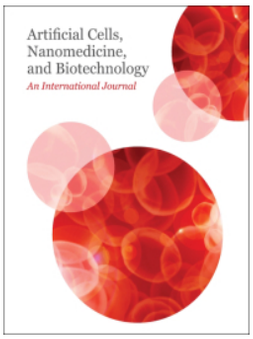

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# Effects of sodium pyruvate on ameliorating metabolic acidosis

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**Abstract**

***Objective:*** **To examine the effects of sodium pyruvate (SP) on metabolic acidosis.** ***Methods:*** **For the *in vivo* experiments, we evaluated effects of SP on an ammonium chloride ($NH_4Cl$)-induced hyperchloremic acidosis rat model. SP was infused at overall doses of 2, 4, and 6 mmol·kg$^{-1}$ for the $SP_1$, $SP_2$, and $SP_3$ groups, respectively. Treatment with sodium bicarbonate (SB) was used as a positive control (2 mmol·kg$^{-1}$), and treatment with normal saline (NS) was used as a volume control (2 mL·kg$^{-1}$). Blood was sampled from the ophthalmic venous plexus for pH, blood gases, electrolytes, glucose, creatinine (Cr), and urea analysis after injection. For the *in vitro* experiment, propionate was applied to induce intracellular acidosis in human endothelial cells. Intracellular pH (pHi) was fluorimetrically measured after the addition of SP.** ***Results:*** **In the *in vivo* study, the pH of $SP_1$ group showed no significant difference compared with that of the NS group. The $SP_2$ and $SP_3$ groups had a higher pH than the NS group ($P < 0.01$). The $SP_3$ group had a higher pH than the SB group ($P < 0.05$) and $SP_1$ group ($P < 0.05$). Moreover, SP treatment ameliorated the abnormality of calcium and decreased the blood potassium levels. The $SP_3$ group had higher glucose levels than $SP_1$ group ($P < 0.05$). No significant differences were observed between all the groups in the plasma Cr and urea levels. In the *in vitro* study, the pHi increased immediately after the addition of SP.** ***Conclusion:*** **The data suggest that intravascular treatment with SP represents a novel therapeutic strategy to ameliorate metabolic acidosis.**

**Keywords:** blood gases, glycometabolism, metabolic acidosis, pyruvate

## Introduction

Metabolic acidosis, a common acid–base disorder, is associated with increased morbidity and mortality due to its depressive effects on cardiovascular function, facilitation of cardiac arrhythmias, stimulation of inflammation, suppression of the immune response, and other adverse effects (Kraut and Madias2010). Lactic acidosis, ketoacidosis, hyperchloremic acidosis, and renal tubular acidosis are the main types of metabolic acidosis in clinic. To evaluate its severity, metabolic acidosis has been divided into three forms based on the levels of blood pH: mild (pH, 7.30–7.36), moderate (pH, 7.20–7.29), and severe (pH, $<7.20$). They correspond to the blood concentration of bicarbonate ($cHCO_3^-$) $>20$ mmol·L$^{-1}$, 10–19 mmol·L$^{-1}$, and $<10$ mmol·L$^{-1}$, respectively (Kraut and Kurtz 2012). The deleterious effects of metabolic acidosis on cellular function result from the decrease in both the extracellular pH (pHe) and intracellular pH (pHi) (Venkatesh et al. 2010). Sodium bicarbonate (SB) therapy is primarily the supportive measure to metabolic acidosis in clinic, when the therapies tailored to the specific disorder are a failure. However, the rationale for using SB to treat metabolic acidosis has been debated for several years. It is reported that SB can cause serious complications including deterioration in systemic hemodynamics, metabolic alkalosis, electrolyte abnormalities, and especially pHi decline (Graf et al. 1985, Kraut and Kurtz 2001, 2006, Shapiro et al. 1989). Though alternative base buffers such as tris-hydroxymethyl (THAM) and Carbicard have been developed, they all have potential adverse effects. THAM therapy can depress breathing and result in hyperkalemia (Nahas et al. 1998), while Carbicard is not currently available in clinics because its effect should be re-examined (Kraut and Madias 2012). Therefore, novel methods are worth studying further for the treatment of metabolic acidosis.

An ideal base therapy should have the ability to eliminate the complications of metabolic acidosis and avoid the potential adverse effects by itself. Pyruvate, known as both an endogenous metabolic substrate and an effective reactive oxygen species (ROS) scavenger (Nath et al. 1994), seems to be an attractive candidate molecule for the treatment of metabolic acidosis. Previous studies have focused on the protective effects of hypertonic sodium pyruvate (SP) on ameliorating organ damage and enhancing survival rate in the animal models of hemorrhagic shock and

Jing Yang and Jing-Xiang Zhao contributed equally to the manuscript.

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ischemic-reperfusion (Petrat et al. 2011, Mongan et al. 2002, Sharma and Mongan 2010). The mechanisms considered here are that SP can inhibit inflammatory mediators such as TNF-α and NF-κB, provide additional energy to the myocardium, improve $Ca^{2+}$ homeostasis, attenuate apoptosis, preserve carbohydrate metabolic pathways and promote lipid metabolism (Kristo et al. 2004, Lee et al. 2004, Lopaschuk et al. 2002, Mongan et al. 2003). Besides its anti-inflammatory and antioxidant effects, the effect of SP on increasing blood pH after resuscitation in rats suffering from lactic acidosis induced by hemorrhagic shock should also be taken into consideration. Mongan et al. used a swine model of hemorrhagic shock to study the protective effect of SP on preventing liver damage. The blood pH values of SP groups were higher than those of NaCl group after resuscitation (Mongan et al. 2002). Petrat et al. used a rat model of severe intestinal ischemia–reperfusion injury to study the protection of SP infusion and found that SP could increase blood pH and base excess (BE) after reperfusion (Petrat et al. 2011). More importantly, it was indicated that pyruvate can increase pHi in isolated failing human myocardium (Hasenfuss et al. 2002).

Above studies suggested that treatment with SP may be a novel strategy to correct lactic acidosis. However, it has been reported that the mortality rate of hyperchloremic acidosis was 29% in intensive care unit (ICU, Gunnerson et al. 2006). This study was designed to investigate the dose-dependent effects of SP on system hyperchloremic acidosis induced by $NH_4Cl$ *in vivo* and intracellular acidosis induced by propionate *in vitro*.

## Materials and methods

### Animals

All experiments were approved by the Institute of Transfusion Medicine, Academy of Military Medical Sciences. All efforts were made to minimize the number of animals used and their suffering. Thirty-nine male Wistar rats (Vital River Laboratories, Beijing, China), weighing between 175 g and 225 g, were adapted to cages for 5 days before experiments. All animals received standard rodent chow and drinking water.

### Induction of metabolic acidosis

Before $NH_4Cl$ (Ammonium Chloride, Sigma, USA) loading, venous blood of all rats was collected from the ophthalmic venous plexus with a heparinized centrifuge tube and analyzed immediately for pH, $PaCO_2$, $PaO_2$, actual $cHCO_3^-$, actual BE, $cK^+$, $cNa^+$, $cCa^{2+}$, $cCl,^-$ and glucose (cGlu) on a blood gas analyzer (ABL 80 CO-OX, Radiometer, Denmark). To induce metabolic acidosis, the rats were administered 0.28 mol·$L^{-1}$ $NH_4Cl$ (containing 0.5% sucrose; Sucrose, Sigma, USA) in drinking water *ad libitum* (Nowik et al. 2010). Six days after $NH_4Cl$ loading, venous blood was collected and analyzed immediately for pH, blood gases, electrolytes, and glucose ($T_0$).

### Grouping and treatment protocol for the *in vivo* study

The rats were randomly assigned to the following five groups based on the solution infused: Group 1 $SP_1$ ($n = 8$), 1 mol·$L^{-1}$ SP (Sigma, USA) solution (pH = 6.37); Group 2 $SP_2$ ($n = 8$), 2 mol·$L^{-1}$ SP solution (pH = 6.32); Group 3 $SP_3$ ($n = 8$), 3 mol·$L^{-1}$ SP solution (pH = 6.36); Group 4 SB ($n = 8$), 1 mol·$L^{-1}$ SB (Sigma, USA) solution (pH = 8.50) for a positive control; and Group 5 NS ($n = 7$), normal saline (0.9% NaCl; Sigma, USA) solution (pH = 7.49) for a volume control. The solution was administered for over 2 min via the tail vein (2 ml·$kg^{-1}$)(Diehl et al. 2001). The dose of SP was selected on the basis of previously published investigations (Shapiro et al 1989, Diehl et al. 2001), and it corresponded to 2 mmol·$kg^{-1}$ of SB, which was chosen based on typical initial clinical dosing for severe acidosis. The doses of SP were 2 mmol·$kg^{-1}$, 4 mmol·$kg^{-1}$, and 6 mmol·$kg^{-1}$ for the $SP_1$, $SP_2$, and $SP_3$ groups, respectively. All fluids were used at room temperature. Blood was sampled from the ophthalmic venous plexus for pH, $PaCO_2$, $PaO_2$, actual $cHCO_3^-$, actual BE, $cK^+$, $cNa^+$, $cCa^{2+}$, $cCl,^-$ and cGlu analysis at 15 min ($T_{15}$), 30 min ($T_{30}$), 60 min ($T_{60}$), 120 min ($T_{120}$), and 240 min ($T_{240}$) after injection.

### Measurement of plasma creatinine (Cr) and urea for the *in vivo* study

Measurement of serum Cr levels and urea levels is helpful in evaluating renal function. At $T_{240}$ point, blood was collected from the ophthalmic venous plexus with a heparinized centrifuge tube and centrifuged at 1448 g for 10 min. The supernatants were used for the measurement of Cr levels and urea levels using an Automatic Analyzer (HITACHI 7020, HITACHI, Japan). Plasma Cr levels were determined using a trinitrophenol method by a Cr detection kit (CRE kit, LEADMAN, China). Urea levels were determined using glutamate dehydrogenase method by a urea detection kit (Urea kit, LEADMAN, China).

### Cell culture

Human endothelial cells of the EA.hy926 cell line was purchased from China Center for Type Culture Collection (CCTCC) and was cultured in complete DMEM medium (DMEM, Gibco, USA), supplemented with 10% fetal bovine serum (FBS, Gibco, USA), 100 U·$mL^{-1}$ penicillin, and 100 μg·$mL^{-1}$ streptomycin (Penicillin-Streptomycin, Gibco, USA) in 5% $CO_2$ at 37°C.

### Intracellular acidosis cell model and measurement of pHi for the *in vitro* study

The pHi was fluorimetrically measured by a dual-excitation (488 nm and 445 nm), single-emission (535 nm) ratiometric technique using the pH-sensitive fluorophore 2,7-biscarboxyethyl-5(6)-carboxyfluorescein-acetoxymethylester (BCECF-AM, Invitrogen, USA) (Young et al. 2011, Huck et al. 2007). There was a positive correlation between BCECF emission ratio (R, $R = F_{(Ex = 488\ nm/Em = 535\ nm)}/F_{(Ex = 445\ nm/Em = 535\ nm)}$) and pHi (Young et al. 2011). Briefly, EA.hy926 cells, grown on glass coverslips, were loaded with the 0.1 μmmol·$L^{-1}$ BCECF-AM in HEPES-buffered Ringer solution (HBRS) (140 mmol·$L^{-1}$ NaCl, 5 mmol·$L^{-1}$ KCl, 1 mmol·$L^{-1}$ MgCl, 1 mmol·$L^{-1}$ $CaCl_2$, 5 mmol·$L^{-1}$ glucose, 10 mmol·$L^{-1}$ HEPES adjusted to pH 7.40) for 30 min at 37°C in darkness (Huck et al. 2007). Then, glass coverslips were

rinsed thrice with HBRS to remove the excess of BCECF-AM and transferred into fresh HBRS. Fluorescence was monitored by a laser scanning confocal microscope (Ultra VIEW VoX 3D Live Cell Imaging System, PerkinElmer, USA). Intracellular acidosis was induced by exposing cells to 30 mmol·L$^{-1}$ sodium propionate (sodium propionate, Sigma, USA), which reduced pHi as uncharged propionic acid (pKa 4.7) diffused into the cell and dissociated to $H^+$ and propionate (De Hemptinne et al. 1983, Spitzer et al. 2000). After 3-min equilibration in HBRS with sodium propionate, 33 mmol·L$^{-1}$, 66 mmol·L$^{-1}$, and 100 mmol·L$^{-1}$ SP was applied for the $SP_1$ group, $SP_2$ group and $SP_3$ group. NS addition was performed as negative control group (NS group). No addition was performed as blank control group (Control group). R values were calculated using the Volocity software (Volocity, PerkinElmer, USA).

### Statistical analysis

Results were expressed as means ± SD. Means of different groups were compared using repeated-measures analysis of covariance with Tukey–Kramer adjustment or one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls test wherever appropriate. When normality and homogeneity of variance assumptions were not satisfied, non-parametric Kruskal–Wallis test was applied. Statistical significance was considered when $P<0.05$. Data were analyzed using Statistical Analysis System software (SAS Version 9.2, SAS Institute, USA).

## Results

### $NH_4Cl$-induced acidosis

Plasma pH (Figure 1), $cHCO_3^-$ (Figure 2A), and BE (Figure 2B) of all groups decreased compared with the baseline values ($P<0.05$), demonstrating that $NH_4Cl$ loading in drinking water for 6 days resulted in induction of metabolic acidosis. Partial respiratory compensation was shown by the decrease in $PaCO_2$ and the elevation of $PaO_2$. No significant differences were observed between $T_0$ values and baseline values in the $PaCO_2$ and $PaO_2$ levels (Table I). Compared with baseline values, plasma potassium concentration (Figure 3A) in all groups showed decreasing trends without statistical significance. However, plasma sodium (Figure 3B), free calcium (Figure 3C), and chloride (Figure 3D) concentrations increased in the rats subjected to $NH_4Cl$ loading compared with the baseline values ($P<0.05$). The weight (data not shown), pH, blood gases, and electrolytes parameters did not differ significantly between groups at $T_0$.

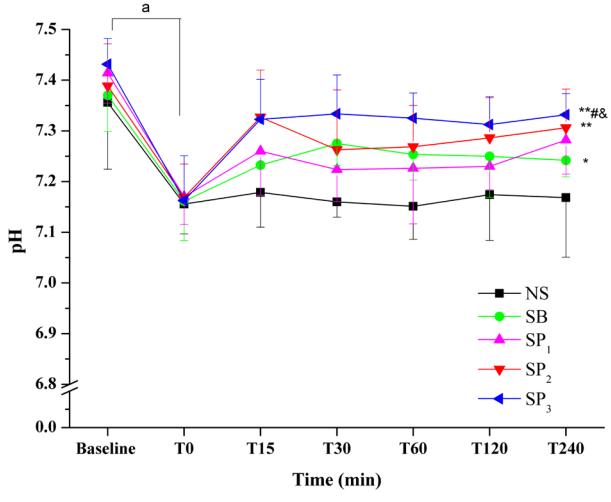


Figure 1. Changes in venous blood pH in response to NH4Cl loading and infusion of solution via tail vein treated with normal saline (NS), sodium bicarbonate (SB) at dose of 2 mmol·kg-1, sodium pyruvate at dose of 2 mmol·kg-1 (SP1), sodium pyruvate at dose of 4 mmol·kg-1 (SP2), and sodium pyruvate at dose of 6 mmol·kg-1 (SP3). Data are presented as means (SD); *$P<0.05$, **$P<0.01$, vs. NS; #$P<0.05$, vs. SB; &$P<0.05$, vs. SP1; $^a P<0.05$, T0 vs. baseline.

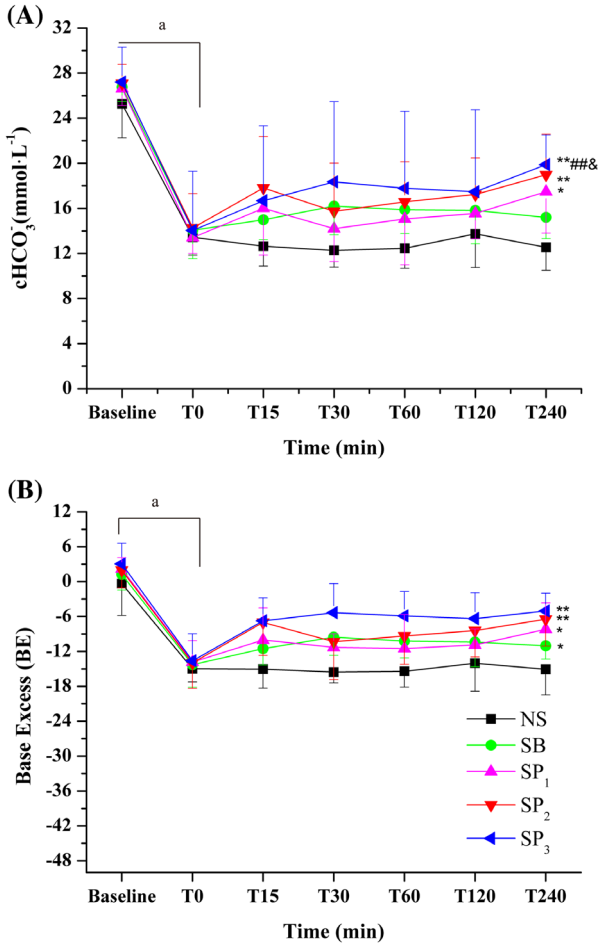


Figure 2. Changes in venous plasma base excess (BE) and concentration of bicarbonate (cHCO3-, mmol·L-1) in response to NH4Cl loading and infusion of solution via tail vein treated with normal saline (NS), sodium bicarbonate at dose of 2 mmol·kg-1 (SB), sodium pyruvate at dose of 2 mmol·kg-1 (SP1), sodium pyruvate at dose of 4 mmol·kg-1 (SP2), and sodium pyruvate at dose of 6 mmol·kg-1 (SP3). Data are presented as means (SD); *$P<0.05$, **$P<0.05$, vs. NS; ##$P<0.01$, vs. SB; &&$P<0.05$ vs. SP1; $^a P<0.05$, T0 vs. baseline.

### Effect of pyruvate treatment on blood pH, BE, and $cHCO_3^-$

The effects of NS, SB, and SP on $NH_4Cl$-induced acidosis were studied in this experiment. Treatment with NS had no significant effect on any of the parameters studied at all the time points. Treatment with SB or SP resulted in a prompt increase in systemic pH (Figure 1), BE, and $cHCO_3^-$ (Figure 2). The pH of $SP_1$ group showed no significant

Table I. Venous Blood $PaCO_2$ and $PaO_2$.

| Parameter (time) | NS ($n=7$) | SB ($n=8$) | $SP_1$ ($n=8$) | $SP_2$ ($n=8$) | $SP_3$ ($n=8$) |
|---|---|---|---|---|---|
| $PaCO_2$ | | | | | |
| Normal | 47.00 ± 11.73 | 47.60 ± 5.77 | 42.71 ± 6.29 | 46.29 ± 7.39 | 41.33 ± 2.07 |
| $T_0$ | 39.86 ± 6.52 | 40.75 ± 5.87 | 40.38 ± 4.00 | 40.25 ± 4.46 | 42.50 ± 4.84 |
| $T_{15}$ | 35.14 ± 2.27 | 36.75 ± 2.92 | 36.19 ± 2.98 | 34.25 ± 3.24 | 36.14 ± 6.28 |
| $T_{30}$ | 35.71 ± 2.29 | 35.75 ± 3.01 | 35.25 ± 4.89 | 35.43 ± 3.99 | 37.25 ± 2.38 |
| $T_{60}$ | 36.86 ± 2.41 | 36.88 ± 1.64 | 36.38 ± 2.88 | 36.57 ± 2.44 | 37.25 ± 3.65 |
| $T_{120}$ | 38.14 ± 1.95 | 36.88 ± 3.27 | 37.29 ± 2.14 | 36.75 ± 1.83 | 37.38 ± 5.71 |
| $T_{240}$ | 35.83 ± 5.46 | 36.40 ± 2.61 | 37.60 ± 1.67 | 38.75 ± 3.20 | 38.40 ± 2.79 |
| $PaO_2$ | | | | | |
| Normal | 38.20 ± 7.56 | 42.40 ± 2.41 | 37.29 ± 3.55 | 38.57 ± 2.51 | 40.83 ± 4.07 |
| $T_0$ | 44.71 ± 5.65 | 42.50 ± 5.21 | 44.00 ± 6.00 | 45.13 ± 2.70 | 43.13 ± 2.85 |
| $T_{15}$ | 38.43 ± 2.64 | 37.50 ± 3.30 | 37.50 ± 6.39 | 35.43 ± 5.35 | 38.14 ± 6.49 |
| $T_{30}$ | 35.14 ± 4.81 | 34.50 ± 2.51 | 36.88 ± 8.68 | 38.71 ± 7.93 | 30.25 ± 3.58 |
| $T_{60}$ | 35.00 ± 5.13 | 34.00 ± 3.02 | 35.63 ± 6.02 | 34.86 ± 6.09 | 30.75 ± 3.28 |
| $T_{120}$ | 33.67 ± 3.44 | 35.00 ± 6.55 | 35.86 ± 3.98 | 33.63 ± 3.38 | 35.71 ± 7.48 |
| $T_{240}$ | 37.33 ± 6.56 | 33.80 ± 4.32 | 30.25 ± 3.20 | 37.50 ± 13.17 | 32.20 ± 5.59 |

Changes in venous plasma $PaCO_2$ and $PaO_2$ (mmHg) in response to $NH_4Cl$ loading and infusion of solution via tail vein treated with NS, sodium bicarbonate at dose of 2 mmol·kg$^{-1}$ (SB), sodium pyruvate at dose of 2 mmol·kg$^{-1}$ ($SP_1$), sodium pyruvate at dose of 4 mmol·kg$^{-1}$ ($SP_2$), sodium pyruvate at dose of 6 mmol·kg$^{-1}$ ($SP_3$). No significant differences were observed between all the groups in the $PaCO_2$ and the $PaO_2$ levels. Data are presented as means ± SD; n is the number of rats.

difference compared with that of the NS group (Figure 1). The $SP_2$ and $SP_3$ groups had a higher pH than the NS group ($P<0.01$) (Figure 1). The $SP_3$ group had a higher pH than the $SP_1$ group ($P<0.05$) (Figure 1). Additionally, the pH values in the $SP_3$ group approached to normal level (7.35–7.46) upon SP infusion at all the time points.

The pH of SB group was higher than that of the NS group ($P<0.05$) (Figure 1). The SP groups took 15 min to reach

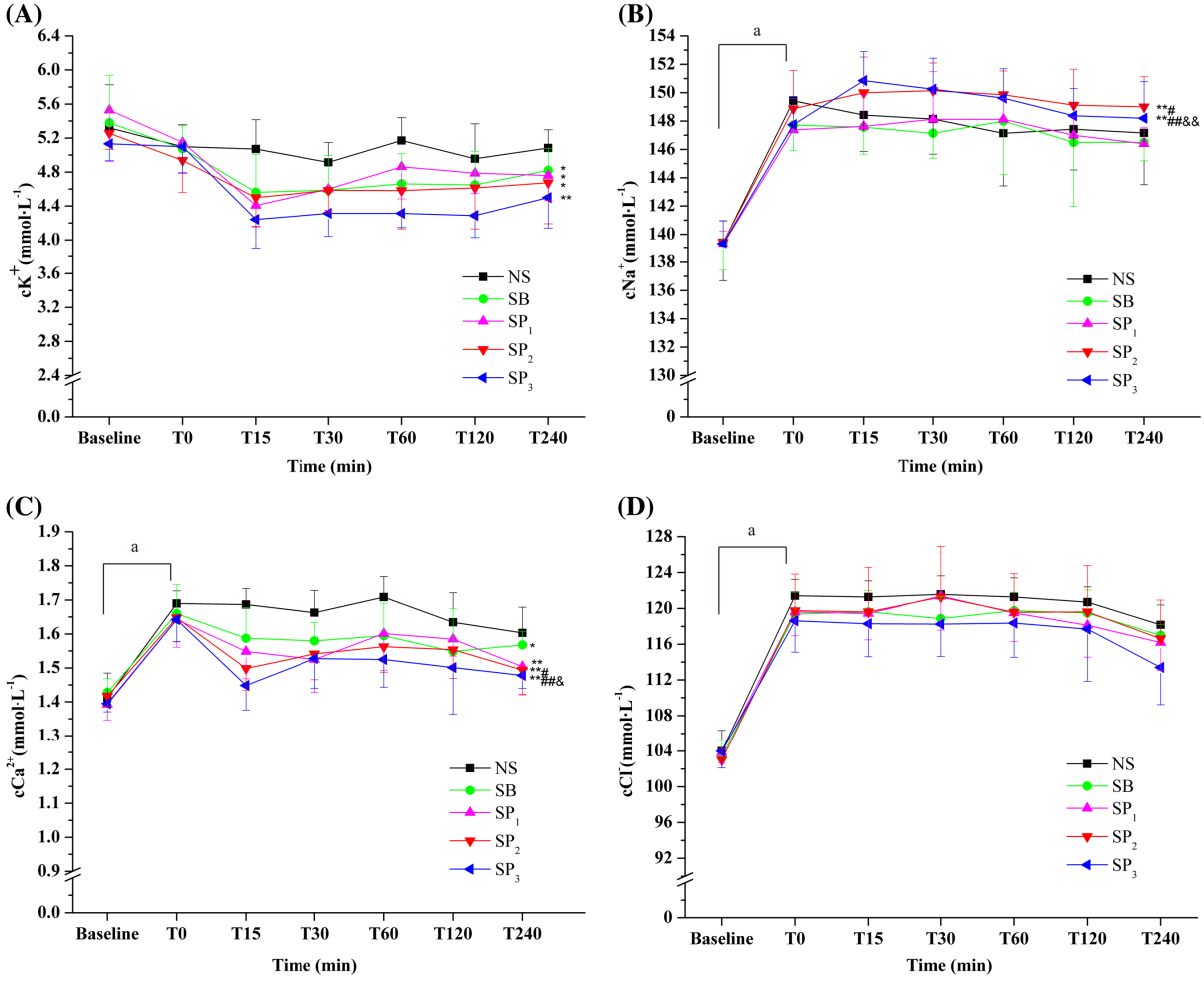


Figure 3. Changes in venous plasma concentration of potassium (cK+, mmol·L-1, A), sodium (cNa+, mmol·L-1, B), calcium (Ca2+, mmol·L-1, C), and chloride (cCl-, mmol·L-1, D) in response to NH4Cl loading and infusion of solution via tail vein treated with normal saline (NS), sodium bicarbonate at dose of 2 mmol·kg-1 (SB), sodium pyruvate at dose of 2 mmol·kg-1 (SP1), sodium pyruvate at dose of 4 mmol·kg-1 (SP2), and sodium pyruvate at dose of 6 mmol·kg-1 (SP3). Data are presented as means (SD); $^{**}P<0.05$, vs. NS; $^{\#}P<0.05$, $^{\#\#}P<0.01$, vs. SB; $^{\&\&}P<0.01$, vs. SP1; $^{a}P<0.05$, T0 vs. baseline.

a peak, while the SB group took 30 min (Figure 1). The pH values in the $SP_3$ group clearly stayed above those of the SB group ($P<0.05$). A notable amelioration of systemic acidosis in the $SP_3$ group lasted for 240 min after administration (Figure 1). The effect of SB on pH was most notable in the first 30 min (pH: $T_0$, $7.16\pm0.08$; $T_{30}$, $7.28\pm0.04$) but then tended to decrease. The BE and $cHCO_3^-$ corresponded to the changes in the values of pH in all groups (Figure 2).

### Effect of pyruvate treatment on blood $PaCO_2$ and $PaO_2$

$PaCO_2$ and $PaO_2$ showed non-significant difference in all groups after administration (Table I).

### Effect of pyruvate treatment on blood electrolytes

Treatment with SP and SB decreased plasma potassium levels (Figure 3A). The SB, $SP_1$, and $SP_2$ groups had a lower potassium level than the NS group ($P<0.05$) (Figure 3A). The $SP_3$ group had a lower potassium level than the NS group ($P<0.01$) (Figure 3A). No significant difference was observed between $SP_1$, $SP_2$, $SP_3$, and SB groups (Figure 3A).

No significant differences were observed between the $SP_1$, SB, and NS groups in the blood sodium levels (Figure 3B). Infusion of $SP_2$ or $SP_3$ resulted in higher blood sodium levels than that of NS ($P<0.01$, Figure 3B). The $SP_2$ group had a higher sodium levels than the SB group ($P<0.05$, Figure 3B). The $SP_3$ group had higher sodium levels than the SB group ($P<0.01$) and the $SP_1$ group ($P<0.01$, Figure 3B).

Infusions of SB and SP were also associated with a decrease in the blood free calcium concentrations that had increased in the acidosis (Figure 3C). The $SP_1$, $SP_2$,and $SP_3$ groups had lower free calcium levels than the NS group ($P<0.01$) (Figure 3C). The SB group had lower free calcium levels than the NS group ($P<0.05$) (Figure 3C). The $SP_2$ group had lower free calcium levels than the SB group ($P<0.05$) (Figure 3C). Moreover, the $SP_3$ group had lower free calcium levels than the SB group ($P<0.01$) and the $SP_1$ group ($P<0.05$) (Figure 3C).

The chloride levels decreased moderately after treatment in all groups, and no significant differences were observed between all the groups (Figure 3D).

### Effect of pyruvate treatment on blood glucose

No significant differences were observed between the $SP_1$, $SP_2$, SB, and NS groups in the glucose levels. The $SP_3$ group had higher glucose levels than the $SP_1$ group ($P<0.05$, Figure 4).

### Effect of pyruvate treatment on plasma Cr and urea levels

No significant differences were observed between all the groups in the plasma Cr and urea levels at $T_{240}$ (Table II).

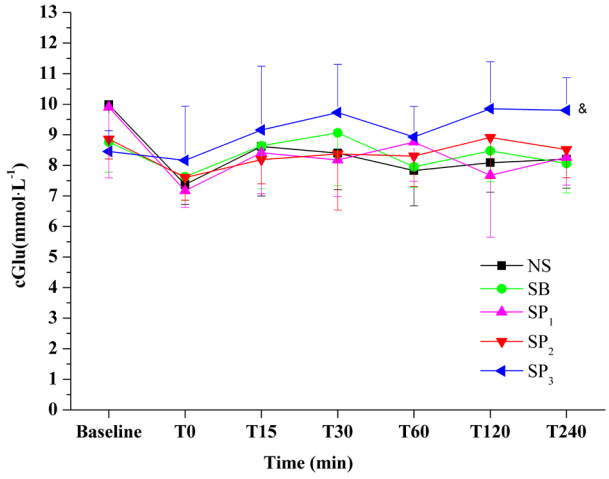


Figure 4. Changes in venous plasma concentration of glucose (cGlu, mmol·L-1) in response to NH4Cl loading and infusion of solution via tail vein treated with normal saline (NS), sodium bicarbonate at dose of 2 mmol·kg-1 (SB), sodium pyruvate at dose of 2 mmol·kg-1 (SP1), sodium pyruvate at dose of 4 mmol·kg-1 (SP2), and sodium pyruvate at dose of 6 mmol·kg-1 (SP3). Data are presented as means (SD); &$P<0.05$, vs. SP1.

### Effect of pyruvate treatment on pHi levels

In the control group, sodium propionate induced a rapid decrease in pHi, corresponding to an average fall of 0.3 units in R values. Following the acidification, the pHi slowly recovered to 85% of its initial values in 3 min. The addition of NS had no effect on pHi, while the addition of SP caused a transient rise in pHi. The SP groups have a higher peak R values than the NS group ($P<0.05$) and the control group ($P<0.05$). No significant differences were observed between the NS group and the control group (Figure 5).

## Discussion

For more than 100 years, SB administration has been considered the most universal therapy for patients with metabolic acidosis (Kraut and Kurtz 2006, Narins and Cohen 1987, Forsythe and Schmidt 2000). However, several studies have suggested that SB administration often increases pHe but transiently decreases pHi in several vital organs such as heart and brain (Arieff et al. 1982, Forsythe and Schmidt 2000, Kraut and Kurtz 2001, Kurtz and Madias 2010, Narins and Cohen 1987, Shapiro et al. 1989). It is due to rapid diffusion of dissolved $CO_2$ originating from exogenous SB into the cell, which results in the overproduction of intracellular $H^+$ (Levraut et al. 1996). The decreased pHi may result in intracellular acidosis that damages organ function. Investigators have focused on the development of agents that could increase both pHe and pHi. Lactated ringer solution and NS, the maintenance therapy with large

Table II. Effect of treatment on Cr and Urea levels.

| Parameter | NS ($n=7$) | SB ($n=8$) | $SP_1$ ($n=8$) | $SP_2$ ($n=8$) | $SP_3$ (n = 8) |
|---|---|---|---|---|---|
| Cr | 45.56 ± 6.65 | 44.7 ± 4.52 | 43.89 ± 4.04 | 39.69 ± 2.61 | 41.35 ± 4.69 |
| Urea | 11.05 ± 1.93 | 9.82 ± 1.65 | 9.56 ± 1.87 | 9.55 ± 1.97 | 10.64 ± 1.11 |

Venous plasma creatinine (Cr, umol·$L^{-1}$) and urea (mmol·$L^{-1}$) levels were determined 240 min after infusion of solution via tail vein treated with NS, sodium bicarbonate at dose of 2 mmol·$kg^{-1}$ (SB), sodium pyruvate at dose of 2 mmol·$kg^{-1}$ ($SP_1$), sodium pyruvate at dose of 4 mmol·$kg^{-1}$ ($SP_2$), sodium pyruvate at dose of 6 mmol·$kg^{-1}$ ($SP_3$). No significant differences were observed between all the groups in the Cr and the urea levels. Data are presented as means ± SD; n is the number of rats.

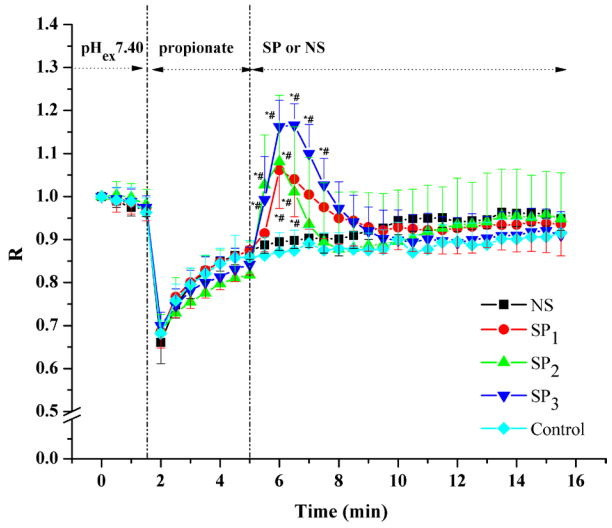


Figure 5. Changes in intracellular pH (pHi) in response to sodium piopronate loading and addition of sodium pyruvate (SP) 33 mmol·L-1 (SP1 group, $n = 45$), 66 mmol·L-1 (SP2 group, $n = 45$) and 100 mmol·L-1 (SP3 group, $n = 45$). NS addition was performed as negative control group (NS group, $n = 45$). No addition was performed as blank control group (Control group, $n = 45$). There was a positive correlation between BCECF emission ratio (R, R = F(Ex = 488nm/Em = 535nm)/F(Ex = 445nm/Em = 535nm)) and pHi. Data are presented as means (SD). *$P < 0.05$, vs. NS; #$P < 0.05$, vs. control.

volume infusions in clinic, didn't change the acid–base balances (Cosenza et al. 2013, Mongan et al. 2002). Thus, in the present study, we chose NS treatment as the negative control referred to in the previous study (Bar-Joseph et al. 1998). The *in vivo* and *in vitro* results showed that treatment with NS has no amelioration effect on metabolic acidosis. The blood pH, bicarbonate, and BE revealed that SP could ameliorate the metabolic acidosis induced by $NH_4Cl$ *in vivo*. In intracellular acidosis cell model, SP was found to increase pHi. Both the *in vivo* and the *in vitro* effects were dose dependent. It seems that SP may be superior to SB in the treatment of acidosis.

The amelioration effect of SP on acidosis did not solely result from its chemical buffering ability. First, the pH of SP solution that infused in our study was from 6.32 to 6.37, while the blood pH was higher than 7.0 even at $T_0$ in acidosis. After treatment with SP, the blood pH increased. Second, pyruvate only has a weaker buffering capacity due to the dissociation constant (pKa) of 2.49 compared with $HCO_3^-$ pKa of 6.36. Thus, exogenous pyruvate may ameliorate acidosis via its biochemical characteristics more than chemical buffering. We propose that it works via consuming intracellular protons in metabolic processes as follows (Zhou 2005):

(1) Oxidation into $CO_2$ and $H_2O$ via the TCA cycle under aerobic conditions;

Endogenous pyruvate is converted into acetyl-CoA catalyzed by the pyruvate dehydrogenase (PDH) complex, which is the main input for the TCA cycle as part of aerobic respiration. Acetyl-CoA can be oxidized into $CO_2$ and $H_2O$ via the TCA cycle, consuming equal molar protons in mitochondria (Oliver et al. 1994). Metabolic acidosis inhibits pyruvate oxidation in liver by decreasing activity of PDH (Toyomizu et al. 1999). Furthermore, Mongan et al. have confirmed that the administration of pyruvate resulted in the prevention of PDH deactivation during acidosis induced by hemorrhagic shock (Mongan et al. 2003, Sharma et al. 2005). Thus, we hypothesis that exogenous pyruvate may correct metabolic acidosis via the TCA cycle by supplying substrate and stimulating the activity of PDH.

(2) Reduction to lactate by lactate dehydrogenase (LDH) under hypoxic conditions;

When oxygen is absent or insufficient, endogenous pyruvate can be transformed into lactate by LDH, which regenerates $NAD^+$ (oxidized form: nicotinamide adenine dinucleotide) from NADH (reduced form: nicotinamide adenine dinucleotide) for a continuous supply for glycolysis. The process consumes equal molar protons from the cytosolic hydrogen pool which may raise pHi and $NAD^+$/NADH ratios. Several studies have shown that exogenous pyruvate loading can increase lactate levels and therefore provide evidence for this mechanism (Petrat et al. 2011, Mongan et al. 2003, Hu et al. 2013). In addition, recent study showed that exogenous pyruvate loading increased $NAD^+$/NADH ratios (Hegde et al. 2010). Hence, we assume that exogenous pyruvate may correct metabolic acidosis by being reduced to lactate.

(3) Gluconeogenesis;

Gluconeogenesis is an energy-requiring process. Exogenous pyruvate can be transformed into glucose by gluconeogenesis, consuming double molar protons (Burns et al. 2001). In our study, glucose concentrations in the $SP_3$ group were higher than those in the $SP_1$ group. It suggests that the appropriate dose of pyruvate may promote gluconeogenesis while increasing systemic pH.

In addition, recent reports show that exogenous pyruvate can enhance hypoxia-inducible factor-1 (HIF-1) activity, which stimulates glycolytic flux by inducing the expression of glucose transporters (Gleadle and Ratcliffe 1997, Maxwell et al. 1997) and glycometabolic enzymes including hexokinase (Mathupala et al. 2001), phosphoglycerate kinase 1 (Semenza et al. 1994), and lactate dehydrogenase A (Semenza et al. 1996). HIF-1 plays a central role in the adaptive regulation of energy metabolism and regulates many target genes. We conjecture that exogenous pyruvate may correct metabolic acidosis through regulating glycometabolism and HIF-1-related signal pathways. Further research is necessary to clarify the exact mechanisms.

Metabolic acidosis has complex effects on various plasma electrolyte levels. In our study, chloride and sodium levels increased significantly with the decreased pH for $NH_4Cl$ loading. After treatment with SP, the plasma sodium levels increased in the high-dose ($SP_2$ and $SP_3$) groups, and there was not significant change in $SP_1$ and SB groups, which corresponded with the concentration of sodium infused. However, no significant differences were observed between the SP and SB groups in the anion gap (AG) which was calculated as follows: $cNa^+ + cK^+ - cCl^- - cHCO_3^-$ ($P > 0.05$, data not shown). This suggests that sodium loading from SP treatment did not change AG levels.

When metabolic acidosis occurs, the plasma free calcium level may increase immediately, because low pH reduces the binding of free calcium to albumin (Pederson 1972). Increased calcium adversely affects the function of most organ systems, especially the kidney, central nervous system, and cardiovascular system. In our study, high plasma free calcium concentrations were also observed in rats with decreased pH. $SP_3$ treatment decreased the plasma free calcium concentration to near-normal levels. This suggests that SP can ameliorate the abnormality of calcium associated with acidosis.

Reports show that metabolic acidosis can increase plasma potassium concentration created by the infusion of mineral acids ($NH_4Cl$ or HCl, Adrogue and Madias 1981, Burnell et al. 1956, Oster et al. 1980). In our study, plasma potassium concentration did not increase significantly, but rather, decreased after 6 days of $NH_4Cl$ loading in drinking water, which corresponds to a recent study by Marta Nowik (2010). The contrary results may be due to the different methods of acid loading, namely the short-duration infusion way (Adrogue and Madias 1981, Burnell et al. 1956, Oster et al. 1980) and the long-duration intake method (Nowik et al. 2010). After treatment, both the SP and the SB groups had more decreased plasma potassium levels. In addition, a low plasma potassium level corresponded with a high pH and $HCO_3^-$ levels. This is because potassium depletion can stimulate $H^+$-$K^+$-ATPase (Doucet and Marsy 1987, Buffin-Meyer et al 1977, Wingo 1989) activity, which may increase $HCO_3^-$ re-absorption and $H^+$ excretion in renal proximal convoluted tubule (Capasso et al. 1987, Hays et al. 1986).

Kidneys play an important role in restoring acid–base balance. In the *in vivo* study, plasma Cr and urea levels increased compared with the normal values in rats because of $NH_4Cl$-loading (Roncal et al. 2007). Meanwhile, the results of Cr and urea levels suggested that SP did not aggravate the renal injury compared with SB and NS.

In summary, in the *in vivo* study, treatment with SP at a dose of 4 and 6 $mmol \cdot kg^{-1}$ provided notable beneficial effects on metabolic acidosis, while 2 $mmol \cdot kg^{-1}$ of SP could alleviate the acidosis partly. In the *in vitro* study, the pHi increased immediately after the addition of SP. Thus, intravascular treatment with SP represents a novel therapeutic strategy to ameliorate metabolic acidosis.

## Limitations

Although the results of this study indicate that SP may ameliorate metabolic acidosis by the regulation of glycometabolism, the mechanisms by which exogenous pyruvate consumes intracellular protons are yet to be determined. Further research is necessary to detect the effect of exogenous pyruvate on pyruvate, lactate, PDH, and LDH levels.

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## Declaration of interest

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