

Original Article

Effects of basic drugs on prognosis of acute lung injury in mice

Liming Jia¹, Junming Ren², Weiwei Zhang¹, Yuehong Qi¹, Lina Zheng¹, Yongqing Guo¹

¹Department of Anesthesiology, The People's Hospital of Shanxi Province, Taiyuan 030012, China; ²Department of Anesthesiology of Shanxi Medical University, Taiyuan 030012, China

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Abstract: The aim of this study was to investigate the effects of basic drugs that alkalizes blood, on prognosis of acute lung injury in mice. Mice were randomized into three groups: Group normal saline, Group THAM, injected with 3.64% tri-(hydroxymethyl) methylamine (THAM), and Group NaHCO₃, injected with 5% NaHCO₃ (n=26, each group). The acute lung injury model was established by intraperitoneal injection of lipopolysaccharide (LPS; 50 mg/kg), followed by infusion of varying concentrations of the above solution into tail vein at the rate of 0.5 ml/h (controlled by micro pump) for over 2 h. Thirty minutes later, 6 mice from each group were randomly selected for blood gas analysis; then, the mice were killed and their lung tissues were sampled for detection of relative indicators, and the remaining mice were observed for signs of mortality for 72 h. Arterial pH, bicarbonate (HCO₃⁻), and BE and mortality of group THAM and NaHCO₃ increased significantly compared to the corresponding parameters of the group normal saline (P<0.05); compared to the group normal saline, group NaHCO₃ had increased blood [Na⁺] and decreased [K⁺] and [Ca²⁺] (P<0.05). Blood [Na⁺] of group THAM decreased while the lactic acid concentration increased (P<0.05) compared to the corresponding values of the group normal saline. Malondialdehyde (MDA) and myeloperoxidase (MPO) activity and wet-to-dry lung weight ratio (W/D) of group THAM and NaHCO₃ increased significantly relative to group normal saline (P<0.05). Compared with the biopsy results of (A), pathological biopsy of (B) and (C) clearly revealed alveolar wall thickening, edema of alveolar epithelial cells, and infiltration of large neutrophils. Alkalizing blood could neither inhibit inflammatory reactions in LPS mouse model nor reduce the mortality rate of mice with acute lung injury, while excessive alkalization of blood could increase mice mortality.

Keywords: Mouse, acute respiratory distress syndrome, alkalizing blood, endotoxin, lung injury

Introduction

Acute lung injury (ALI) is a clinical syndrome that occurs in response to various characteristic pathological changes in lung tissue structures, such as alveolar capillary endothelial cell and alveolar epithelial cell injury; extensive pulmonary edema and microatelectasis are hallmarks of ALI. The pathophysiological changes mainly appear as increasing pulmonary shunt and decreased lung compliance. The clinical manifestations include hypoxemia, increased respiratory rate, and bi-pneumal diffuse infiltration in X-ray. The mortality rate of ALI was as high as 39% [1-6], and certain studies have shown that neutrophils played important roles in the pathogenesis of ALI [7-9], the activation of which would release oxygen free radicals, proteases, leukotriene, and other proinflamma-

tory molecules, thus initiating the inflammatory cascade, and eventually leading to ALI.

An in vitro study has shown that alkalizing blood could weaken neutrophil's adhesion and accelerate their apoptosis [10-12]; however, blood alkalization treatment for ALI has not been reported in vivo thus far. This study established an ALI model and investigated the effects of alkalized blood on the pathogenesis of ALI.

Materials and methods

Animals and grouping

Seventy-eight healthy female Kunming mice, 4-6 weeks old, weighing 18-22 g, were provided by Experimental Animal Center of Shanxi Medical University. The mice were randomly divided into three groups of 26 mice each:

group normal saline, receiving normal saline (NS); group THAM receiving 3.64% tri(hydroxymethyl) methylamine (THAM); group NaHCO_3 , receiving 5% NaHCO_3 ; the treatments were injected into the tail vein. Animals were provided free access to food and water for 3 days before the experiments. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Shanxi provincial people's hospital.

Experimental methods

First, two pilot experiments were performed to determine the mortality of endotoxin-induced ALI in mice and the effects of THAM and NaHCO_3 at different gradient doses on plasma pH in ALI mice. The targets of mortality and plasma pH of ALI mice within 72 h were 40% and ~7.5, respectively. Based on the results of these 2 pilot experiments, the ALI mice were divided into three groups as mentioned above. We compared the mortality rates among the three groups, aiming to investigate the mechanisms of basic drugs on neutrophils in ALI mice.

Pilot experiment 1: Determining the mortality of endotoxin-induced ALI in mice

Twenty-four mice were randomly divided into three groups and received intraperitoneal injection of 0.2 ml of NS and 0.2 ml of 40 and 50 mg/kg lipopolysaccharide (LPS; dissolved in phosphate-buffered saline solution). The mortality rates were monitored for 3 days; the mice that survived were killed for lung tissue sampling to confirm the occurrence of ALI.

Pilot experiment 2: Determining the target pH value

The THAM and NaHCO_3 doses that could alkalinize the plasma pH of ALI mice to 7.5 were evaluated. Eighteen mice were randomly divided into two groups of 9 mice each; the two groups were further subdivided into three subgroups, with 3 mice in each subgroup. The mice were anesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg), and received intraperitoneal injection of LPS (50 mg/kg). Each THAM subgroup received infusion of 3.64% THAM solution through an infusion pump

into tail vein 30 minutes after LPS administration at a rate of 0.3, 0.5, and 0.8 ml/h, respectively, for 2 consecutive hours; the NaHCO_3 group was also divided into three subgroups, which received infusion of 5% NaHCO_3 similar to the THAM group. Since NaHCO_3 infusion could cause hypokalemia, 0.16% KCl was added to 5% NaHCO_3 to avoid hypokalemia. Thirty minutes after infusion, arterial blood was sampled from the heart for blood gas analysis.

Effects of blood alkalization on mortality

Seventy-eight mice were randomly divided into three groups: group normal saline, group THAM, and group NaHCO_3 , with 26 mice in each group. According to the pilot experiment 1, all mice were anesthetized with intraperitoneal injection of sodium pentobarbital (40 mg/kg) and then received intraperitoneal injection of LPS (50 mg/kg), 30 minutes later. According to the pilot experiment 2, group THAM was infused with 3.64% THAM, group NaHCO_3 was infused with 0.16% KCL-containing 5% NaHCO_3 , and group normal saline received NS infusion; all the treatments were infused into the tail vein through an infusion pump for 2 consecutive hours at an infusion rate of 0.5 ml/h. After infusion, 6 mice from each group were randomly selected for arterial blood sampling from the heart for blood gas analysis 30 minutes after respective treatments. The remaining mice were placed back into their cases and observed for signs of mortality for 72 h, with the observation frequency being once every 3 h for the first 24 h, and once every 12 h, thereafter.

Mortality

The mortality rate of the experimental groups was monitored for 72 h after the above-mentioned interventions.

Malondialdehyde (MDA) and myeloperoxidase (MPO) activity

After the experiment, the left lung of a mouse was collected to prepare 10% lung tissue homogenate, which was then centrifuged and the supernatant was evaluated for MDA and MPO content [7-10].

Determination of lung wet/dry ratio

The superior right lung lobe tissues were sampled, wiped-off of liquid from the surface, the

Effect of alkalizing blood on prognosis of acute lung injury

Table 1. Arterial blood gas analysis

Item	THAM	NaHCO ₃	Normal saline
pH	7.42±0.07 ^Δ	7.48±0.09 ^Δ	7.17±0.05
HCO ₃ ⁻	30.24±5.42 ^Δ	34.40±8.30 ^Δ	24.84±4.30
BE	8.41±0.15 ^Δ	8.51±0.34 ^Δ	-4.52±0.20
Na ⁺	128.30±8.14 ^Δ	165.30±8.08 ^Δ	136.50±9.21
K ⁺	3.38±0.08 [*]	2.84±0.05 ^Δ	3.45±0.11
Lactic acid	6.44±0.05 ^Δ	7.24±0.12 ^Δ	2.46±0.25
PCO ₂	54.33±8.12 [*]	55.83±9.91 [*]	49.00±6.19
PO ₂	73.83±10.26 [*]	71.33±8.05 [*]	81.20±14.80
Glucose	4.84±0.21 [*]	5.10±0.34 [*]	4.62±0.54

Note: Compared with group Normal saline: ^ΔP<0.05; ^{*}P>0.05 (n=6, mouse numbers in each group).

Table 2. Mortality rates of each group

Item	THAM	NaHCO ₃	Normal saline
Survival (n)	5	3	11
Death (n)	15	17	9
Sum (n)	20	20	20
Mortality rate (%)	75 ^Δ	85 ^Δ	45

Note: Compared with group Normal saline: ^ΔP<0.05. (n=20, mouse numbers in each group).

Table 3. Detection indicators of each group

Group	MDA activity (u/g)	MPO activity (u/g)	W/D
THAM	2.64±0.41 ^Δ	1.13±0.22 ^Δ	0.115±0.004 ^Δ
NaHCO ₃	2.70±0.10 ^Δ	1.68±0.43 ^Δ	0.163±0.076 ^Δ
Normal saline	1.20±0.74	0.83±0.19	0.087±0.004

Note: Compared with group Normal saline: ^ΔP<0.05; (n=6, mouse numbers in each group).

wet weight was measured, tissues were incubated in 90°C oven to constant weight, the dry weight was measured, and then, the wet/dry weight ratio was calculated [7-10].

Morphological examination

The inferior right lung lobe tissues were sampled, fixed in 4% formalin, followed by paraffin-embedding, hematoxylin-eosin staining, and examined for pathological changes under light microscope [7-10].

Statistical analysis

All experimental data were processed with SPSS11.5 software; the mortality of the three

groups were compared with chi-square test of fourfold table exact probability method, the results of blood gas analysis, lung wet/dry ratio, MDA and MPO activity of the 3 groups were compared with ANOVA, with P<0.05 considered as significant difference.

Results

Pilot experiment 1

Compared with mice receiving intraperitoneal injection of NS, the mice receiving intraperitoneal injection of LPS (40 mg/kg and 50 mg/kg) were more prone to trembling, shortness of breath, anorexia, convulsions, and drowsiness. Of the 8 mice that received intraperitoneal injection of LPS (40 mg/kg), 2 died within 3 days, with the mortality rate being 25%; of the mice receiving intraperitoneal injection of LPS (50 mg/kg), 4 died within 3 days, with the mortality rate being 50%.

Pilot experiment 2

After infusion of 5% NaHCO₃ at the rate of 0.3, 0.5, and 0.8 ml/h, the pH values of arterial blood were 7.359±0.060, 7.492±0.098, and 7.606±0.106, respectively; while the pH values after infusion of 3.64% THAM were 7.219±0.063, 7.48±0.079, and 7.55±0.116, respectively.

The mortality rate of group normal saline was 45% within 72 h (95% CI, CI: 33.20%~76.80%), while those of group THAM and NaHCO₃ were 75% and 85%, respectively, (95% CI, CI: 56.02%~93.98%; 95% CI, CI: 56.02%~93.98%); significant differences in mortality rates between group THAM and NaHCO₃ were observed.

Arterial blood gas analysis

Arterial pH and HCO₃⁻BE of group THAM and group NaHCO₃ significantly increased compared to that of group normal saline (P<0.05); compared with group normal saline, group NaHCO₃ had significantly elevated plasma [Na⁺] and decreased [K⁺] (P<0.05), while group THAM had significantly reduced plasma [Na⁺] and elevated plasma lactic acid concentration (P<0.05). However, pCO₂, pO₂, blood glucose

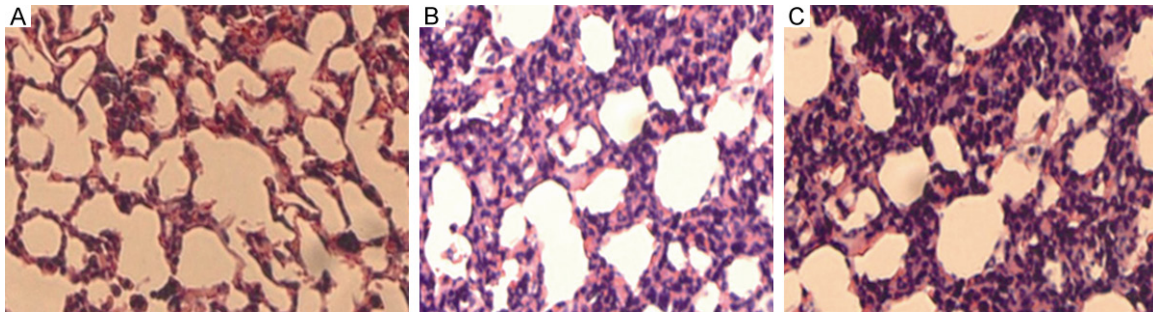


Figure 1. Note: A: Group Normal saline; B: Group THAM; C: Group NaHCO₃.

levels, and hematocrit among the three groups were not significantly different ($P>0.05$; **Table 1**).

Mortality rates

Compared with the mortality rates of group normal saline, the mortality rates of group THAM and NaHCO₃ increased significantly ($P<0.05$; **Table 2**).

MDA and MPO activity and W/D

Compared with group normal saline, group THAM and NaHCO₃ had significantly elevated MDA and MPO activity and W/D ($P<0.05$; **Table 3**).

Morphological examination

Compared with the biopsy results of (A), pathological biopsy of (B) and (C) revealed alveolar wall thickening, edema of alveolar epithelial cells, and infiltration of large neutrophils (**Figure 1**).

Discussion

ALI or acute respiratory failure is caused by various factors with respiratory distress, refractory hypoxemia, and non-cardiogenic pulmonary edema being the main features. It is one of the common clinical critical diseases, with the mortality rate of 39%. The 72-h mortality rate observed in this study was consistent with that reported in the literatures [13-16].

This study showed that intravenous infusion of THAM and NaHCO₃ could not reduce the mortality in LPS-induced ALI mice; on the contrary, compared with NS infusion, infusion of NaHCO₃ and THAM increased the mortality rates of ALI mice. The results showed that alkalizing the

blood inside body could neither promote apoptosis of neutrophils, nor inhibit inflammatory mediators; therefore, this treatment could not inhibit the inflammatory responses.

The pathological changes in lung tissues could help diagnose ALI. In pilot experiment 1, pathological biopsy clearly revealed alveolar wall thickening, edema of alveolar epithelial cells, and infiltration of large neutrophils. These pathological changes are the main hallmarks of ALI. Although the pilot experiment showed that intraperitoneal injection of 40 mg/kg or 50 mg/kg LPS could induce ALI, these treatments produced different mortality rates. The mortality rates associated with intraperitoneal injection of 40 mg/kg LPS was 25% within 72 h, while that of intraperitoneal injection of 50 mg/kg LPS was 50%. It was reported that the mortality rate of ALI was 49%; therefore, this study selected 50 mg/kg LPS to induce ALI.

In vitro studies have reported that increasing plasma pH to 7.5 [17-20] could effectively reduce neutrophils' adhesion and accelerate their apoptosis [21-23], thereby disrupting the functions of neutrophils. In pilot experiment 2, infusion of NaHCO₃ or THAM into tail vein increased plasma pH to 7.5 in ALI mice; the experiments showed that 2-consecutive-hour infusion of 3.64% THAM and 5% NaHCO₃ at 0.5 ml/h, could increase plasma pH to 7.5 in ALI mice.

In this study, two different base preparations, namely THAM and NaHCO₃, were used. They had different effects on plasma serum [Na⁺] and [K⁺]. THAM could increase plasma [K⁺] but not plasma [Na⁺] whereas NaHCO₃ could decrease plasma [K⁺] and increase plasma [Na⁺]; these differential effects were mainly owing to their different roles in regulating elec-

trolytes. This study showed that these two different base preparations had differential effects on mortality and plasma pH in ALI mice, suggesting that the clinical application of base preparation should be carefully considered and strictly mastered the indications, in order to avoid the base preparation infusion-triggered life-threatening hypokalemia. In this study, 0.16% KCL was added to avoid the hypokalemia-induced death of ALI mice.

It was previously reported that granulocytes, especially neutrophils played important roles in the occurrence and development of ALI [24, 25]. Increasing plasma pH could weaken the functions of neutrophils; therefore, we examined the effects of alkalizing blood on ALI. However, the results of this experiment were not satisfactory. Instead, alkalizing the blood of ALI mice led to harmful results; blood alkalization through infusion of THAM and NaHCO_3 led to higher mortality rate in ALI mice most likely owing to elevated pH as well as other factors involved in *in vivo* experiments. The underlying reason warrants further research. The increased mortality rate observed in this study could also be attributable to the differential effects of treatments (NS, NaHCO_3) on plasma lactic acid levels and $[\text{Na}^+]$, $[\text{K}^+]$, and $[\text{Ca}^{2+}]$. The single buffer system in *in vitro* experiments could not truly reflect the overall moderating effects in animals; the findings of this study were consistent with those of the reports that the infusion of NaHCO_3 had no beneficial effect on ALI and lactic acidosis.

Previous studies have reported that although NaHCO_3 infusion could lead to plasma electrolyte imbalance in ALI mice, it did not enhance pCO_2 . This abnormal phenomenon could be attributable to the ALI model used in this study as well as to blood gas analysis performed 3 h after LPS induction; the compensatory mechanism of gas exchange is not damaged during this time.

The purpose of this experiment was to evaluate the protective effects of alkalizing blood on ALI mice, by administrating LPS at a dose above its LD50. Compared with the mortality rate associated with intraperitoneal injection of LPS (87.5%) [26, 27], the mortality rate of group normal saline group (50 mg/kg group) was 45%. The difference in mortality rate between these two groups might be owing to anesthesia and

fluid resuscitation. Certain studies had shown that pentobarbital sodium could aggravate LPS-induced hypotension; however, it was also reported to relieve LPS-induced organ dysfunction, thereby affecting the mortality rates of LPS-induced ALI. The infusion of NS solution improved organ perfusion and oxygen delivery. The infusion of pentobarbital sodium and NS solution significantly reduced the mortality rate in LPS-induced ALI mice.

Our research showed that the infusion of THAM improved arterial blood pH, HCO_3^- , and base excess compared to NS, while the data showed no statistical significance, which might possibly be owing to sample size and other factors. The 72-h mortality rate of group THAM was higher, indicating that alkalizing blood did not decrease the mortality rate of ALI mice. The experimental results showed that the blood alkalization therapy was harmful towards ALI mice, but the underlying mechanisms warrant further studies.

Conclusions

THAM and NaHCO_3 could alkalize the blood in ALI mouse; however, alkalizing blood had no inhibitory effects on inflammatory responses in ALI mice, and did reduce the mortality rate. NaHCO_3 solution was not suitable for the treatment ALI mainly owing to its side effects.

Disclosure of conflict of interest

None.

Address correspondence to: Yongqing Guo, Department of Anesthesiology, The People's Hospital of Shanxi Province, Taiyuan 030012, China. Tel: +86-351-4960427; Fax: +86-351-4960184; E-mail: lmyqcn@163.com

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Effect of alkalizing blood on prognosis of acute lung injury

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