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Contribution of NHE3 and dietary phosphate to lithium pharmacokinetics

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Abstract

Lithium is one of the mainstays for the treatment of bipolar disorder despite its side effects on the endocrine, neurological, and renal systems. Experimentally, lithium has been used as a measure to determine proximal tubule reabsorption based on the assumption that lithium and sodium transport go in parallel in the proximal tubule. However, the exact mechanism by which lithium is reabsorbed remains elusive. The majority of proximal tubule sodium reabsorption is directly or indirectly mediated by the sodium-hydrogen exchanger 3 (NHE3). In addition, sodium-phosphate cotransporters have been implicated in renal lithium reabsorption. In order to better understand the role of sodium-phosphate cotransporters involved in lithium (re)absorption, we studied lithium pharmacokinetics in: i) tubule-specific NHE3 knockout mice (NHE3^{loxloxPax8Cre}), and ii) mice challenged with low or high phosphate diets. Intravenous or oral administration of lithium did not result in differences in lithium bioavailability, half-life, maximum plasma concentrations, area under the curve, lithium clearance, or urinary lithium/creatinine ratios between control and NHE3^{loxloxPax8Cre} mice. After one week of dietary phosphate challenges, lithium bioavailability was ~30% lower on low versus high dietary phosphate, possibly the consequence of a smaller area under the curve after oral administration. This was associated with lower lithium clearance after oral administration and lower urinary lithium/creatinine ratios on low versus high dietary phosphate. Collectively, renal NHE3 does not play a role in lithium pharmacokinetics; however, dietary phosphate could have an indirect effect on lithium bioavailability and lithium disposition.

Keywords

Lithium; kidney; bipolar; proximal tubule; urine; excretion

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Author contribution statement

TR and LT conceived and designed the work. LT, JX, JDR, and TR contributed to the acquisition, analysis, or interpretation of data for the work. All authors drafted the work or revised it critically for important intellectual content and approved the final version of the manuscript.

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Conflict of interest

The authors declare that they have no conflicts of interest.

1. Introduction

Lithium, despite its renal (Poulsen et al., 2017), endocrine (Salata and Klein, 1987) and neurological (Ghadirian and Lehmann, 1980) side effects, is still frequently used for the treatment of bipolar disorder. Lithium has a narrow therapeutic range and is required to have steady-state plasma levels between 0.6 – 1.2 mmol/L for maintenance treatment and 0.8 – 1.5 mmol/L for acute management (Geddes and Miklowitz, 2013). Lithium toxicity occurs when levels are >1.5 mmol/L, and levels >3.5 mmol/L are potentially fatal. In addition to its clinical use in humans, lithium has been used since the seventies as a marker of proximal tubular reabsorption in mice (Huls et al., 2007; Veiras et al., 2017), rats (Miracle et al., 2007; Thomsen and Leyssac, 1986), dogs (Abildgaard et al., 1986; Shalmi et al., 1992), and humans (Petersen et al., 1974; Thomsen and Olesen, 1984). An ideal marker of proximal tubule Na^+ reabsorption should fulfill the following criteria: (i) it's reabsorption should be proportional to Na^+ and H_2O reabsorption along the proximal tubule, (ii) no reabsorption/secretion further downstream of the proximal tubule should occur, (iii) no impact on glomerular filtration rate, and (iv) be freely filtered. The proximal tubule is responsible for the majority of renal Na^+ and H_2O reabsorption (60% – 75%) and the Na^+/H^+ exchanger isoform 3 (NHE3) plays a vital role, both directly and indirectly, in this process (Biemesderfer et al., 1993; Girardi and Di Sole, 2012). Experiments in NHE3 knockout mice identified that proximal tubule Na^+ reabsorption was reduced by 63% when studied *in vivo* and 46% when studied *in vitro* (Fenton et al., 2017; Ledoussal et al., 2001; Schultheis et al., 1998). Micropuncture studies in rats identified that in the proximal tubule, lithium is transported at the same rate as Na^+ and H_2O (Hayslett and Kashgarian, 1979). Supporting this are pharmacological studies employing acetazolamide, a proximal tubule carbonic anhydrase inhibitor, which results in a significant increase in fractional lithium clearance (Kersten and Braunlich, 1987; Kirchner, 1987; Steele et al., 1976). However, in addition to the proximal tubule, lithium reabsorption occurs up to 18% in the loop of Henle/thick ascending limb (Atherton et al., 1991; Hayslett and Kashgarian, 1979; Unwin et al., 1994). While there has been functional evidence that in the connecting tubule and collecting duct, lithium transport by the epithelial Na^+ channel, ENaC, contributes to the development of nephrogenic diabetes insipidus (Christensen et al., 2011; Vallon et al., 2009), micropuncture studies did not identify significant lithium transport beyond the early distal tubule versus the urine (Hayslett and Kashgarian, 1979). However, Na^+ restriction, a condition where ENaC is highly active, in combination with amiloride (ENaC blocker) was able to uncover a contribution of ENaC to lithium reuptake into the body (Kirchner, 1987).

In addition to NHE3, a multitude of other Na^+ -dependent transport proteins are expressed in the proximal tubule (Zhuo and Li, 2013). The studies evaluating their role for lithium transport are sparse. One of the proteins that has been studied is the Na^+ -phosphate cotransporter. Initial studies in human erythrocytes identified that the Na^+ -phosphate cotransporter accepts both Na^+ and lithium as substrates (Elmariah and Gunn, 2003). Parathyroid hormone (PTH) is an important regulator of renal phosphate reabsorption (Thomas et al., 2017; Wagner et al., 2014) and because of its effects on NHE3 (Collazo et al., 2000; Fan et al., 1999), proximal tubular Na^+ and H_2O reabsorption in general. Consistent with this, administration of PTH extract to lithium-loaded humans reduces

lithium reabsorption (Steele et al., 1975). In rats, administration of PTH or foscarnet (an antiviral drug that non-selectively inhibits Na⁺-phosphate cotransport) increased lithium clearance (Uwai et al., 2014; Uwai et al., 2018). Of note, non-selective inhibition of Na⁺-glucose cotransporters, also highly expressed in the proximal tubules (Gorboulev et al., 2012; Vallon et al., 2011), did not affect lithium clearance (Uwai et al., 2014).

Taken together, the lithium transport protein(s) in the proximal tubule have so far not been identified. Based on the prominent role of NHE3 in the proximal tubule for Na⁺ and water reabsorption, we hypothesized that lack of NHE3 (tubule-specific NHE3 knockout, NHE3^{loxloxPax8Cre}) results in changes in lithium disposition and/or urinary excretion. In addition, we studied the impact of dietary phosphate, which is well known to regulate the expression of proximal tubule Na⁺/phosphate cotransporters (Npt2a and Npt2c) (Tenenhouse et al., 2003) and the intestinal Na⁺/phosphate cotransporter (Npt2b) (Capuano et al., 2005) on lithium disposition in mice. Our data demonstrate comparable pharmacokinetics of lithium in the absence of renal NHE3. However, dietary phosphate has direct impact on (i) lithium bioavailability and (ii) urinary lithium excretion.

2. Materials and methods

2.1 Animals and ethics

All animal experimentation was conducted in accordance with the Guide for Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD) and was approved by the local Institutional Animal Care and Use Committee. The generation and phenotype of tubule-specific NHE3 knockout mice using Pax8-Cre transgenic mice has been described previously (Fenton et al., 2015; Fenton et al., 2017). Mice were housed under a 12:12-hour light-dark cycle in isolated ventilated cages with free access to standard rodent chow (TD, 2018; Envigo, Madison, WI) and tap water. Age-matched, 3- to 6-month old male and female mice (control, NHE3^{loxlox}) or tubule-specific NHE3 knockout mice (NHE3^{loxloxPax8Cre}) were used for experiments.

2.2 Lithium administration and blood collections

2.2.1 Intravenous administration (IV).—Lithium chloride (LiCl 5 mmol/kg, 2 µl/g body weight, Fisher Scientific, Pittsburgh, PA) (Wraae, 1978), dissolved in sterile water, was administered intravenously under brief isoflurane anesthesia. Blood was collected in 10 µl minicaps (Hirschmann Laborgeräte, Eberstadt Germany) by tail nick immediately before injection (baseline) and at 3, 5, 7, 10, 20, 30 minutes and 1, 2, 4, 6, 8 and 24 hours. Plasma was separated by centrifugation and used for subsequent analysis.

2.2.2 Oral gavage.—Lithium chloride, dissolved in sterile water, was given to mice via oral gavage (5 mmol/kg, 1% of body weight). In oral gavage experiments blood was collected at baseline, 15, 30, 45 minutes and 1, 2, 4, 6, 8 and 24 hours. In both experiments, IV and oral gavage, urine was collected at baseline, where the bladder was emptied, and 30 minutes after lithium administration.

2.3 Low and high dietary phosphate manipulations

Mice were randomized to either low phosphate (<0.01% phosphate; TD.08601, Envigo, USA) or high phosphate diet (1.5% phosphate; TD.140038, Envigo) (Fenton et al., 2014). Apart from phosphate, diets were otherwise identical in composition. After one week on either low or high phosphate diet, mice were anesthetized for blood collection to determine plasma phosphate and calcium levels.

2.4 Analysis of plasma and urine samples

All clinical chemistry was performed using commercially available assays that were modified to work with small volumes (Rieg et al., 2004; Rieg et al., 2005). Lithium concentrations in plasma and urine were determined using Infinity™ Lithium Liquid Stable Reagent (Thermo Fisher Scientific, Middletown, VA, USA; detection limit 0.04 mmol/L), urinary creatinine was determined using Infinity™ Creatinine Liquid Stable Reagent (Thermo Fisher Scientific), phosphate and calcium in plasma and urine were determined using Inorganic Phosphorous Reagent and Calcium Arsenazo III Reagent, respectively (Pointe Scientific, Canton, MI, USA).

2.5 Lithium pharmacokinetics

Pharmacokinetic data analysis was performed using GraphPad Prism (La Jolla, CA). Peak plasma concentration (C_{max}) was calculated by Prism from the concentration-time profile data. The area under the curve (AUC) was calculated by the trapezoid rule. The area, therefore, is $X*(Y1+Y2)/2$ and Prism uses this formula repeatedly for each adjacent pair of points defining the curve. Half-life ($T_{1/2}$) was calculated using a two-compartment model of two-phase exponential decay. The extent of biological availability (F), defined as the fraction of the oral dose which reaches the systemic circulation, was determined from the AUC after oral gavage and IV administered lithium doses ($F = AUC_{(gavage)}/AUC_{(IV)}$). The lithium dose administered divided by the AUC gave the mean plasma lithium clearance after IV administration ($CL = Dose/AUC$); for oral administration the dose was corrected by bioavailability ($CL = Dose \times F/AUC$).

2.6 Statistical analyses

The data are expressed as mean $\pm$ SEM. Two-way ANOVA was used for comparison of several experimental curves and procedures followed by Tukey or Bonferroni post-hoc tests (all data analyzed via GraphPad Prism or SigmaPlot, San Jose, CA, USA). Significance was considered at $P < 0.05$.

3 Results and discussion

3.1 Effect of lithium administration on plasma lithium levels and urinary excretion in control and NHE3^{loxlox}Pax8^{Cre} mice

In order to determine lithium pharmacokinetics, we studied mice after IV and oral administration of lithium chloride. Before lithium administration, plasma lithium levels were below the detection limit of the used assay. Lithium is a nutritionally essential trace element (Terao, 2015) and consumption with food and water results in plasma lithium levels ~0.3

$\mu\text{mol/L}$ (Bochud et al., 2007), which is a factor of $\sim 10,000$ lower compared to the recommended plasma concentrations required for the treatment of psychiatric disorders (0.6 and 1.2 mmol/L). IV injection caused a rapid increase in plasma lithium concentrations in control (Fig. 1a) and NHE3^{loxloxPax8Cre} mice (Fig. 1b). Twenty-four hours after injection, plasma lithium returned to baseline levels in both genotypes. Our results are comparable to other studies using comparable lithium doses (Can et al., 2011; Wraae, 1978); however, our more frequent blood collections clearly identified, by employing a 2-compartment model, that lithium pharmacokinetic follows a 2-phase exponential decay with a terminal $T_{1/2}$ of 6.5 ± 1 hours in control mice and 6.5 ± 1 hours in NHE3^{loxloxPax8Cre} mice (Fig. 1a, b). Two-compartment models for lithium disposition have been described before in humans, with erythrocytes being representative for the tissue compartment (Poust et al., 1976). Erythrocytes express the Na^+/H^+ exchanger isoform 1 (NHE1) (Duhm and Becker, 1977), and expression of human NHE1 in *Xenopus leavis* oocytes identified that NHE1 mediates Na^+/Li^+ countertransport (Busch et al., 1995). Our data also confirm a significantly shorter $T_{1/2}$ in rodents (Giles and Bannigan, 1997; Wood et al., 1986) compared to humans (Timmer and Sands, 1999) and other species (Manuelian et al., 2015; Rosenthal et al., 1986). The $\text{AUC}_{0-24\text{h}}$ (Fig. 1c) and lithium clearance (Fig. 1d) were not significantly different between control and NHE3^{loxloxPax8Cre} mice. Urine collected at baseline did not show measurable lithium concentrations in either genotype. After 30 minutes, urinary lithium/creatinine ratios were not significantly different between genotypes after IV bolus injection (Fig. 1e). We chose to study urinary lithium/creatinine ratios after 30 minutes when the plasma lithium concentration was still in the distribution phase, and we could successfully obtain urine. Considering that 25% of the cardiac output reaches the kidneys and that the proximal tubule is the major site of lithium reabsorption, we expected that we would see the biggest differences in urinary lithium excretion at this early timepoint (Uwai et al., 2014). Time-concentration curves for lithium after oral gavage are shown in (Fig. 2a, b); 24-hours after administration, plasma lithium returned to baseline levels in both genotypes. The terminal $T_{1/2}$ was 8.3 ± 1 hours in control mice and 6.7 ± 1 hours in NHE3^{loxloxPax8Cre} mice. After oral gavage of lithium, C_{max} was reached within 30 minutes independent of genotype (Fig. 2c). The $\text{AUC}_{0-24\text{h}}$ (Fig. 2d) and lithium clearance (Fig. 2e) were not significantly different between control and NHE3^{loxloxPax8Cre} mice. Of note, one study in rats showed that there was surprisingly no C_{max} after oral gavage, an effect that was independent of rats having free access to food or not (Olesen et al., 1976). The reason for this remains elusive. Urinary lithium/creatinine ratios were not significantly different between genotypes (Fig. 2f). Consistent with a significantly smaller $\text{AUC}_{0-24\text{h}}$ and lithium clearance after oral gavage (see above), urinary lithium/creatinine ratios were significantly lower in the oral gavage groups compared to their respective IV administration ($P < 0.05$), possibly a consequence of lower lithium AUC up to the 30-minute time point. NHE3^{loxloxPax8Cre} mice have absent NHE3 expression in the proximal tubule and $>85\%$ reduced NHE3 expression in the thick ascending limb (Fenton et al., 2015; Fenton et al., 2017). Under normal conditions, lithium is highly reabsorbed by the proximal tubule, possibly in parallel with Na^+ and H_2O , and a reduction in lithium reabsorption would indicate reduced proximal tubular transport. Mice lacking P-glycoprotein exhibit increased amino acid, calcium, protein, and glucose excretion, together indicative of proximal tubular dysfunction (Huls et al., 2007). Consequently, lithium clearance was significantly increased in this model. Based on the

prominent role of NHE3 for Na⁺ and H₂O reabsorption, we would have expected that NHE3^{loxloxPax8Cre} mice show a similar phenotype of increased lithium clearance, or possibly an even more pronounced phenotype considering the absence of NHE3 in the thick ascending limb, if NHE3 mediates lithium transport. However, our studies do not indicate a contribution of NHE3 for lithium disposition even though NHE3 is a close family member of NHE1, which transports lithium very well (Busch et al., 1995). Supporting these findings are *in vitro* data from PS120 fibroblast cells transfected with either NHE1, NHE2 or NHE3. When PS120 cells expressed NHE1 or NHE2, extracellular lithium induced intracellular alkalization; however, this effect was completely absent when cells expressed NHE3 (Kobayashi et al., 2000). Taken together, the importance of NHE3 for renal lithium transport appears to be minimal, if at all.

For the first time, to our knowledge, lithium bioavailability was determined in mice by comparing IV and oral routes of administration. High lithium bioavailability (~80%) was observed after oral dosing in mice; however, was not significantly different between genotypes (Fig. 2g). Other studies focused on comparing bioavailability of different lithium preparations, e.g. lithium carbonate versus lithium citrate (Guelen et al., 1992) or extended and immediate release lithium formulations (Arancibia et al., 1986). Estimated bioavailability was calculated to range from 60–90%, depending on formulation (Ware et al., 2016), which is assumed to be equivalent in humans (Grandjean and Aubry, 2009).

3.2 Lithium disposition in mice treated with low and high phosphate diet

In addition to studying the contribution of NHE3 for lithium disposition, we also studied the role of dietary phosphate challenges, and therefore changes in Na⁺-dependent phosphate cotransporter expression (Fenton et al., 2014; Lanaspá et al., 2013; Tenenhouse et al., 2003), for lithium disposition. We chose this “natural” maneuver because dietary phosphate intake in humans can vary widely depending on meat intake versus a vegetarian diet (Moe et al., 2011) and the amount of phosphate-containing beverages consumed (Calvo and Uribarri, 2013), which would have secondary consequences on lithium pharmacokinetics in humans. In addition, patients with chronic kidney disease usually suffer from hyperphosphatemia and require special dietary restrictions (Cannata-Andia and Martin, 2016). After 10 days of dietary phosphate challenges, plasma phosphate levels (Fig. 3a) and urinary phosphate/creatinine ratios (Fig. 3b) were ~0.9 mmol/L and ~1200-fold higher on high compared to low dietary phosphate ($P<0.05$). Vice versa, plasma calcium levels (Fig. 3a) and urinary calcium/creatinine ratios (Fig. 3b) were ~0.25 mmol/L and ~33-fold lower on high compared to low dietary phosphate ($P<0.05$). The lower plasma calcium on high dietary phosphate is possibly a consequence of decreased active vitamin D and reduced intestinal calcium absorption (Portale et al., 1986) as well as calcium/phosphate deposits in soft tissues (Brown and Razzaque, 2015). The lower urinary calcium excretion is possibly a consequence of elevated PTH levels causing significantly increased renal calcium reabsorption (Lau and Bourdeau, 1995).

3.3 Lithium disposition in response to dietary phosphate challenges

Lithium pharmacokinetic after IV administration showed terminal $T_{1/2}$ of 8.4 ± 2 hours on low phosphate diet and 6.4 ± 1 hours on high phosphate diet (Fig 4a, b). AUC_{0-24h} (Fig. 4c)

and lithium clearance (Fig. 4d) were not significantly different between dietary conditions after IV administration. However, dietary phosphate directly impacted urinary lithium/creatinine ratios: low dietary phosphate decreased, and high dietary phosphate increased urinary lithium/creatinine ratios. After oral gavage of lithium, $C_{\max}$ was reached within 45–60 minutes (Fig. 5a–c). The terminal $T_{1/2}$ was 9.7 ± 1 hours on low and 10.5 ± 1 hours on high dietary phosphate. The AUC_{0-24h} (Fig. 5d) and lithium clearance (Fig. 5e) were slightly but significantly lower on low compared to high dietary phosphate. Consistent with IV administration, a similar pattern was observed for urinary lithium/creatinine ratios (Fig. 5f). Comparing low and high dietary phosphate intake unraveled a possible contribution of the amount of dietary phosphate on lithium bioavailability. On low dietary phosphate, lithium bioavailability was 30% lower compared to high dietary phosphate (Fig. 5g), indicating for the first time that dietary phosphate could indirectly impact lithium bioavailability. Currently, the mechanism for how lithium enters the systemic circulation after oral administration is unclear. Our data argue against a role of intestinal Npt2b for lithium absorption because Npt2b expression is inversely regulated by dietary phosphate (Capuano et al., 2005; Sabbagh et al., 2011). If lithium would be transported by Npt2b, one would expect the opposite: high lithium bioavailability on low phosphate diet and low bioavailability on high phosphate diet. It remains to be determined which other transport protein(s) and/or paracellular transport contribute to lithium absorption. Of note, data on intestinal lithium uptake by glucose transporters are ambiguous: in hamster small intestine, lithium was shown to stimulate glucose entry (Bihler and Adamic, 1967), while in rat intestinal loops, glucose transport was inhibited in the presence of lithium (Rosensweig et al., 1965).

Approaches to selectively study the contribution of Na^+ /phosphate cotransporters for renal lithium handling are difficult to interpret. Foscarnet, a pyrophosphate analog, is used for the treatment of viral infections and has been postulated to inhibit Na^+ /phosphate cotransporters. Only one group has chosen to employ this approach and reported that foscarnet increased renal lithium excretion and lithium clearance (Uwai et al., 2014; Uwai et al., 2016, 2018), an effect that was proposed to be mediated by Npt2a. However, a direct link between foscarnet and Npt2a is lacking. In addition, clinically, foscarnet has been reported to cause hyper- as well as hypophosphatemia, the former incompatible with inhibition of Na^+ /phosphate cotransporters (Gerard and Salmon-Ceron, 1995). PTH administration was shown to increase lithium clearance (Uwai et al., 2014; Zhang et al., 1999). However, since PTH redistributes Npt2a and NHE3 to lysosomes and the base of the microvilli, respectively (Fenton et al., 2014; Yang et al., 2004), these studies do not allow us to distinguish which of these protein(s) mediate(s) lithium transport. Of note, our studies in $NHE3^{loxlox}Pax8^{Cre}$ mice do rule out a contribution of NHE3 since all measured pharmacokinetic parameters were not significantly different from control mice.

Our study is not without limitations. So far, we only have been able to provide indirect evidence about the involved lithium transport protein(s) and/or mechanisms. It is possible that other transport protein(s) are the apical entry pathway of lithium and NHE1 on the basolateral side the exit pathway. NHE1 is expressed in segments that are involved in lithium transport: the proximal tubule (Parker et al., 2015), thick ascending limb (Watts and Good, 1999) and distal tubule (Bobulescu and Moe, 2006). Along those lines, if nephron segments

further downstream of the proximal tubule can compensate when fractional lithium delivery out of the proximal tubule is high remains to be determined.

4 Conclusion

In summary, our data indicate that renal NHE3 is not required for lithium disposition, possibly indicating that other transport mechanisms in the proximal tubule/thick ascending limb mediate lithium reabsorption. Modulating the amount of dietary phosphate unraveled a possible contribution of renal Na⁺-phosphate cotransporters, but not the intestinal phosphate cotransporter Npt2b, for lithium transport. However, the former cannot be responsible for lithium reabsorption in the thick ascending limb. We also provide new evidence that dietary phosphate possibly modulates lithium bioavailability, having direct implications how dietary phosphate consumption in humans could impact lithium plasma levels by possibly affecting lithium bioavailability.

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Figure 1. Lithium pharmacokinetic after intravenous administration in the presence and absence of renal NHE3 (NHE3^{loxloxPax8Cre})

Two compartment 2-phase exponential decay of lithium in control (A) and NHE3^{loxloxPax8Cre} mice (B). Lack of renal NHE3 does not affect (C) area under the curve up to 24 hours (AUC_{0-24h}), (D) lithium clearance or (E) urinary lithium/creatinine ratios determined after 30 minutes. Data are expressed as mean±S.E.M, *n*=6/genotype.

Figure 2. Lithium pharmacokinetic after oral gavage in the presence and absence of renal NHE3 (NHE3^{loxloxPax8Cre})

Concentration -time curves of lithium in control (A) and NHE3^{loxloxPax8Cre} mice (B). Lack of renal NHE3 does not affect (C) maximal plasma concentrations (C_{max}) after 30 minutes, (D) area under the curve up to 24 hours (AUC_{0-24h}), (E) lithium clearance corrected by lithium bioavailability, (F) urinary lithium/creatinine ratios determined after 30 minutes or (G) lithium bioavailability. Data are expressed as mean ± S.E.M. $n=6$ /genotype.

Figure 3. Responses to dietary phosphate challenges

(A) Plasma phosphate and calcium levels after one week of low or high dietary phosphate challenges. (B) Corresponding urinary phosphate/creatinine and calcium/creatinine ratios. Data are expressed as mean $\pm$ S.E.M. $n=6$ /group. * $P < 0.05$ versus low dietary phosphate.

Figure 4. Role of dietary phosphate on lithium pharmacokinetics after intravenous administration

Two compartment 2-phase exponential decay of lithium after mice were challenged for one week with low (A) or high (B) dietary phosphate. No differences were observed in (C) area under the curve up to 24 hours (AUC_{0-24h}) and (D) lithium clearance. Urinary lithium/creatinine ratios determined after 30 minutes (E) show changes that go in parallel with dietary phosphate content. Data are expressed as mean $\pm$ S.E.M. $n=5-7$ /group. * $P<0.05$ versus low dietary phosphate.

Figure 5. Role of dietary phosphate on lithium pharmacokinetics after oral gavage

Concentration-time curves of lithium after mice were challenged for one week with low (A) or high (B) dietary phosphate. No differences were observed in (C) maximal plasma concentrations (C_{max}) after 45–60 minutes. (D) Area under the curve up to 24 hours (AUC_{0-24h}) and (E) lithium clearance were significantly lower on low compared to high dietary phosphate. Urinary lithium/creatinine ratios (F) determined after 30 minutes show changes that go in parallel with dietary phosphate and (G) lithium bioavailability was significantly lower on low compared to high dietary phosphate. Data are expressed as mean $\pm$ S.E.M. $n=5-7$ /group. * $P<0.05$ versus low dietary phosphate.