ORIGINAL ARTICLE

Staying at the crossroads: assessment of the potential of serum lithium monitoring in predicting an ideal lithium dose

Em uma encruzilhada: potencial do nível plasmático de lítio como preditor da dose ideal

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Abstract

Objective: *Lithium has been successfully employed to treat bipolar disorder for decades, and recently, was shown to attenuate the symptoms of other pathologies such as Alzheimer's disease, Down's syndrome, ischemic processes, and glutamate-mediated excitotoxicity. However, lithium's narrow therapeutic range limits its broader use. Therefore, the development of methods to better predict its dose becomes essential to an ideal therapy.* ***Method:*** *the performance of adult Wistar rats was evaluated at the open field and elevated plus maze after a six weeks treatment with chow supplemented with 0.255%, or 0.383% of lithium chloride, or normal feed. Thereafter, blood samples were collected to measure the serum lithium concentration.* ***Results:*** *Animals fed with 0.255% lithium chloride supplemented chow presented a higher rearing frequency at the open field, and higher frequency of arms entrance at the elevated plus maze than animals fed with a 50% higher lithium dose presented. Nevertheless, both groups presented similar lithium plasmatic concentration.* ***Discussion:*** *different behaviors induced by both lithium doses suggest that these animals had different lithium distribution in their brains that was not detected by lithium serum measurement.* ***Conclusion:*** *serum lithium concentration measurements do not seem to provide sufficient precision to support its use as predictive of behaviors.*

Descriptors: *Lithium chloride; Brain, lithium levels; Test, anxiety; Open field test; Serum, lithium levels*

Resumo

Objetivo: *Além de ser usado há décadas para tratar distúrbio bipolar, o lítio, mais recentemente, demonstrou-se eficaz para Alzheimer, síndrome de Down, processos isquêmicos e excitotoxicidade mediada por glutamato. Contudo, a estreita janela terapêutica do lítio limita seu uso. Portanto, o estabelecimento de métodos preditivos de dose torna-se importante.* ***Método:*** *O desempenho de ratos Wistar adultos foi avaliado no campo aberto e labirinto em cruz elevado após seis semanas de tratamento com uma ração suplementada com 0,255% ou 0,383% de cloreto de lítio ou ração normal. Coletou-se amostras de sangue para dosagem plasmática do lítio.* ***Resultados:*** *Os animais alimentados com a ração com 0,255% de cloreto de lítio fizeram mais* rearing *no campo aberto e tiveram uma maior freqüência de entradas nos braços do labirinto elevado que os animais que ingeriram a dose mais alta. Apesar disso, verificou-se níveis plasmáticos de lítio semelhantes em ambos os grupos.* ***Discussão:*** *A variação nos comportamentos destarte a presença de níveis plasmáticos semelhantes sugere que as diferentes doses produziram diferentes concentrações cerebrais não detectadas pela medida plasmática.* ***Conclusão:*** *Medidas da concentração plasmática de lítio não permitem prever de forma completa seus efeitos comportamentais.*

Descritores: *Cloreto de lítio; Cérebro, níveis de lítio; Teste, ansiedade; Teste, campo aberto; Soro, níveis de lítio*

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Introduction

Since its original proposal as a therapeutic means for the treatment of gout in 1817 and its more recent use in psychiatric patients in 1949 a long clinical experience has been developed with the use of lithium chloride. Yet, even for a compound used over such a long time there are still reports reminding of or describing for the first time aspects associated with its clinical use in neuropsychiatric patients.[1,2] Recently, It was reported that children may be more susceptible to the consequences of overestimation of lithium dose, as lithium levels reach a steady-state balance faster in youths, leading to earlier toxicity.[3] In addition, it was pointed that a delayed and slower equilibrium between lithium concentration at extracellular space and the inner compartment could result in delayed cardiac lithium poisoning.[1] These recent discoveries highlight that there are a number of features on the clinical use of lithium that are yet to be revealed.

Lithium salts have been successfully used to treat especially the classical bipolar disorder.[4-7] In spite of this, the narrow therapeutic range for the safe use of lithium induces physicians to avoid prescribing it, resulting in reduced consumption times of lithium salts. Conversely, as new studies demonstrate an increasing potential for the treatment of other pathologies the interest in using lithium salts was rekindled, being in vogue again. Recently, the use of lithium has been proven beneficial over animal models of Alzheimer's disease, Down's syndrome,[8] lesions produced by ischemic processes and glutamate-mediated excitotoxicity,[9,10] in patients affected by Canavan disease,[11] and in subjects with a high risk to develop psychosis.[12] Moreover, lithium seems to protect neurons[13] and stimulate the proliferation and migration of neural stems cells.[12,14,15] This current expanding use of lithium resumes a need for methods able to monitor the lithium levels, in order to adjust its dosage to reach therapeutic effects, besides avoiding its toxicity.

Lithium's therapeutic window for human clinical use is between: 0.6 to 1.2 mMol/L serum levels. Thus lithium plasmatic levels below the minimum limit result in a higher than acceptable relapse risk of manic-depressive symptoms, while serum levels above the maximal limit produce intoxication.[16,17] Although the security range has been established, this does not exclude the occasional toxic consequences of lithium. Neurological features may be encountered early, including drowsiness, slurred speech, psychomotor slowing, and impaired memory and, in severe cases, seizures, coma and death.[18] Given that lithium is exclusively eliminated by kidneys, its therapy is associated with a progressive decline in creatinine clearance and an estimated interval of 20 years until end-stage renal failure.[19,20]

The toxicity of lithium is related to its distribution, which is determined, mainly, by passive diffusion modulated by bicarbonate. Lithium can be also transported by ionic pumps with ATP consumption, and through channels coupled to the Na+ driving force that in turn is maintained by the associated Na+/K+ pump.[21] The active transport of lithium allows its concentration at specific systems and organs of the body, such as central nervous system (CNS) and muscles.[22] Actually, lithium levels vary even in the CNS, where there is a higher concentration in the brain than in the spinal cord,[23] and the lithium distribution through brain structures may also vary according to the administered doses.[24] In this way, measurements of lithium in a system may not necessarily predict its levels in another one. Therefore, this work aimed at evaluating the accuracy and relevance of the plasmatic lithium measurement aiming to estimate CNS levels in well-controlled laboratory conditions.

Method

Three-month-old Wistar rats, of both genders, were housed under standard laboratory conditions with light/dark cycle (12/12h), lights on at 7:00 AM, and food and water were provided ad *libitum*. All experimental protocols were approved by the Animal Care and Use Ethics Committee of Universidade Federal de São Paulo. Animals were distributed to experimental groups according to the different treatments over a period of six weeks. Thus, animals fed with conventional rat chow (Nuvilab, Brazil) constituted the control group (n = 10); animals that received a 0.255% rich lithium chloride (LiCl) rat chow constituted the 0.255 group (n = 8); and finally, group 0.383 was composed by 8 animals fed with a 0.383% LiCl rich feed. The 0.255% lithium supplemented feed has already been demonstrated to result in a plasmatic level within the therapeutic ranges.[25] Here we chose to compare the plasmatic levels and the behavioral effect of this dose with a 50% higher dose (0.383%). The body weight of all animals was evaluated over the six-week period. At the end of the treatment, the animals were tested at an open field and elevated plus maze task. And finally, blood samples were collected through a small incision on the tail vein, for measuring lithium plasmatic concentration.

1. Treatment

The lithium rich feed was produced by mixing conventional commercial chow dust with a watery lithium chloride solution of known concentration. After that the rat chow pellet was extruded and dried in an oven.

2. Open field task

After a habituation period, the exploratory behavior of all animals was analyzed at an 87 cm diameter wooden open field surrounded by a 20 cm high polyvinyl chloride wall. The apparatus's floor was divided in twelve compartments by lines painted at the surface of a wooden disk.

In the beginning of the task, the animal was positioned in the center of the apparatus, and was allowed to freely explore it for three minutes. The number of times the animals crossed a line, i.e. compartments crossed, was measured as a quantification of horizontal locomotor activity. The vertical locomotor activity was assessed by measurement of the rearing frequency. The time spent in freezing and grooming behavior was also recorded (as an indication of fear and anxiety emotional states).

3. Elevated plus maze

The maze apparatus was comprised by two arms surrounded by 30 cm high wooden walls that cross with two open arms, resulting in a central compartment. Both arms were 49 cm in length and 10cm in width and were placed 50 cm above the floor.

After a five-minute task, the elevated plus maze allows to investigate the anxiety emotional state of rats through the quantification of the time spent in each arm. This apparatus also allows the acquisition of data from horizontal locomotor activity by means of quantifying the frequency of entrance in each compartment.

4. Plasmatic lithium level measurement

Finally, blood samples of all animals were collected through a small incision on tail vein, after ether anesthesia. Then, the plasmatic lithium levels of these blood samples were measured by the Crown-Ether method.

5. Statistical analyses

Data, that fit into a normal distribution, as feed ingestion and body weight, were analyzed by one-factor repeated measure ANOVA. Data that did not fit a normal distribution (Open field, elevated plus maze and plasmatic lithium level) were analyzed by Kruskal-Wallis, followed the post-hoc test of Multiple Comparison of Mean Ranks – MCMR. For all statistical procedures, it was adopted a significance level (α) of 0.05.

Results

1. Feed consumption and body weight progression

Groups treated with both doses of LiCl-rich feed presented a reduced chow consumption as compared to the control group ($p = 0.03$). However, in spite of the food ingestion attenuation caused by lithium intake, no difference was found between the higher (group 0.383) and lower doses (group 0.255), as indicated in Figure 1.

Both groups treated with lithium rich chow showed a diminished body weight gain as compared with control animals (repeated measures ANOVA, $p \leq 0.0001$ followed by Tukey-Kramer, $p \leq 0.05$). As shown in Figure 2, lithium-treated animals gained mass more slowly than control rats, from the third treatment week onwards, resulting in a lower body weight at the end of lithium administration.

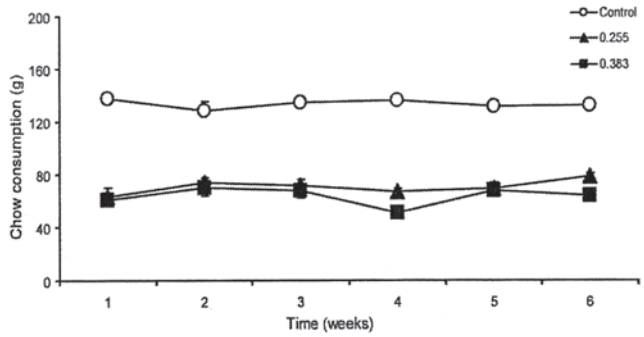

Figure 1 - Food consumption over time for the 3 groups
Lithium diet addition implicated in diminished food ingestion for both 0.255 and 0.383 groups as compared to controls. Note that despite the initial lower chow ingestion for both lithium-treated groups we observed no variation of the amount ingested over the duration of the experiment (One way repeated measure ANOVA, (p = 0.032).

2. Open field

Animals fed with higher lithium dose chow (group 0.383) showed a smaller (horizontal) locomotor activity through central compartments of the open field as compared to the control group (Figure 3B) ($p = 0.024$, Kruskal-Wallis followed by MCMR post-hoc test). In spite of a 2.4 times smaller locomotion presented by group 0.383 relative to group 0.255, this did not reach statistical significance ($p = 0.0587$). In addition, group 0.383 showed a significant 45% smaller rearing frequency than the control group did and a significant 55% reduction as compared to the 0.255 group (Figure 3C). Moreover, group 0.383 presented a 5.2 and 3 times longer mean freezing duration than the control and 0.255 groups did, respectively (Figure 3D), but this, however, only achieved significance between group 0.383 and controls ($p = 0.044$, Kruskal-Wallis followed by MCMR post-hoc test). In contrast, the comparison between animals that received the smaller dose of LiCl and control animals produced only a trend ($p = 0.079$) in increasing the duration of freezing.

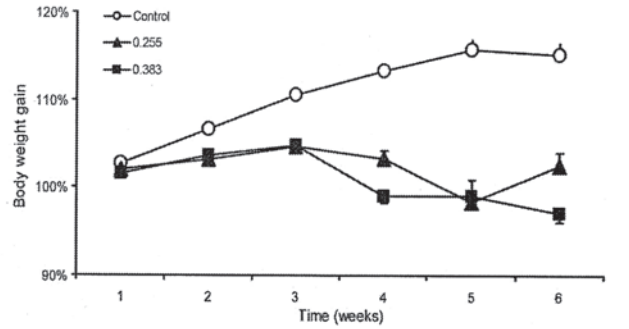

Figure 2 - Control animals had a significant weight gain over the duration of the experiment.
In contrast, addition of lithium to the diet resulted in a lack of significant body weight variation over the duration of the experiment that achieved statistical significance after the fifth week of treatment (One factor repeated measure ANOVA, $p \leq 0.0001$).

3. Elevated plus maze

While the 0.383 group presented the same total entrance (open plus closed arms) frequency as control animals, the 0.255 group, in spite of having received a smaller dose of lithium, showed a greater absolute number of entries than did both other groups ($p = 0.008$, Kruskal-Wallis followed by MCMR post-hoc test), as illustrated by Figure 4A. Moreover, animals fed with the 0.255% lithium-rich diet demonstrated a reduced relative entrance frequency in the open arms (Figure 4B), as compared to the control and 0.383 groups ($p = 0.03$, Kruskal-Wallis followed by MCMR post-hoc test). Nevertheless, such a difference should be carefully analyzed, given that there was no difference among the relative time spent in the open arms by any experimental group (Figure 4C - Kruskal-Wallis, $p > 0.05$). Therefore, the effects induced by the treatment with the smaller dose of lithium chloride should be considered as a disturbance on animal's behavior (thus not similar to the human mood disorder), rather than be classified as a parallel to human anxiety.

4. Plasmatic lithium level measurement

At the end of the six-week treatment, the plasmatic lithium level of the groups treated with both doses of LiCl was significantly higher than that of control animals ($p = 0.013$, Kruskal-Wallis followed by MCMR post-hoc test). However, in spite of having received lithium doses with 50% difference, groups 0.255 and 0.383 yielded the same lithium serum concentration (Figure 5, $p = 0.73$).

Discussion

1. Feed consumption and body weight progression

Animals fed with both doses of LiCl showed reduced food ingestion, what confirms data already collected,[26] but the chow consumption by both lithium-treated groups was always the same, excluding the possibility that the same lithium serum levels could result from distinct amount of drug ingestion. Given that lithium intake can result in visceral illness,[27-29] it was expected that lithium-treated animals would present an attenuated body weight gain, as it is indeed confirmed in Figure 2.

2. Open field task

A central zone locomotion difference was encountered between control and 0.383 groups. Given that, the total locomotion of all groups was equal (Figure 3B, $p = 0.2346$), and such a divergence should be considered as a signal of avoidance during the exploration

of the apparatus, rather than altered horizontal locomotion. The lack of divergence in total locomotion among the groups presented here is in disagreement with already published studies,[27,29,30] which demonstrated a suppressive effect of lithium salts over the horizontal locomotor activity. In the work of Smith,[27] rats developed a lithium plasmatic level similar to that shown here (0.66 mMol/L ± 0.05), although they clearly showed a smaller locomotion after treatment with 1.5 mMol/L lithium for four days. It is likely that the nature of the lithium treatment employed here resulted in a heterogeneous lithium distribution through brain structures. Therefore, the animals evaluated in the present study showed more variability in open field variables analyzed, masking a possible effect of lithium on the evaluated parameters. While there are studies in which rats had received a precise amount of lithium salts by gavage,[27] or by intraperitoneal administration,[30] here, lithium was supplied in supplemented chow. This chow was freely available for the rats to ingest. Obviously the consumption rate can thus be more variable. Moreover, the difference in the duration of the lithium treatment could explain why the treatment employed here failed in corroborating the suppressive effect of lithium salts over horizontal locomotor activity, as demonstrated in the above mentioned studies. Such papers shown a suppressive effect of lithium upon acute drug administration,[30] or have sustained the lithium treatment for a shorter time[27] than performed here, suggesting a possible habituation to lithium's effects during a long consumption time.

Despite the lack of a suppressive effect of lithium treatment over the horizontal locomotion, the group fed with the higher lithium dose explored the central compartment of the apparatus less often than the control group did. Moreover, the high-dose lithium-treated group also showed longer freezing time in this test than control animals. These behavioral consequences could be seen as suggestive of an anxiogenic effect of this high-dose treatment. Yet this finding was not confirmed by the more specific test for the assessment of anxiety (the elevated plus maze). Therefore, the smaller time spent in the central compartment of the open field test by the lithium-treated group under the higher dose does not unequivocally equate with greater anxiety, and might be more conservatively rated as an avoidance state

The higher dose of lithium reduced the vertical locomotor activity, as shown by a diminished rearing frequency of the 0.383 group. This suppressive action of lithium strengthens the data on the already known effect of lithium.[27,30,31] Interestingly, the doses of lithium did not produce the same behavioral effect in both groups of lithium-fed animals, in spite of the similar changes observed in the lithium serum levels of both groups. Therefore, lithium serum measurement did not allow the prediction of its levels in the brain.

3. Elevated plus maze task

The smaller open/total arms entrance rate of the 0.255 group as compared to control animals suggests a preference of the lithium-

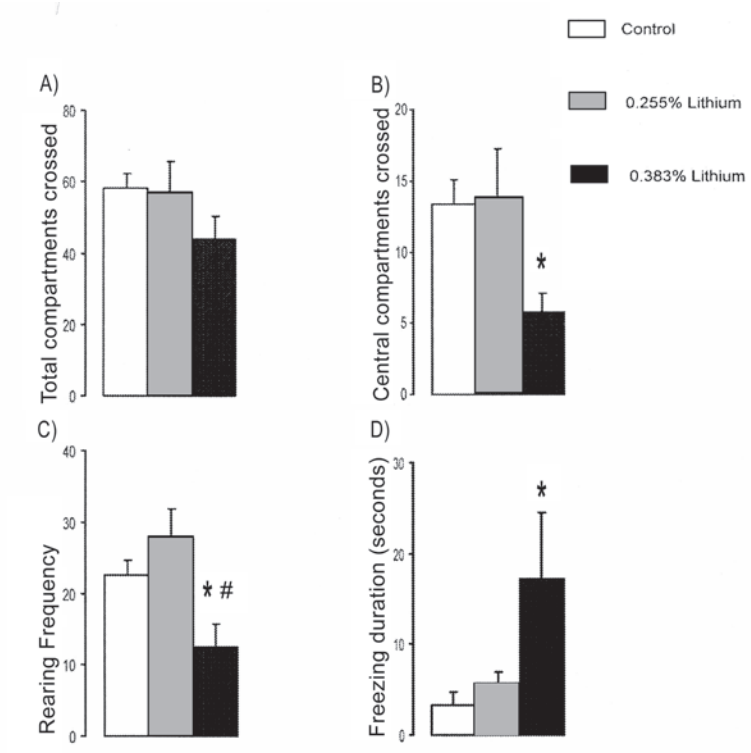

Figure 3 - Performance of the animals in the open field task.
In A note the diminished total locomotion of the 0.383 group as compared to the other groups that however did not reach statistical significance (Kruskal-Wallis, p = 0.23). In B see a clear reduction of central locomotion by group 0.383 as compared to controls. In C note the diminished rearing frequency of group 0.383 as compared to both other groups. Finally, in D note the greater freezing time of animals in group 0.383 as compared to controls. Data expressed as mean ± SEM. * different from controls and # different from the 0.255 group, Kruskal-Wallis followed by Multiple Comparison of mean ranks post-hoc test, p < 0.05).

treated rats for the closed – and thus, less exposed – arms. This sign of anxiety was not confirmed as such given the lack of an increase of time spent in the closed arms (the "safer" compartment). Despite the well known mood stabilizing properties of lithium over emotional disorders, its anxiolytic or anxiogenic effects over emotional state of normal animals are less explored. Wood and colleagues, in agreement with our current findings, did not find an anxiogenic consequence of the treatment with lithium carbonate supplemented chow for 35 days in adult rats.[32] On the other hand, Youngs and colleagues argued that adolescent rats (45-day old, rather than 3 months of age as employed in the present study) maintained an anxiogenic effect of lithium for some weeks after the end of treatment,[33] in spite of having been fed with lithium carbonate supplemented chow for a shorter time than here. Regardless of the above discrepancies or similarities with our current findings, we should remark that here we observed a dissimilar outcome for the two lithium-treated groups in the elevated plus maze despite similar plasmatic lithium levels. Animals that received the lower lithium dose (the 0.255 group) presented a greater number of entries in both open and closed arms as compared to others groups. Such a difference might be an indicative of a greater horizontal activity produced by the lower lithium dose in this test rather than an indication of anxiety. Nevertheless, this enhancement in horizontal locomotion must be carefully interpreted, because the same effect was not observed in the open field task. Moreover, this effect is in disagreement with already-published reports, which found no change in the elevated plus maze as a consequence of the treatment of Sprague-Dawley rats with 0.24% lithium carbonate supplemented chow for 35 days.[32] Therefore here again, lithium plasmatic measurements did not seem to clearly indicate what would be the lithium levels in specific brain structures related to the analyzed behavior.

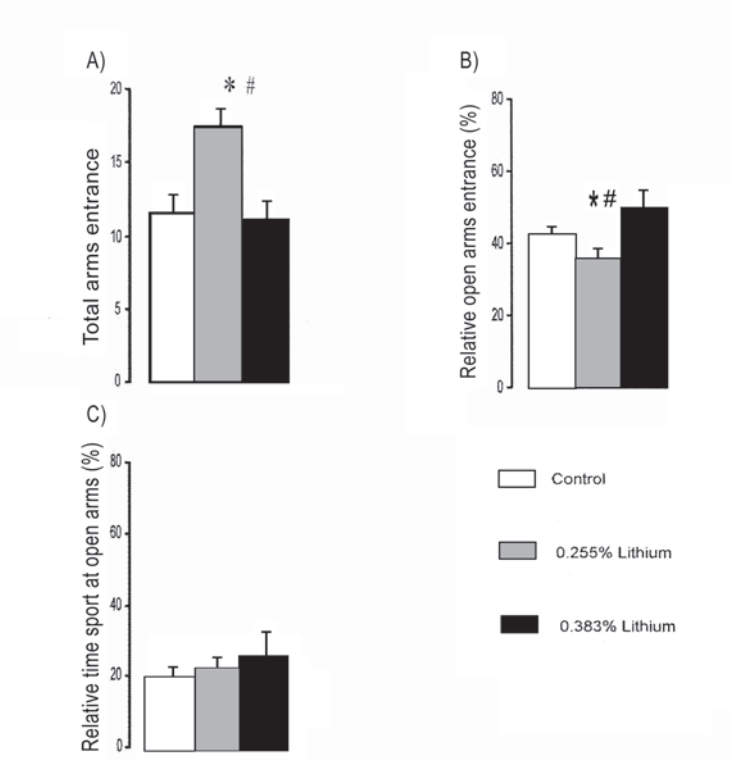

Figure 4 - Performance of the animals in the elevated plus maze.
In A observe the augmented entry in the arms of the maze by group 0.255 animals as compared to both controls and 0.383 group. A) The lower lithium dose produced an increase in total arms entrance frequency, suggesting an enhancement in locomotor horizontal activity; B), entrance in the open arms/entrance in closed and open arms was significantly less in the low lithium dose group as compared to controls and the other lithium group; C) time spent in the open arms/time spent in the open and closed arms did not differ among the 3 tested groups. * indicates a difference from controls; # indicates a difference between the two lithium treatments (0.255 and 0.383 groups) Kruskal-Wallis followed by Multiple Comparison of Mean Ranks post-hoc test, $p \leq 0.05$

4. Plasmatic lithium concentration

Lithium is not a perfect drug due to the occurrence of short- and long-term side effects. However, it is the most effective drug to treat classical bipolar disorder, whose consequences may be even worse than lithium's toxicity. Without some form of therapy, up to 20% of bipolar disorder patients will commit suicide,[34] and lithium treatment reduces this rate by a factor of eight. Therefore, lithium therapeutic effects compensate its toxic effects making it an alternative that should be better understood rather than so easily discarded.

In spite of new methods that have been developed to better estimate a precise dose of lithium,[2,3,5,17,35-39] many of these technologies are based in the serum level measurement. However, lithium does not have a uniform distribution through body compartments. In fact, its concentration in the brain, where it exerts its therapeutic actions, is higher than in the blood.[6,7,24] It is very difficult to estimate CNS lithium levels, as serum and red blood cell concentration does not closely correlate with brain levels, with the coefficient for the strongest correlation (between serum and brain) remaining at 0.66.

Some authors have already pointed that lithium serum concentration did not satisfactorily reflect the tissue level, and therefore, could not predict the occurrence of toxicity.[1,40] Here, some behavioral effects of two doses of lithium (after a six-week treatment) were measured at the same time that plasmatic lithium concentrations were obtained. According to the data presented above, both lithium doses resulted in similar plasmatic levels, although rat chows had different lithium concentrations and similar food consumption levels. Nevertheless, these two groups with equal lithium plasmatic levels, showed significantly different behavioral responses in some of the behavioral tests evaluated here. This suggests that the different lithium concentration of the ingested food

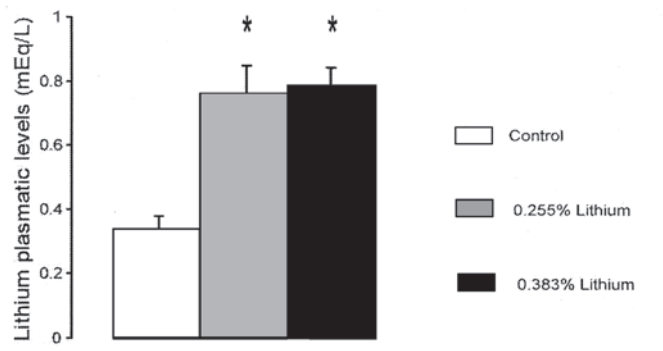

Figure 5 - Plasmatic concentration of lithium at the end of six weeks treatment.
Both lithium-treated groups presented lithium levels higher than controls and between therapeutically ranges. However, 0.383 group showed the same lithium serum concentration as 0.255 group, in spite of the former animals had been fed with a 50% higher lithium rich feed and having a similar consumption level. * Different from controls (Kruskal-Wallis $p = 0.014$ followed by Multiple Comparison of Mean Ranks post-hoc text, $p \leq 0.05$)

resulted in different brain concentrations and/or distributions, and, although undetectable by serum monitoring, may lead to distinct behavioral responses.

The experimental evidence presented here, in well-controlled laboratory conditions, corroborate the notion that lithium serum measurement does not provide an estimative of brain lithium level that is precise enough to predict its effects over the CNS.

Conclusion

The measurement of serum lithium concentration, which is the diagnostic method currently used to monitor lithium levels in bipolar patients, did not detect changes in brain lithium levels, despite an increase of 50% in the lithium dose ingested. Behavioral tests, however, pointed to different cognitive performances, suggesting that the groups had different lithium brain concentrations or distributions that were sufficient to change their cognitive state. Therefore, serum monitoring seems to be inaccurate to ensure the safe use of lithium. New methods to predict the best lithium dosages for patients are required to allow the growing clinical use of lithium salts.

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Disclosures

Writting group member	Employment	Research grant[1]	Other research grant or medical continuous education[2]	Spekear's honoraria	Ownership interest	Consultant/ Advisory board	Other[3]
Thiago Lima	UNIFESP	FAPESP	---	---	---	---	---
Marcela Blanco	UNIFESP	FAPESP	---	---	---	---	---
Jair Guilherme dos Santos	UNIFESP	FAPESP	---	---	---	---	---
Carolina Coelho	UNIFESP	FAPESP	---	---	---	---	---
Luiz EMello	UNIFESP	FAPESP CNPq	FAPESP CNPq	---	---	---	---

** Modest*
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For more information, see Instructions to Authors.

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