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Lithium Toxicology: Effect of Isotopic Composition on Lethality and Behavior

G. J. ALEXANDER

Neurotoxicology Research Unit, New York State Psychiatric Institute, New York, NY 10032

K. W. LIEBERMAN

Psychobiology Study Unit, Department of Psychiatry and Department of Biochemistry Cornell University Medical College, New York, NY 10021

M. OKAMOTO

Department of Pharmacology, Cornell University Medical College, New York, NY 10021

P. E. STOKES

Psychobiology Study Unit, Department of Psychiatry and Department of Medicine (Endocrinology) Cornell University Medical College, New York, NY 10021

AND

E. TRIANA

Psychobiology Study Unit, Department of Psychiatry Cornell University Medical College, New York, NY 10021

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ALEXANDER, G. J., K. W. LIEBERMAN, M. OKAMOTO, P. E. STOKES AND E. TRIANA. *Lithium toxicology: Effect of isotopic composition on lethality and behavior.* PHARMAC. BIOCHEM. BEHAV. **16**(5) 801–804, 1982.— Naturally occurring lithium consists largely of the stable lithium-7 isotope but it contains 7.6% of a second stable isotope, lithium-6. Biological effects of these two isotopes were not identical. When administered in isotopically pure form, the choride of lithium-6 was more toxic after acute intake than the chloride of pure lithium-7: its LD_{50} in Swiss-Webster mice was 13.2 mEq/kg as opposed to 15.9 mEq/kg for the salt of the heavier isotope and 14.9 mEq/kg for that of the natural mixture of the two isotopes. A single injection of one or the other pure isotope in a constant dose, 14.5 mEq/kg led to a 90% mortality in mice given lithium-6 but only 10% in mice given lithium-7. Side effects such as hypoactivity, ataxia, intense perspiration and diarrhea appeared within 10 min after administration of toxic doses of lithium-6 but more gradually after lithium-7. The differences between the isotopes in toxicity and rate of appearance of effects on spontaneous motor activity were significant at the $p<1\%$ level.

Lithium Isotopes Stable isotopes LD_{50} Behavior Hypoactivity Lethality Toxicology LiCl

CHRONICALLY administered salts of lithium (Li) have been highly effective in preventing recurrences of mania in patients with bipolar affective disorders and may be effective in depressions [3,5]. The necessity for prolonged treatment has called our attention to the toxic side effects of lithium.

Lithium exists in two stable (i.e., non-radioactive) isotopic forms, lithium-6 (Li-6) and lithium-7 (Li-7). The nucleus of the former consists of 3 protons and 3 neutrons, of the latter of 3 protons and 4 neutrons. The isotope mixture occurring naturally (Li-N) consists largely of Li-7 (92.4%) with a small amount of Li-6 (7.6%) [4]. It has been tacitly assumed that there are no significant differences between the therapeutic (or toxic) effects of the two isotopes. However, our recent work has demonstrated that Li-6 penetrates human erythrocytes *in vitro* [7, 8, 9] and rat erythrocytes *in vivo* [1] faster than Li-7, its half-life in cats is shorter [11] and its effect on spontaneous motility in rats more rapid. In exploratory studies in which toxic doses were administered intraperitoneally (IP) to mice, the mortality rate due to Li-6 was 15–20% higher than that of Li-7 chloride [1,2].

We now describe studies which show that the toxicity of isotopically pure Li-6 chloride in mice is higher than that of Li-7 chloride and that the behavioral effects of toxic doses of Li-6 appear before those of Li-7.

METHOD

Isotopically pure Li-6 and Li-7 chlorides (both 99.9%) were obtained from the Oak Ridge National Laboratory, Oak Ridge, TN. Natural Li-N chloride containing the usual mixture of stable isotopes was purchased from Fisher Scientific Corporation, Springfield, NJ. Mice, Swiss-Webster albino males, 20–30 g, were purchased from the breeder, Flora O'Grady, Bronx, NY. They were maintained on Teklad Rat/Mouse Chow and tap water ad lib, 10 per cage. Assembled in groups of 10, they were injected IP double blind, at 1 p.m. with a single dose, ranging from 12.0 to 17.5 mEq/kg (i.e., 494–750 mg/kg), of Li-6, Li-7 or Li-N chloride dissolved in deionized water.

Mortality served as the sole criterion in the first experiment involving 160 animals. Results were plotted and the dose which resulted in death of 50% of the subjects, LD_{50}, was estimated and confirmed by linear regression statistics. In an additional experiment, 40 mice in 4 groups were injected with either 13 or 16 mEq/kg of Li-6 or Li-7. Survival time of mice in this experiment was recorded. A third series, 20 mice, received either 13 mEq/kg of Li-6 or 16 mEq/kg of Li-7.

One group of mice received an injection of 14.5 mEq/kg of Li-6 and another group 14.5 mEq/kg of Li-7, the approximately mean dose of the two respective LD_{50}'s. Survival time was recorded.

In the next series of experiments behavior of lithium-treated mice was observed. Ten mice received IP injections of 14.5 mEq/kg of Li-6, 10 of Li-7 and 10 of deionized water. These animals were observed continuously for one hour and the observations recorded at 10 min intervals. The extent of spontaneous motor activity was rated blindly as approximately normal (1), decreased ($^1/_2$) or nil (0). An additional group of 30 mice was similarly treated and rated for 10 min periods at 1, 2, 3, 24, 48 and 72 hr. In the final series, 20 mice were treated with 13 mEq/kg of Li-6, 20 with 16 mEq/kg of Li-7 and 20 with deionized water. As before, half the animals were observed for one hour at ten min intervals and the remainder at 1, 2, 3, 24, 48 and 72 hr.

Statistical analysis to compare the distribution of animals in each of the treatment categories was carried out with the chi square test for differences among the totals of all time points during the first hour after 0 time and the Fisher exact test for each time point, with multiple pair comparisons between groups. Longitudinal analysis, for a given treatment group, was also performed in the same way. The use of a standard contingency table analysis for the longitudinal investigation was justified, even though the same animals were being tested at each time point thus resulting in a dependence among table entries, because we felt that a quantitative measure (p-value) might prove useful in identifying time-related differences.

RESULTS

Mouse Mortality After Acute Intake of LiCl

Acute treatment of mice with a single dose of LiCl showed that isotopically pure Li-6 was more toxic than either pure Li-7 or the naturally occurring mixture of isotopes. Pure Li-7 was somewhat less toxic than Li-N. Animals died mostly between 20 and 72 hr, several died in the first few hours and none after 72 hr. The LD_{50} for IP Li-6 chloride in our SW mice was 13.2 mEq/kg, for Li-N chloride 14.9 mEq/kg and for Li-7 chloride 15.9 mEq/kg ($p<0.01$).

TABLE 1

EFFECTS OF 14.5 mEq/kg OF ISOTOPICALLY PURE Li-6 OR Li-7 CHLORIDE

	Isotope	
	Li-6	Li-7
Appearance of toxicity within 30 min		
Decrease in motility	70%	30%
Change in motility, %/min	2.3 ± 1.9	1.0 ± 0.8
Presence of ataxia or tremors	9/10	3/10
Presence of diarrhea	10/10	4/10
Mortality in 72 hrs	9/10	1/10
Time of death (hrs after injection)	20, 24, 27, 40, 48, 55, 72, 72, 72	48

Swiss-Webster mice, 10 per group, injected intraperitoneally with Li-6 or Li-7 chloride and observed double blind. Control batch injected with deionized water showed no consistent changes in any of the parameters.

Further support for these LD_{50} values was obtained in the experiment in which an additional 40 mice were given single injections of doses close to the putative LD_{50}'s of the respective pure isotopic lithium chlorides. An injection of 16 mEq/kg of either isotope was lethal 50% for Li-7-treated and 100% for Li-6-treated mice and produced a mortality pattern in which the first two Li-7 animals died at 23 hr after injection and others at 39, 72 and 72 hr, but in which the first Li-6 animals died at 2 and 5 hr, 4 more died within 23 hr (at 8, 20, 21 and 23 hr) and the others at 24, 40, 48 and 72 hr.

An injection of 13 mEq/kg of either isotope produced no mortality in the Li-7 group and 5 deaths in the Li-6 group, at 5, 20, 23, 40 and 72 hr, confirming the LD_{50} for Li-6. Repetition of the latter experiment with 10 additional mice given 13 mEq/kg of Li-6 and 10 given 16 mEq/kg of Li-7 led again to 50% mortality in each group, with 2 of 10 Li-7 mice dead at 24 hr, one more at 48 hr and 2 more at 72 hr, and with 3 of 10 Li-6 mice dead at 24 hr, one at 48 hr and one at 72 hr.

Acute Toxicity in Mice: Behavioral Changes

A single injection of 14.5 mEq/kg of either isotopic LiCl led to a sharp differentiation between the effects of the two isotopes. This concentration was lethal for most mice given Li-6 but only for one in 10 mice given Li-7 (Table 1). The time of death underscored this difference: the single Li-7 mouse death occurred at 48 hr but 4 Li-6 mice died earlier, one died at 48 hr and 4 more died later.

Treatment with 14.5 mEq/kg of Li-6 or Li-7 produced immediate differential behavioral effects. Within 5–10 min treated mice began to display gross signs of toxicity: a decrease in spontaneous locomotor activity, tremors, loss of body tonus, ataxia, loss of reflex responses and a rapid water loss: diarrhea, perspiration and increase in urination. During the first 30 min more of these early toxic symptoms were observed in Li-6 than Li-7 mice (Table 1). In 10 min motility in the Li-6 group decreased 50% but only 20% in the Li-7 group (Table 2). After 30 min the decreases were 70% and

TABLE 2

MEAN MOTILITY IN MICE INJECTED WITH ISOTOPICALLY PURE Li-6 OR Li-7 CHLORIDES

Time min	Li-6			Li-7	
	Placebo	13.0 mEq/kg	14.5 mEq/kg	14.5 mEq/kg	16.0 mEq/kg
0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
10	1.00 ± 0.00	0.55 ± 0.20	0.50 ± 0.20	0.80 ± 0.20	0.55 ± 0.22
20	1.00 ± 0.00	0.15 ± 0.23	0.35 ± 0.45	0.70 ± 0.30	0.40 ± 0.23
30	1.00 ± 0.00	0.20 ± 0.24	0.30 ± 0.40	0.70 ± 0.40	0.25 ± 0.25
40	0.95 ± 0.15	0.20 ± 0.33	0.20 ± 0.20	0.40 ± 0.40	0.15 ± 0.30
50	1.00 ± 0.00	0.30 ± 0.33	0.10 ± 0.20	0.25 ± 0.25	0.05 ± 0.15
60	1.00 ± 0.00	0.15 ± 0.05	0	0.20 ± 0.20	0.10 ± 0.20

Swiss-Webster mice, 10 per group. Spontaneous motility of individual animals was rated as normal (1), decreased (1/2) or nil (0) and averaged. Values shown are means ± standard deviations; for example, at 30 min Li-6 group treated with 13.0 mEq/kg showed the following activities: 1/2, 0, 0, 0, 0, 1/2, 1/2, 0, 0, 1/2 (0.20 ± 0.24), and Li-7 group treated with 14.5 mEq/kg. 1, 1, 0, 1/2, 0, 1, 1, 1/2, 1, 1 (0.70 ± 0.40).

30%, respectively. The average slope of the decreases in motility with time during the first 3 ten-minute periods was 2.1±1.9%/min for Li-6 and 1.0±0.8%/min for Li-7. Thus, the average rate of appearance of toxicity was faster after Li-6 than after Li-7.

During the period between 10–60 min after injection we observed each of 20 mice 6 times, for a total of 120 observations, 47% of them indicating complete absence of spontaneous motor activity and 31% a sharp decrease in such activity. Treatment with Li-6 had a significantly greater effect: more than twice as many observations of total immobility were made in Li-6 than Li-7 mice (68% vs 32%). Conversely, of the 26 observations of unchanged motor activity 73% were made in Li-7 mice and only 27% in mice treated with Li-6. Within the Li-6 group during the first hour, complete absence of motility was seen 63% of the time and normal motor activity only 12% of the time. By contrast, in the Li-7 group, the percentages were 30 and 32%, respectively. The chi square test for motility differences for all points between 10–60 min taken together was highly significant ($p<0.001$). Chi square test was not applicable to comparisons at individual time points but the Fisher exact test showed significant difference at 30 min ($p<0.02$).

Recovery occurred faster in the Li-7 group. At 3 hr, motility in the Li-6 mice was still 75% below normal, while all Li-7 mice recovered some activity. In 24 hr 9 Li-7 mice and 4 Li-6 mice recovered (2 Li-6 mice died and 4 more were only partly mobile). In 72 hr one Li-6 mouse recovered.

As expected, the differential isotope effect on behavior was less pronounced when mice were treated with their respective LD_{50} doses. At 10 min after an IP injection, motility decreased 45% in mice treated with 13 mEq/kg of Li-6 or 16 mEq/kg of Li-7. At 20 min, motility of Li-7 mice was down 60%, of Li-6 mice 85%. At 30 min, the difference was minimal (75% vs 80% decrease) and after 40 min the Li-7 group became more affected. Symptoms of severe ataxia appeared in a few Li-6 (40%) and Li-7 (20%) mice during the 30 min after injection and no significant difference in the number of mice showing signs of water loss (diarrhea, severe perspiration) was observed (7–8 in each group).

Recovery rate after one hour was slightly greater for Li-7 than Li-6 mice, the difference being smaller than in the case of animals treated with the median 14.5 mEq/kg dose. In 3 hr 4 Li-7 and 6 Li-6 mice were still immobile. In 24 hr 2 Li-7 and 3 Li-6 mice died, while the rest recovered some motility. In 72 hr 5 mice in each group died, the rest recovered.

Analysis of the effects of treatment with 13–16 mEq/kg across time excluded time 0, since motility at 0 time was significantly different from at least 3 of the other time points within each lithium group. Due to small sample size motility ratings were condensed to a dichotomy (1 vs 0 + 1/2). All lithium-treated mice were significantly less mobile than controls ($p<0.001$). Li-6 mice treated with 13 mEq/kg showed a breakpoint between 10 and 20 min: their motility at 10 min differed from that for all other time points ($p<0.006$). For the group treated with 14.5 mEq/kg of Li-7 the breakpoint occurred between 30 and 40 min: motility ratings at 10, 20 and 30 min differed from those at 40, 50 and 60 min ($p<0.001$). No longitudinal differences were found in the other groups.

DISCUSSION

Our data show that the two stable naturally occurring isotopes of lithium have different toxicities: Li-6 is quantitatively more lethal than Li-7. Its LD_{50} was 3 mEq/kg lower than the LD_{50} of the heavier isotope. Treatment with a dose of 14.5 mEq/kg, approximately median between the two LD_{50}'s, led to a 90% mortality in mice treated with Li-6 but only 10% in those treated with Li-7. In addition, Li-6 exerted its toxic behavioral effects more rapidly than Li-7 and Li-6 animals became quiescent earlier than the corresponding Li-7 animals. At a dose of 14.5 mEq/kg, the initial rate of appearance of toxic effects was 2.3 times greater for Li-6 than for Li-7. Even after administration of the respective LD_{50}'s the behavioral effects seen during the first 30 min were more pronounced for Li-6 than Li-7.

Theoretical considerations of ionic charge and mass and their ratios suggested that the rate of transport of the two stable lithium ions across biological membranes may be different [7, 8, 9]. Previous *in vitro* and *in vivo* findings in hu-

mans and in animals [1, 7, 8] were consistent with these considerations. In addition, more rapid influx and higher intracellular concentrations of Li-6 than Li-7 in plasma, red blood cells and cerebrospinal fluid were found in cats after acute single doses of the isotopes [11]. In the current work we have shown that treatment of mice with toxic doses of the two isotopes led to a quantitative differential isotope effect which was mirrored not only in the death rate but also in the rate of appearance of physiological and behavioral changes such as loss of spontaneous motility. In all cases, decreased motility occurred more rapidly in animals pretreated with Li-6 than Li-7.

The different toxic and behavioral effects of the two stable lithium isotopes may reflect either an increased rate of entry and higher cellular levels attained by Li-6 or a quantitatively different effect of Li-6 on intracellular metabolic process or both, because of the physicochemical differences between ions of the two isotopes. The difference between isotopes may be a significant factor in the therapeutic usefulness and clinical toxicity of natural lithium. We are currently investigating whether the Li-6 isotope accounts for a disproportionate amount of the side effects observed during treatment with natural lithium in humans [10].

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