Pharmacology Biochemistry & Behavior, Vol. 19, pp. 257–261, 1983. © Ankho International Inc. Printed in the U.S.A.

Strain Dependent Rate of Li^+ Elimination Associated with Toxic Effects of Lethal Doses of Lithium Chloride in Mice

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Received 21 November 1982

EL-KASSEM, M. AND S. M. SINGH. *Strain dependent rate of Li^+ elimination associated with toxic effects of lethal doses of lithium chloride in mice.* PHARMACOL BIOCHEM BEHAV **19**(2)257–261, 1983.—Strain differences in response to the administration of two lethal doses (700 and 900; mg/kg) of lithium chloride were studied in eight week old males from six genetic strains of mice. Two parameters were considered: (a) toxicity (time to death) and (b) hypothermia. Lithium distribution in the body (blood, seven tissues, excreta and urine) were evaluated for each strain following IP injection of 200 mg/kg dose of LiCl. The strain differences were significant for toxicity. The order of susceptibility of the strains was 129/ReJ>S.W.>C3H/S>DBA/2 = Balb/c>C57/6J with a 15-fold difference between the most susceptible and the least susceptible strain at the 900 mg/kg dose. Strain differences for hypothermic response at both doses were not significant. Significant strain differences were also observed for lithium distribution in different parts of the body, excreta and urine. The concentration of Li^+ found in urine and excreta was positively correlated with resistance (time to death at 900 mg/kg LiCl) to the toxic effect of lithium. The lithium concentration in blood, muscle and lung on the other hand reflected a negative correlation with toxicity. The susceptibility of a strain could be characterized by its inherent lithium excretory ability, particularly through urine. It may suggest an involvement of membrane transport mechanisms in determining toxicity to lithium compounds.

Lithium chloride Toxicity Hypothermia Pharmacodynamics Strain differences

LITHIUM compounds have been used in the treatment of manic disorders since 1949 [12]. They have a rather low therapeutic index [8] and may be toxic to some individuals at recommended doses [22]. They are clearly toxic to all individuals at higher doses and the lethal dose may vary among individuals. The understanding of the factors affecting lithium toxicity and pharmacodynamics is, therefore, important for proper use of this salt as a drug. Although the mode of action of lithium is unknown [12], it induces variable responses both in humans and laboratory animals [13]. It has been suggested that genetic factors may play a role in various lithium induced responses (e.g., behavioral, clinical, toxicity, etc.) in man and laboratory animals [3, 5, 11, 17, 18, 19, 20]. The present study was carried out to establish the role of genetic determinants and their possible mode of action in determining lithium induced toxicity. We used six inbred strains of mice. The inbred lines offer a convenient model system to evaluate the role of genetic contributors in drug action and pharmacodynamics and have been extensively used in such studies. We first determined the toxicity and hypothermia induced by two lethal doses of LiCl. The distribution of Li^+ in different tissues, blood, excreta and urine was subsequently determined following IP injection of LiCl to evaluate the role of genetically influenced Li^+ pharmacodynamics in causing toxicity.

METHOD

Eight week old males weighing 20–30 g from six strains of mice (BALB/c, C57BL/6J, C3H/S, DBA/2, 129/ReJ and S.W.) were used for this study. BALB/c, C3H/S and DBA/2 strains were obtained from Canadian Breeding Farms, St. Constant, Charles River, Quebec, while C57BL/6J, 129/ReJ and S.W. strains were obtained from The Jackson Laboratory, Bar Harbor, ME. The mice were isolated in individual plastic cages in a thermostatically controlled room (23°C) on a 14:10 hr light-dark cycle (lights on at 0800; off at 2200 hr) one week prior to lithium injection. A commercial mouse chow diet (Purina Canada Inc.) and water were continuously available during experimentation.

Lithium chloride toxicity and its induced hypothermic response were determined in mice given intraperitoneal injections of one of two lethal doses (700 mg/kg or 900 mg/kg) at room temperature. Lithium distribution in the body was determined one hour after an intraperitoneal injection of 200 mg/kg LiCl. All injections had a constant volume of 1 ml. Each dose was given to a group of six mice from each strain with the same dose of sodium chloride being given to two control mice for comparison. Injections were always given between 1000 and 1400 hr to avoid cyclical (circadian) variations [9]. The drop in rectal temperature following Li treatment was recorded using a thermistor probe connected to a

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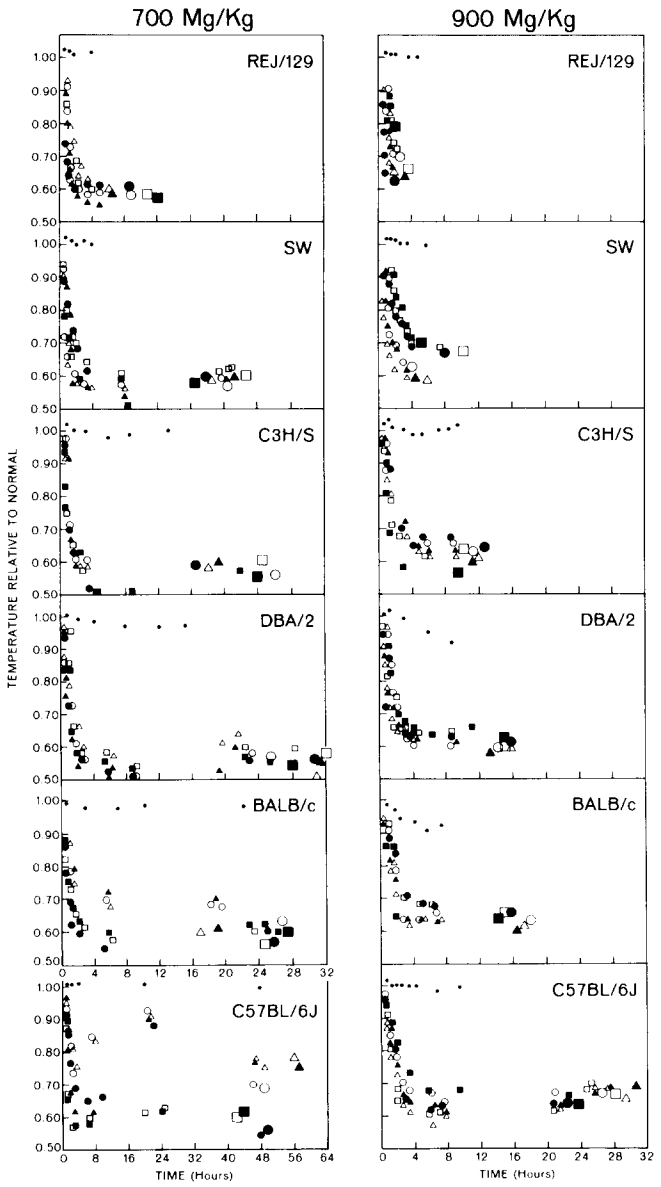

FIG. 1. Hypothermic response to LiCl in six genetic strains of mice at two lethal doses (700 and 900 mg/kg). Six mice (○, ●, □, ■, △, ▲) per strain were treated with two control mice injected with NaCl (·). Larger symbols indicate time to death. Relative temperature is the temperature at time t divided by the temperature at time zero.

YSI-telethermometer. The time of death following 700 and 900 mg/kg doses was noted for each individual. Animals used for lithium distribution study were killed by cervical dislocation. Samples from eight tissues (liver, kidney, heart, lung, muscle, blood, brain and eye), urine (directly extracted from the bladder) and all the excreta recovered over one hour were collected. Lithium concentrations were determined by atomic absorption spectrophotometry [1] for each sample. Analyses of variance were carried out to evaluate the significance of differences between strains in toxicity (time to death), hypothermic response and lithium distribution in the body. Comparison between pairs of groups were made by Duncan's Multiple Range test. Statistical rank correlations were used to evaluate the relationship between toxicity and other variables studied.

RESULTS

Toxicity

In general there are two approaches to the evaluation of toxicity of a given compound on a biological system. The first approach uses LD_{50} reflected in the form of a dose lethal to 50% of the individuals tested, while the other evaluates the effect of a lethal dose. In the second approach, time to death is among the suitable indicators used for comparing group or individual differences. We used the second approach and recorded time to death of six individuals from each of six strains following injection of two lethal doses (700 mg/kg and 900 mg/kg). Results are given in Fig. 1. When time to death was analysed by analysis of variance, the F value yielded significant between strain differences at both doses, 700 mg/kg; $F(5,30)=62.47$ and 900 mg/kg; $F(5,30)=118.73$, while within strain differences were not significant. The time to death (mean and S.D.) at two lethal doses of LiCl shows the relative susceptibility of different strains (Table 1). Here, although the relative susceptibility of most strains stays the same at the two doses, the strain differences are much more pronounced at the higher dose. The differences between the most resistant strain (C57BL/6J) and the most susceptible strain (129/ReJ) reflected a 6-fold difference at the 700 mg/kg and a 15-fold difference at the 900 mg/kg dose of LiCl. It may be noted from Fig. 1 and the S.D. values given in Table 1 that within strain differences are very small indeed. These strain differences therefore may reflect on the inherent property of these lines.

Hypothermia

Changes in body temperature following IP injection of two lethal doses (700 mg/kg and 900 mg/kg) were recorded for six male mice per strain. NaCl of the same dose was used as control on two mice in each set. The normal body temperature was variable (35°C–38°C) among individuals and not strain specific. In order to make appropriate comparisons (within and among strains) for the hypothermic response, the drop in temperature over time was standardized by use of relative temperature (temperature at time t divided by temperature at time zero; prior to the IP injection). The rate of the temperature drop was similar among strains. Most of the decline in temperature occurred within the first hour following LiCl treatment, with lower levels reached at the 700 mg/kg dose than at the 900 mg/kg dose for all strains. The minimum temperature was reached just before death at both doses. When the minimum relative temperature was analysed by analysis of variance it gave a nonsignificant F value for strain differences at both LiCl doses. However, at both doses DBA/2 had the lowest relative temperature followed by C3H/S.

Distribution of Li^+ in the Body

Table 2 shows the levels of Li^+ in eight tissues plus excreta and urine one hour following injection of a 200 mg/kg dose of Licl in different strains of mice that were also evaluated for the toxic effects of this salt. In general, about 10% of the Li^+ is present in excreta and 50–80% is eliminated

TABLE 1

TOXICITY (TIME TO DEATH) FOLLOWING TWO LETHAL DOSES OF LITHIUM CHLORIDE

LiCl Dose		Strains 129/ReJ	S.W.	C3H/S	BALB/c	DBA/2	C57BL/6J
700 mg/kg	Mean (hr.)	8.4	19.7	20.4	23.4	30.5	49.8
	S.D.	2.7	2.3	3.7	4.1	3.5	6.2
900 mg/kg	Mean (hr.)	1.8	6.1	10.7	15.9	14.5	26.5
	S.D.	0.6	2.4	1.4	1.0	0.6	3.5

TABLE 2

LITHIUM DISTRIBUTION IN THE BODY ONE HOUR AFTER INTRAPERITONEAL INJECTION OF 200 mg/kg LITHIUM CHLORIDE

Tissues/ Strains	Lithium concentration (mmol/kg) (Mean ± SD) Liver	Kidney	Heart	Lung	Muscle	Blood*	Brain	Eye	Excreta	Urine
129/ReJ	1.448	4.567	3.962	4.940	1.903	4.351	0.478	1.586	3.843	24.880
	±0.083	±0.173	±0.126	±0.256	±0.068	±0.603	±0.028	±0.265	±0.789	±5.634
S.W.	1.481	3.973	2.945	4.245	1.861	3.910	0.482	1.532	5.095	27.833
	±0.209	±0.450	±0.985	±1.231	±0.277	±0.725	±0.133	±0.212	±2.065	±15.685
C3H/S	2.735	4.771	3.108	3.364	1.783	3.370	0.512	1.396	6.118	27.517
	±0.493	±0.143	±0.440	±0.286	±0.204	±0.391	±0.023	±0.063	±0.781	±2.117
DBA/2	2.441	4.689	4.368	4.112	1.914	3.935	0.558	1.573	5.312	30.779
	±0.382	±0.363	±0.270	±0.341	±0.124	±0.302	±0.055	±0.079	±1.407	±4.678
BALB/c	2.427	4.162	4.398	3.905	1.774	3.818	0.537	2.107	4.800	35.529
	±0.177	±0.379	±0.264	±0.450	±0.098	±0.550	±0.066	±0.234	±0.322	±3.264
C57BL/6J	1.172	3.590	2.566	2.937	1.552	2.244	0.464	1.070	14.133	95.600
	±0.106	±0.237	±0.221	±0.246	±0.286	±0.234	±0.027	±0.221	±1.874	±5.025
r†	−0.049	−0.569	−0.269	−0.845‡	−0.834‡	−0.859‡	−0.037	−0.330	0.841‡	0.858‡

*Lithium concentration in the blood is measured in mmol/l.
†Coefficient of correlation between toxicity at 900 mg/kg dose and lithium concentration following 200 mg/kg dose.
‡Significant correlation ($p<0.05$) based on t-statistic at 4 df. Others not significant.

through urine. The remainder is distributed in different tissues. The Li^+ distribution in the samples studied was not evenly distributed in all strains. In the most susceptible strain (129/ReJ) about 55% of the Li^+ is eliminated through excreta (7.4%) and urine (47.9%). On the other hand, in the most resistant strain (C57BL/6J), over 87% of the Li^+ observed is found in excreta (11.2%) and urine (76.3%). Other strains also show a similar pattern and the rank correlation between time to death and Li^+ in excreta ($r=+0.841$) and urine ($r=+0.858$) over all strains is significant ($p<0.05$). The total Li^+ in eight organs ranged from 12.5% in C57BL/6J to 44.7% in 129/ReJ, and reflects a negative rank correlation with time to death. Furthermore, when the Li^+ level of individual organs is assessed in relation to time to death of different strains, it yielded a significant ($p<0.05$) negative correlation for three tissues only (lung, $r=-0.845$; muscle, $r=-0.834$ and blood, $r=-0.859$). These results suggest that the relative resistance of a strain to a lethal dose of Li^+ is associated with their ability to eliminate Li^+ from the body, particularly through urine, and to retain lower amounts of the salt in different tissues.

DISCUSSION

Inbred strains have been widely used in pharmacogenetic research. Inbreeding leads to effective homogenization of the genotype of animals within strains, and, because the homogenization is largely stochastic, genetic differences exist between and among strains. Thus the demonstration of strain differences in a trait constitutes prima facie evidence of genetic influence on that trait. Variable response to drugs and chemicals will depend on the fate of the drug in the body. Features such as the binding of the drug with a given protein, formation of a particular metabolite and the active transport of the drug may depend on the product of a single gene and follow a monogenic pattern of inheritance. On the other hand, kinetic parameters such as plasma half-life or clearance of a chemical often depend on several simultaneously operating factors. Most pharmacokinetic data, therefore, will have the tendency of being multigenic and multifactorial [10].

Lithium salts are among the commonly used drugs known to induce variable clinical and behavioural responses [12] and may be lethal even at seemingly low doses [22]. The concentration of lithium is seemingly high in blood shortly after IP injection. It passes through other organs such as liver, heart and kidney and eventually accumulates in the brain several hours after the injection [16]. The effect of lithium on brain and brain tissues is well recognized, and forms the basis of its use in mental disorders. However the actual mechanism involved remains obscure. Furthermore,

lithium also competes with such cations as Na^+ and Mg^{2+} in biological systems at sites which carry them [2,14]. The potential for such cation-cation antagonism may involve modifying conductance and possibly membrance transports. We attempted evaluation of two responses: toxicity (time to death) and hypothermia produced by two lethal doses of lithium chloride in six genetic strains of mice. Although the effect of lithium on thermoregulation has been previously investigated in rats [21] and mice [6,13], the mode of action of lithium in thermoregulation remains unknown. The effect of lithium on thermoregulation is suggested to be due to alterations in the hypothalamic Ca^{2+}-Na^{2+} ratio [7,21]. In this context Edelfors [4] has shown that lithium could replace a substantial fraction of intracellular sodium in the brain. Although our data does not contribute to the clarification of this hypothesis, they do point out that the effect of lithium in thermoregulation is immediate and that the drop in temperature is unrelated to the lethality.

In our evaluation of the toxic effect of lithium, we used time to death at lethal doses rather than the often used LD_{50} estimates. The time to death is a sensitive indicator for comparing variable toxicity and corresponds well with other measures of toxicity including LD_{50}, especially if repeated on more than one lethal dose in a replicated trial. The order of susceptibility of the mouse strains at the 700 mg/kg and 900 mg/kg doses was almost identical (129/ReJ>S.W.>C3H/S> DBA/2 = Balb/c>C57BL/6J at the later dose). Smith also found the order of lithium susceptibility in four of these strains to be C3H/S > DBA/2 > Balb/c > C57BL/6J based on LD_{50} values [20] and lithium carbonate induced effects of activity-suppression [18].

Possible causes of toxicity and lethality following Li^+ IP administration may fall in three categories; its action on brain, hypertonicity caused by Li^+ in peritoneal space and retention of Li^+ in the body. The action of Li^+ on brain has been considered in some detail by others [15,19]. We are able to exclude the hypertonicity of LiCl in peritoneal space as a possible cause in determining lithium toxicity, as IP injections of equivalent NaCl did not cause any toxic effect and lethality. These results follow some earlier reports [13], but do not rule out possible peripheral toxic effects of lithium. For example, Li^+-induced diarrhea may result in sodium loss. This can reduce lithium excretion due to the fact that Li^+ transport is closely related to Na balance [4]. The eventual outcome of Li^+ retention may mean increased toxicity. Smith [20] observed that the toxicity of lithium in inbred mice cannot be fully predicted by the level of lithium in blood and tissues. He suggested that perhaps some genetic factors with yet undefined function may be important in determining lithium toxicity.

From this study, which evaluated lithium levels in seven tissues (liver, kidney, heart, lung, muscle, brain and eye) and blood along with excreta and urine (Table 2), we are able to reflect on the possible functional role of genetic determinants in causing lithium toxicity. In this context, the lithium level studied in excreta and urine are of utmost significance. The strong positive rank correlation between the lithium elimination from the body particularly through urine and time to death following 900 mg/kg IP injection of lithium, suggests the involvement of a strain specific membrane transport mechanism as a possible cause of differential lithium toxicity. Although the involvement of membranes in determining toxicity is suggested, to our knowledge this is the first investigation that provides the evidence for such a hypothesis for lithium related toxicity. Our findings of significant strain differences in the concentration of lithium in blood and other tissues given the same dose of LiCl follows this hypothesis. Here, the Li^+ concentration in blood, muscle and lung is negatively correlated with time to death in different strains of mice and suggests that genetic factors involved in elimination of the salt, possibly through membrane transport systems, are important factors in influencing the pharmacodynamics of lithium in mice and ultimately in determining the relative susceptibility for the toxic effects of lithium. It may be pointed out, however, that although the retention of Li^+ is an important factor, other genetic factors involved in determining the toxic effects of lithium can not be ruled out.

ACKNOWLEDGEMENTS

This research was supported by a Natural Sciences and Engineering Research Council, Canada grant to S.M.S. We thank Drs. John Steele and David Ogilvie for technical assistance and Dr. Roger Green for statistical advice.

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