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SHORT REPORT

Potential involvement of intracellular pH in a mouse model of amyotrophic lateral sclerosis

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This study tested possible involvement of intracellular acidification as a secondary pathogenic factor in ALS. As one of cell death mechanisms, apoptosis is known to be involved in motor neuronal death in ALS. Cancer research has revealed that the activity of apoptosis strongly depends on intracellular pH (pHi), enhanced with low pHi and inhibited with high pHi (1). In ALS, excessive glutamate and calcium overloading are two up-stream factors for apoptosis. Other experiments have shown that neuronal pHi can be acidified by glutamate in a calcium dependent manner (2,3). We thus hypothesized that an acidified pHi may occur in the motor neurons of ALS and accelerate the disease progression. Therefore, a mild intracellular alkalinization may be beneficial to the disease. The hypothesis was tested by two chemicals, sodium bicarbonate ($NaHCO_3$) and ammonium chloride (NH_4Cl) for their major effects on adjusting pH. $NaHCO_3$ provides base buffer for the body both extracellularly and intracellularly. NH_4Cl is known to acidify extracellular pH after being largely metabolized in liver, but to alkalize pHi when dissociated ammonia diffuses into cells and combines with hydrogen proton (4). If these two chemicals with opposing effects on pHe both ameliorate symptoms in ALS mice, most likely the beneficial effects come from the common pHi alkalizing abilities.

G93A-SOD1 (B6SJL-Tg-SOD1*G93A, Jackson Lab, MA) mice were bred in a hemizyous manner and maintained at the Center for Comparative Medicine of Northwestern University. All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee and were in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals. The G93A-SOD1 mice were randomly assigned into either experimental (14,17 and 16 mice for 50 mM NH_4Cl, 30 and 100 mM $NaHCO_3$, respectively) or untreated (14 mice) groups. To avoid metabolic alkalosis and acidosis, low concentrations of $NaHCO_3$ (30 and 100 mM) and NH_4Cl (50 mM) were chosen to add into drinking water with 0.005−0.01% (w/v) sucrose to improve taste. An improved method was employed to determine the end stage. At the beginning of the treatment (P85), all testing mice were trained to walk on a rotarod (Ugo Basile, Rotarod 7650, Italy) until they could stably stay for 4 min. The rotarod time was then monitored and the end stage was determined when the mice fell within 5 s in three consecutive tests with 2-min intervals. Motor function was evaluated by rotarod performance over time. Meanwhile, body weight, muscle weakness and paralysis, and urine pH were monitored. The results were analyzed by Student's *t*-test, one-way and two-way ANOVA and post hoc (Dunnett) on SPSS by comparing to untreated groups. A *p*-value less than 0.05 was considered significant. Numerical data values are presented as mean ± SEM.

Results

Sixty-one female mSOD1 mice were tested and the results are listed in Table I. The one-way ANOVA test showed a significant difference in this survival test ($p = 0.003$). Further post hoc tests showed significance in the NH_4Cl group compared to the controls (151.93 ± 4.28 days, $p = 0.002$), which was a 13% increase in lifespan, while the 30 mM $NaHCO_3$ group extended the lifespan to 145.29 ± 2.23 days ($p = 0.047$). No significant difference was found comparing the effect on mice of NH_4Cl to those treated with either 30 or 100 mM $NaHCO_3$. Also,

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Table I. Lifespan of G93A-SOD1 mice.

	n	Mean	Standard deviation	Standard error	Post hoc test*
Untreated	14	133.86	14.81	3.96	–
30 mM $NaHCO_3$	17	145.29	9.18	2.23	0.047
100 mM $NaHCO_3$	16	139.06	12.01	3.00	0.558
50 mM NH_4Cl	14	151.93	16.00	4.28	0.002

*One-way ANOVA, $p = 0.011$; Levene homogeneity of variances test insignificant; Dunnett (two-sided) test.

$NaHCO_3$ did not produce a dose-dependent response from 30 to 100 mM. The survival patterns in all groups were plotted in a Kaplan-Meier plot (Figure 1A). All treated groups showed right shifts compared to the control group, but only NH_4Cl treatment was statistically significantly different to control (log rank, $p = 0.021$).

The motor function was analyzed in 50 mM NH_4Cl and control groups, and both deteriorated gradually with progression of the disease (starting from day: 104.9 ± 5.4 for NH_4Cl; 103 ± 3.1 for control; Student's t-test, $p > 0.05$). As shown in Figure 1B, NH_4Cl treatment prolonged the overall lifespan, but did not significantly improve the motor function over time. Consistent with this, all tested mice showed weight loss, tremor, and paralysis of muscles without statistical differences between the groups. Slight shifts in urine pH were found (6.08 ± 0.042 for 50 mM NH_4Cl; 6.69 ± 0.063 for 100 mM $NaHCO_3$; and 6.36 ± 0.05 for untreated controls) with significant difference ($p = 0.023$) between the NH_4Cl and $NaHCO_3$ groups. However, these pH values were still within the normal ranges determined by the control group (Figure 1C).

Discussion

$NaHCO_3$ and NH_4Cl are two chemicals used to produce metabolic alkalosis and acidosis in experiments at much higher concentrations (5,6). In this study, these two chemicals in lower concentrations led to mild alkalizing and acidifying effects, respectively, on urine pH without manifest side-effects, suggesting the mild pH changes in blood and extracellular space (pHe) in the whole body. These contrasting pHe changes are unlikely to be causal for the similar therapeutic effects from both groups. Increase in pHe may enhance calcium influx through the NMDA receptor (7), which may augment excitotoxicity of ALS.

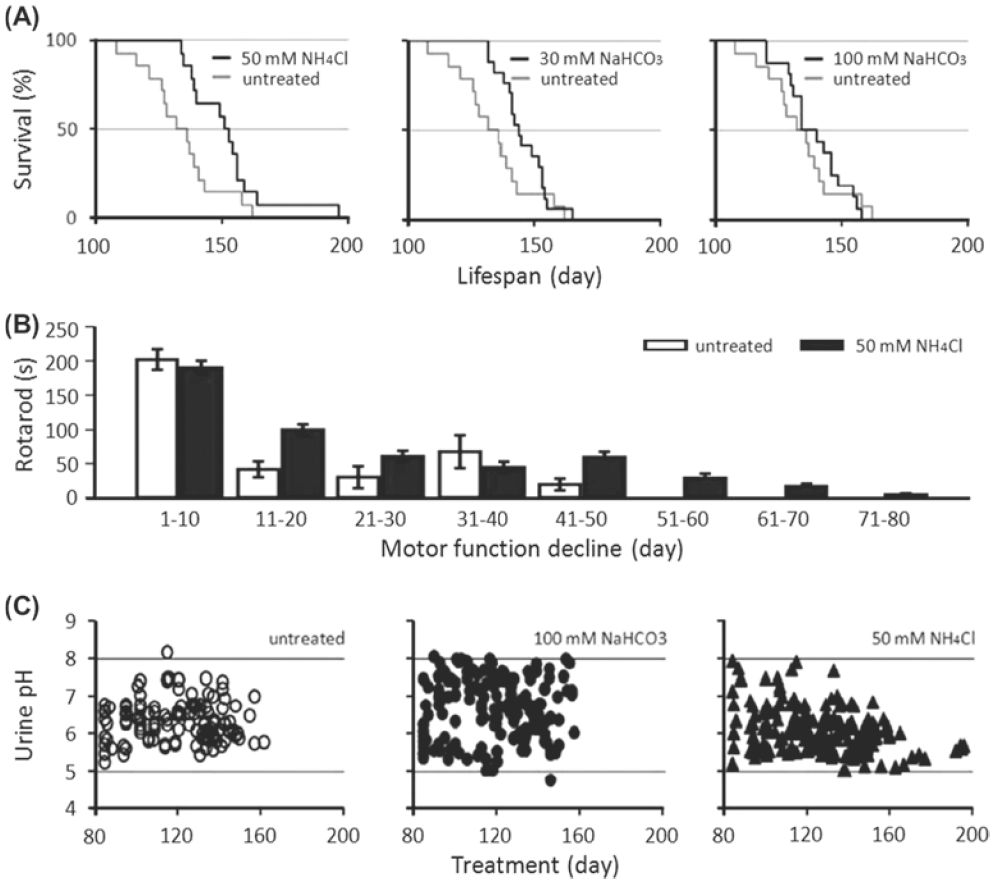

Figure 1. (A) Kaplan-Meier survival plots display the survival patterns of the mSOD1 mice in each group as indicated. (B) The histogram shows progressive decline of motor function (y-axis: rotarod time) over time (x-axis: binned by 10 days after rotarod time fell below 4 min) in control and 50 mM NH_4Cl treated mice. No significant difference was found between these two groups when analyzed by two-way ANOVA ($p = 0.053$). (C) Scattered plots show all measured urine pH over time in three major groups as indicated. The two lines in the individual plot mark normal range of urine pH.

Recently, up-regulated motor neuronal acid-sensing ion channels (ASICs) were found in both ALS patients and G93A-hSOD1 mice (8), suggesting the decrease in pHe may exacerbate the degeneration of ALS motor neurons as well. In addition, abnormal pHe may increase excitotoxicity of ALS motor neurons through inhibiting the glycine receptor (9). Thus, the benefits of $NaHCO_3$ and NH_4Cl are likely due to their alkalinizing effect on pHi. As many pathogenic pathways are involved in ALS, alkalinizing pHi appears to be more closely related to a survival mechanism of ALS, because the main effect observed here was the significantly prolonged lifespan. Further study to examine the proposed mechanism underlying the therapeutic effect will involve direct measurement of pHi shifting in motor neurons by techniques such as magnetic resonance spectroscopy. This proof-of-concept study showed 13% enhancement in the lifespan of G93A-hSOD1 mice, which is one of the best results in ALS animal experiments under similar conditions (10). Thus, our results suggest an overlooked biochemical factor, which might be implicated in ALS progression and deserves further investigation.

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