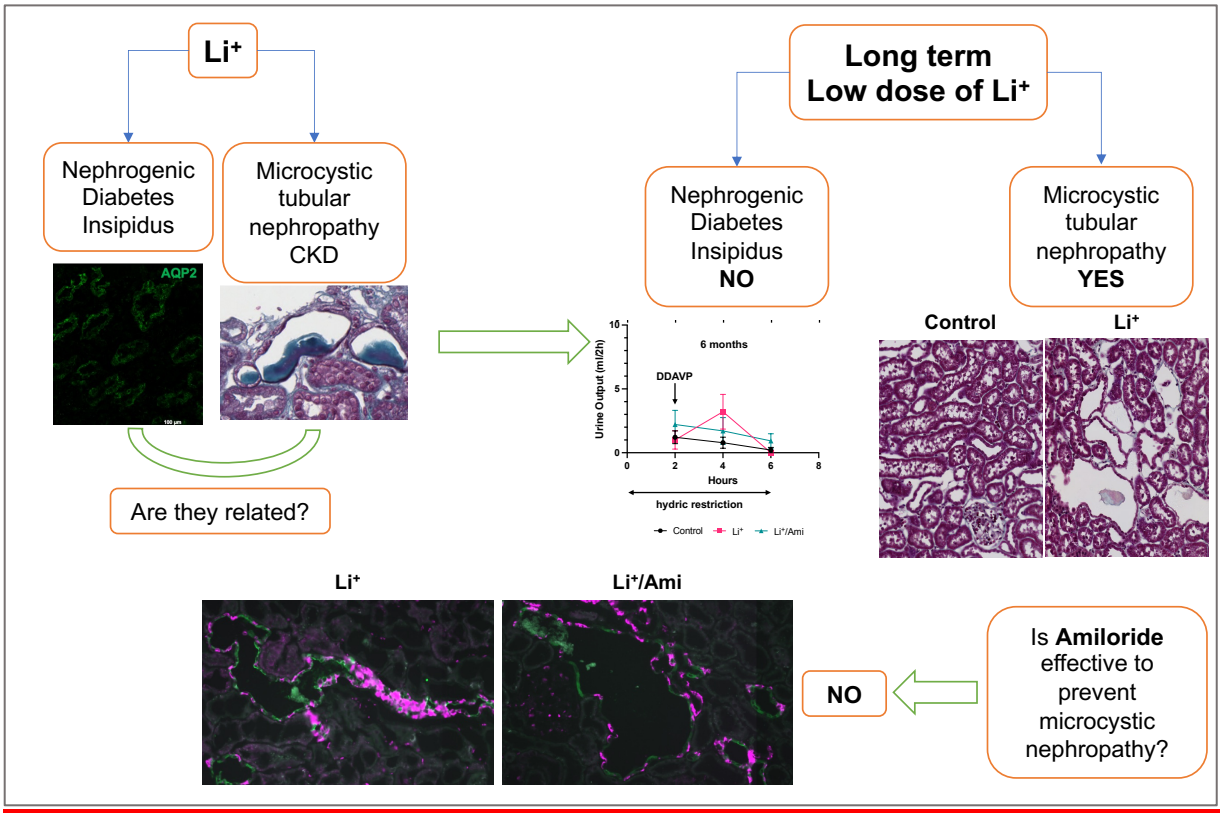


Upper panel, rRelative AQP2 mRNA expression in the cortex, the outer stripe of the outer medulla (OSOM), the inner stripe of the inner medulla (ISOM) and the inner medulla (IM) in control rats, rats treated with lithium (Li^+) and rats treated with lithium and amiloride (Li^+/Ami). Lower panel, relative Pendrin log mRNA expression in the cortex, the outer stripe of the outer medulla (OSOM), the inner stripe of the inner medulla (ISOM) and the inner medulla (IM) in control rats, rats treated with lithium (Li^+) and rats treated with lithium and amiloride (Li^+/Ami). * $p<0.05$, ** $p<0.01$, *** $p<0.001$. RPS23: ribosomal protein S23.

Graphical abstract

Lithium treatment is the main treatment of bipolar disorder but can induce renal adverse events, including nephrogenic diabetes insipidus (NDI) and microcystic chronic kidney disease (CKD). We found that in a long-term treatment rat model, low exposition to lithium prevented NDI, but not microcysts, which were predominantly composed of collecting ducts principal cells, conversely to hypertrophic zones of the collecting duct that were mainly bordered by intercalated cells.


Li+
Nephrogenic Diabetes Insipidus
Microcystic tubular nephropathy CKD
AQP2
Are they related?
Long term Low dose of Li+
Nephrogenic Diabetes Insipidus NO
Microcystic tubular nephropathy YES
6 months
DDAVP
Urine Output (ml/2h)
Hours
hydric restriction
Control
Li+
Li+/Ami
Control
Li+
Li+/Ami
Cortex
AQP2 AE1 Ki67
NO
Is Amiloride effective to prevent microcystic nephropathy?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