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Polyamines Improve Anti-Blood Lactate Accumulation in an Acidosis Rat Model

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Lactic acidosis (lactate accumulation and pH downfall) occurs in a number of clinical conditions and has deleterious effects on the patient's survival. Sodium bicarbonate and tromethamine are administrated in these conditions. However, these compounds adjust only the blood pH and do not affected the lactate level. In this study, administration of polyamines was hypothesized as a novel approach for treatment of lactate accumulation. For this purpose, the impact of different polyamines on the experimental model of acidosis was evaluated. In this study, the rats were divided into different groups and lactic acidosis type B was induced in them. Blood lactate was measured before and after acidosis in rats along with polyamines administration. Statistical analysis showed that polyamines such as putrescine, cadaverine and spermidine had significant effects on the lactate level, whereas sodium bicarbonate and tromethamine had no effect on it. These findings supported the advantageous effects of polyamines in treatment of lactate accumulation and can have implications for treatment of acidosis in human. Therefore, polyamines are proposed as an effective treatment for lactic acidosis.

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Introduction

Lactic acidosis, a disorder characterized by accumulation of lactic acid in the blood (to 5 mmol/L or higher), results in a lowered pH (below 7.35) in the muscle and blood. These conditions occur most commonly in tissue hypoxia; however, it may also result from liver impairment, respiratory failure, burn trauma, neoplasms, and cardiovascular disease. Different reasons lead to lactic acidosis including: *i)* genetic conditions: diabetes mellitus and deafness, glucose-6-phosphatase deficiency, fructose 1,6-diphosphatase deficiency, pyruvate dehydrogenase (PDH) deficiency and pyruvate carboxylase deficiency; *ii)* drugs: phenformin, metformin, isoniazid, nucleoside reverse transcriptase inhibitors, potassium cyanide (cyanide poisoning); *iii)* other reasons: hypoxia and hypoperfusion, hemorrhag, ethanol toxicity, sepsis, shock, hepatic disease, diabetic ketoacidosis, muscular exercise, regional hypoperfusion (bowel ischemia, marked cellulites, etc), non-Hodgkin's and Burkitt lymphomas [1].

Sodium bicarbonate (Na-bicarb), tromethamine (Thm) and dichloroacetate are the drugs that have been administrated in lactic acidosis condition. Although Na-bicarb administration to treat metabolic acidosis has been controversial, Na-bicarb treatment is no longer recommended. This is due to the concern of increased CO_2 concentration in the blood in the event of impaired pulmonary blood flow and ventilation; thereby it prevents CO_2 elimination [2-6].

Putrescine (Put: 1,4-diaminobutane), cadaverine (Cad: pentane-1,5-diamine), spermidine (Spd: N-(3-aminopropyl) butane-1,4-diamine) and spermine (Spm: N,N'-bis (3-aminopropyl-1,4-butanediamine) are simple aliphatic multivalent cations with amine functional group as essential constituents of all mammalian cells. These are well known as biogenic polyamines (BPs). One or more of these compounds are present in every living cell [7]. All prokaryotic and eukaryotic cells synthesize Put and Spd. Spm synthesis, however, is largely confined to nucleated eukaryotic cells. In animals and most plants, Put can be synthesized directly by decarboxylation of ornithine via enzyme ornithine decarboxylase. Polyamines (PAs) act as an activator of the enzyme PDH at physiological concentrations of Mg^{2+} [8].

In this study, the effects of different BPs of Spd, Cad, and Put on the simulated acidosis induced in rat (by administration of phenformin) were investigated. In this regard, lactate enhancement in the blood sample of acidosis rat was compared between different groups treated with different PAs or Na-bicarb and Thm.

Materials and methods

Chemicals

Phenformin hydrochloride (Phen-HCl), spermidine hydrochloride (Spd-HCl), cadaverine hydrochloride (Cad-HCl), and Putrescine hydrochloride (Put-HCl) were purchased from Sigma-Aldrich. Tris buffer (2-Amino-2-hydroxymethyl-propane-1, 3-diol) was purchased from Merck. Thm solution was prepared from Tris buffer (0.3 M), adjusted to a pH of approximately 8.6 with glacial acetic acid. Na-bicarb injection (8.4% w/v acetic substance) was received from Hospira, Inc., USA. Male Sprague dawley rats were received from Animal House of Shiraz University of Medical Sciences, Shiraz, Iran. The animals were housed in special cages at a controlled temperature (24±2°C) and humidity (40% to 70%) with weekly floor exchange. They had free access to water and standard pelleted laboratory animal diets. A 12:12 light: dark cycle was followed in the mentioned Animal House Center. All animals received care in compliance with standard animal ethics of Iran. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Shiraz University of Medical Sciences (Permit Number: 6734). 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. Administration was started at 4 weeks of age with approximately 250-300 g body weight and all efforts were made to minimize suffering.

Induction and treatment of lactic acidosis

Five groups of six male Sprague dawley rats weighting 250-300 g were selected to attain the condition of acidosis induction. These groups were administrated to a gavage dose of Phen-HCl. Phen-HCl was freshly prepared in double distilled water (ddH_2O) and the dosage level was 400

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mg/Kg/day. The dose volumes were calculated based on the latest body weight of animals (~4 mL/Kg). In order to investigate the treatment effect of PAs, immediately after Phen-HCl administration, Spd-HCl, Cad-HCl and Put-HCl at a concentration of 0.06 nmol dissolved in 250 µL ddH2O) was injected to three groups of animals. The PA injection was performed by the saphenus vain injection. The positive control group received only a single dose of Phen-HCl without any treatment. Another group of animals (six male Sprague dawley rats weighting 250-300 g) was fed with ddH_2O instead of phenformin as a control.

In order to measure the lactate levels in the animals' blood, blood samples were collected after anesthetizing the animals by ketamin and diazepam, and the blood samples were obtained by cardiac puncture and collected in sodium fluoride sample tubes for lactate assay tests. On the first day, before induction of acidosis (at the beginning of the acidosis induction) and after 24 h, the blood samples were obtained.

Treatment with Thm and Na-bicarb

In order to calculate the level of treatment dose of Na-bicarb and Thm, the amount of excess base was measured in the animal groups (same as the previous section). Arterial blood samples were collected 24 h after treatment in heparinzed sample tubes. The amount of excess base was measured, using OPTICCA-TS from OPTIMedical. Dosage level of Thm and Na-bicarb was calculated for the positive control group based on the following formula:

Na-bicarb Solution (mL of 8.4% solution) = 0.2 × Body weight (kg) × Base Deficit (mEq/L)

Thm Solution (mL of 0.3 M solution) = 1.1 × Body Weight (kg) × Base Deficit (mEq/L)

Then, three groups of male Sprague dawley rats weighting 250-300 g were selected to attain the condition of acidosis induction (based on previous method). Immediately after a single dose of phen-HCl, the animals were treated with an injection dose of Na-bicarb and Thm. On the first day, before induction of acidosis (at the beginning of the acidosis induction) and after 24 h, the blood samples were obtained by cardiac puncture and collected in sodium fluoride tubes for blood lactic acid measurement. Control and positive control groups were received ddH_2O and a single dose of Phen-HCl without any treatment, respectively.

Blood lactic acid assay

Blood samples were stored in an ice bath. The plasma was then separated by centrifugation at 30 min. Lactic acid content in the samples was measured by means of an enzymatic method of L-lactate Randox kit. 5 µL of the sample was mixed with 500 µL of enzyme reagent, and incubated at 37°C for 5 min. The enzyme reagent included 0.4 mmol/L 4-aminophenazone + peroxidase (≥ 1000U/L) + lactate oxidase (≥ 600 U/L) + ascorbate oxidase (≥ 10000U/L), dissolved in 6 mL working buffer (Pipes buffer 100 mmol/L, pH 7.2 + N-ethyl-N-(2-hydroxy-3-sulphopropyl) m-toluidine, 2.1 mmol/L + sodium azide 1 g/L). Then, the absorbance at 550 nm was recorded using a BS-200 Mindray automatic analyzer. The absorbance values were measured against a blank sample including 5 µL of ddH_2O mixed with 500 µL of enzyme reagent. The standard sample was prepared; 5 µL of a standard solution (maintained in the kit) was mixed with 500 µL of enzyme reagent. The concentration of L-lactate was obtained as follows:

$$\textbf{L-lactate concentration (mg/dL)} = [Abs_{sample} / Abs_{standard}] \times \textbf{Standard concentration (mg/dL)}$$

Statistical analysis

Mean and standard deviation (SD) of lactate levels in each group were calculated. Lactate level of before and after treatment and the ratio of the average of lactate level after treatment /average of lactate before treatment were presented as mean ±SD. Statistical significance of the ratio value of different groups was evaluated using one way analysis of variance (ANOVA) and Duncan test.

Results and discussion

Our result in a previous study [9] showed that the administration of a single dose of Phen-HCl (400 mg/Kg/day) in rats induces acidosis after 24 h. In the present study, lactic acidosis was induced in rat via Phen-HCl gavage. The concentrations of PAs were selected based on the previous results [9]. Figure 1 shows the average lactate levels of blood samples (before and 24 h after acidosis induction) for the control (that took only water), positive control (that took a single dose of Phen-HCl without any treatment), and Spd, Cad and Put groups (that took a single dose of Phen-HCl along with a treatment dose of Spd-HCl, Cad-HCl, and Put-HCl, respectively). After 24 h of treatment, the lactate levels in the positive control and control groups were increased 2.04±0.31 and 1.04±0.09 times, respectively. The results show that the blood lactate levels of the positive control group were almost two times that of the control group; this is defined as the lactic acidosis condition [10]. In addition, the results show that enhancements in lactate levels after 24 h were inhibited in Put, Cad and Spd groups, compared to the positive control group. Because of the non-normal distribution of the values of the lactate concentration, the average ratios of the lactate levels (24 h after treatment relative to the before treatment) were calculated for a statistical analysis. Table 1 represents base excess values of the animal bloods in different groups, and using the value of the positive control group, the injection doses of Na-bicarb and Thm were calculated. Table 2 shows the results of the average ratios of lactate levels (24 h after treatment relative to before treatment) in the control, positive control, and other groups treated with Spd-HCl, Cad-HCl, Put-HCl. Statistical analysis indicates that the results of these groups are placed in three categories with significant differences (df=7; F=42.5; $P<0.001$). Statistical analysis (Table 2) showes also no statistically significant difference between the control and Put groups. Treatment of the animals using Put-HCl and Cad-HCl inhibits completely the lactate enhancement in acidosis animals. In addition, the results show that in the case of Spd-HCl treatment, this ratio is equal to 1.3, indicating the significant inhibition of lactate enhancement. Table 3 shows the results of the average ratios of the lactate levels (24 h after treatment relative to before treatment) in the control, positive control, and other groups treated with Na-bicarb, and Thm. The results indicate that Na-bicarb and Thm had no effect on lactate enhancement. Statistical analysis (Table 3) shows no statistically significant difference between the positive control, Na-bicarb and Thm treatment groups (df =3; F=25.7; $P<0.001$).

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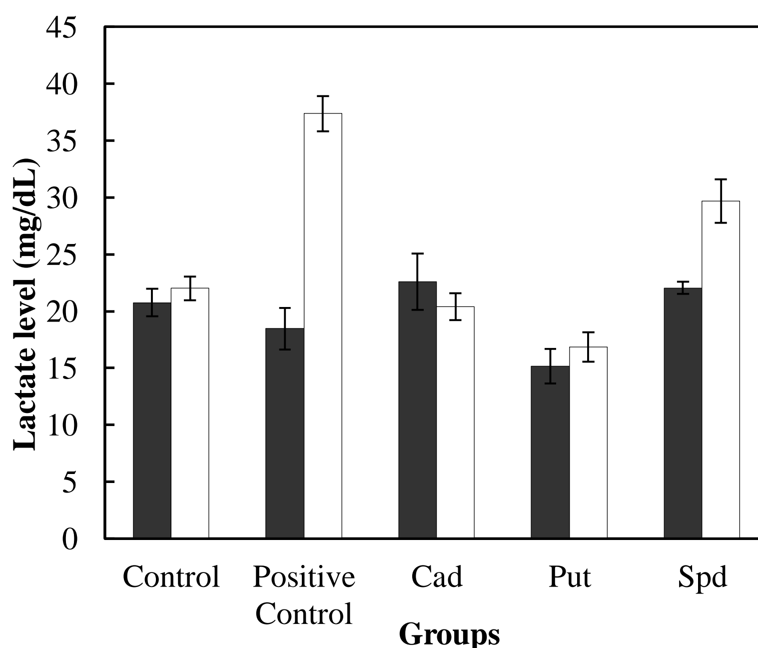

Fig. 1. The average lactate levels (mg/dL) of blood samples for the groups of rats (control, positive control, Cad, Put, Spd). Lactate level has been measured before acidosis induction (■) and 24 h after treatment (□) with 400 mg/kg/day Phen-HCl and 0.06 nmol PAs. The results are expressed as means ± SD.

Based on the presented results, PAs significantly treat lactate enhancement in acidosis rats. PAs are aliphatic amines with three or four methylene carbon chains connecting the amino and/or imino groups [11]. PAs, with protonation of their amine groups reduce the proton concentration of the medium, and prevent the blood pH decrement during acidosis conditions [9]. Moreover, PAs do not carry additional chemical species that cause negative side effects, such as sodium ions in the case of Na-bicarb. On the other hand, some studies have shown PAs stimulate the activity of PDH and citrate synthase [12, 13]. Deficiency of PDH can cause lactic acidosis. Activity of PDH is regulated by PDH phosphatse and PDH kinase [12-15]. In this regard, PA act as activator of phosphatase. Phosphatase activates PDH with dephosphorylation [16]. PAs activate phosphatase, resulted in activation of PDC. PDC with three catalytic components of PDH, dihydrolipoyl transacetylase, and dihydrolipoyl dehydrogenase, catalyzes the oxidative decarboxylation of pyruvate. Deficiency in the pyruvate oxidation process causes pyruvate accumulation, conversion to lactate and finally induces lactic acidosis type B [17].

Table 1. The values of HCO_3^- and base excess in the bloods of different groups of rat.

Groups	HCO_3^- (mmol/L)	Base excess (mmol/L)
Control	29.3±1.46	4.9±0.24
Positive control	24.6±1.23	0.4±0.02
Cad	27.6±1.38	2.9±0.14
Put	27.3±1.36	1.4±0.07
Spd	30.4±1.52	5.3±0.26

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Table 2. Ratio of lactate levels in different groups of rat administrated with PA.

Groups	Ratio of lactate level* (as mean±SD)
Control	1.08±0.05[ab]
Positive control	2.08±0.42[c]
Cad	0.91±0.10[a]
Put	1.11±0.05[ab]
Spd	1.34±0.11[b]

*Ratio of lactate level is the average of lactate value after treatment divided by the average of lactate before treatment.
Note: Same alphabets (a, b, c, and ab) indicates that there is not statistically significant difference between values at P<0.001.

BPs in the treatment of acidosis is effectiveness with a very low treatment dose. Endogenous synthesis, interconversion pathways and dietaries are the main sources of PAs in human body [18]. Although blood PA levels increase in response to long-term BP intake, some studies also confirmed that short-term BPs intake do not induce acute changes in their contents in blood and cells [19-20]. A study showed only about 20 percent of perfused putrescine in human was recovered in blood [21]. PAs levels are changed during the stress conditions, diabetes and cancer as a result of the activity alterations of PAs regulator enzymes. Therefore, PAs levels in blood act as a marker of stress conditions [22-27]. Due to these changes in the blood levels of PAs, it cannot select the best PA for the acidosis treatment.

Table 3. Ratio of lactate levels in different groups of rat administrated with Na-bicarb, and Thm.

Groups	Ratio of lactate level* (as mean±SD)
Control	1.08±0.05[a]
Positive control	2.08±0.42[b]
Na-bicarb	2.15±0.17[b]
Thm	2.11±0.15[b]

*Ratio of lactate level is the average of lactate value after treatment divided by the average of lactate before treatment.
Note: Same alphabets (a and b) indicates that there is not statistically significant difference between values at P<0.001.

Conclusion

Na-bicarb and Thm are currently employed in the lactic acidosis disorders with adjustment of the blood pH. However, they do not have any effect on the lactate increscent. The aim of the present study was the discovery of a treatment route with the lowest side effect to prevent lactate accumulation. Our findings suggest that BPs (including Put, Cad and Spd) have a significant effect on lactate level reduction with inhibition of lactate enhancement. Therefore, BPs as natural and biocompatible compounds with no harmful effect may be the best treatment for the patients suffering from lactate accumulation and lactic acidosis. In this regard, our results indicated that in equal doses of administrated BPs represent a positive effect on the manner Put>Cad>Spd, and Put is the best one.

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Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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