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# Lactic acidosis treatment by nanomole level of spermidine in an animal model

Mozhgan Sedigh-Ardekani [a,b,c], Mohammad Ali Sahmeddini [d], Naghmeh Sattarahmady [a,b,e,*], Hossein Mirkhani [f,e]

[a] Department of Medical Physics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[b] Department of Nanomedicine, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran
[c] Pharmaceutical Science Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[d] Shiraz Anesthesiology and Intensive Care Research Center, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[e] Nanomedicine and Nanobiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[f] Department of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

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ABSTRACT

Lactic acidosis occurs in a number of clinical conditions, e.g. in surgeries, orthotopic liver transplant, and anesthetic agent administration, which has deleterious effects on the patient's survival. The most rational therapy for these patients, the sodium bicarbonate administration, cannot prevent those accompanying deficiencies and may actually be harmful. In addition, tromethamine adjusts the blood pH, it does not affect the lactate accumulation. Therefore, discovery of a therapeutic agent is still a major unsolved problem. In this study, the rats were divided into different groups and lactic acidosis type B was induced in them. Then, the effect of different injection doses of spermidine (0–20 nmol) on lactic acidosis was analyzed by measuring the lactate level and pH in the rat blood samples. The results showed that spermidine effectively and simultaneously inhibited the lactate and pyruvate accumulations, and also adjusted the pH of bloodstream. On the other hand, it has been shown (Damuni et al., 1984; Rahmatullah and Roche, 1988) that spermidine increases the activity of phosphatase, leading to prevention of lactate accumulation. The results indicate that administration of only nanomole level of spermidine may be the best treatment in the liver transplant and other patients suffering from lactic acidosis type B.


## 1. Introduction

Lactic acidosis in human is expressed as enhanced blood lactate concentration (to 5 mmol $L^{-1}$ or higher) along with a decrease in arterial pH (below 7.35). Two categories of lactic acidosis are types A and B. Type A lactate acidosis resulted from mitochondrial dysfunction and arrest of ATP production causes to lactate accumulation. Disorders such as hypotension (cardiac failure, sepsis, hypovolemia) or weakened oxygenation (hemoglobin transfer disorders) may result in type A acidosis (Allen and Holm, 2008; Vitin et al., 2010). In type B, as underutilization of lactate, oxygen delivery is sufficient; however, carbohydrate metabolism or mitochondrial functions are changed. Type B is divided into three subdivisions of B1 (decreasing lactate clearance), B2 (drugs/toxins interfere with oxidative phosphorylation) and B3 (mitochondrial defects) (Vitin et al., 2010; Blackshear et al., 1974). Type B lactic acidosis arises in some diseases such as diabetes mellitus, liver failure, sepsis, and neoplasia (Allen and Holm, 2008; Vitin et al., 2010) and in the presence of some drugs such as phenformin. Phenformin, a drug with antihyperglycemic effect, induces lactic acidosis type B due to inhibition of gluconeogenesis (Fulop and Hoberman, 1976). Upper abdominal surgery, especially orthotopic liver transplant, and anesthetic agents administration lead to improving lactic acidosis (Scale and Harvey, 2011; Murthy, 2007; Fung et al., 1999; Cooper et al., 1990; Mathieu et al., 1991). Therefore, lactic acidosis compensation is a complex clinical problem. To date, some approaches have been proposed for the treatment of lactic acidosis conditions, especially during orthotopic liver transplant. The historic approach to treatment of acidemia in lactic acidosis condition is the aggressive injection of sodium bicarbonate.

*Abbreviations:* PDC, pyruvate dehydrogenase complex; PDH, pyruvate dehydrogenase; DCA, dichloroacetic acid.

* Corresponding author at: Department of Medical Physics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. Fax: +98 711 234 93 32.
*E-mail addresses:* nsattar@sums.ac.ir, sattarahmady@yahoo.com (N. Sattarahmady).

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Although sodium bicarbonate induces normal pH, it does not compensate lactate accumulation, tissue oxygenation and aggravates intracellular acidosis (Scale and Harvey, 2011; Murthy, 2007; Fung et al., 1999; Cooper et al., 1990; Mathieu et al., 1991). Therefore, the main problem of acidosis is unsolved. Another treatment of acidemia condition is attained by tromethamine (THAM). THAM is an amino alcohol with acid-buffering capacity (Hoste et al., 2005; Papandreou et al., 2011) and the $NH_2$ functional group in THAM adjusts acidemia via binding to hydrogen ions. Although THAM is rapidly cleared by the kidney, great amounts of THAM increase the blood pH and decrease the blood $CO_2$ level (Vitin et al., 2010; Damuni et al., 1984). Also, dichloroacetic acid (DCA), as an organic acid, is applied for the treatment of lactic acidosis. The mechanism of action is the DCA effect on the pyruvate dehydrogenase (PDH) activity by inhibition of pyruvate dehydrogenase kinase (PDH kinase). Increase in the PDH activity may lead to a decrease in pyruvate accumulation and lactate production (Allen and Holm, 2008; Papandreou et al., 2011; Damuni et al., 1984; Nyhan et al., 2002). DCA is an analog of pyruvate. DCA with inhibition of PDH kinase stimulates PDH and decarboxylation of pyruvate. However, DCA is not effective in the treatment of severe acidosis in rat which has been induced by phenformin (Sheu et al., 1981).

Biogenic polyamines are present in all living organisms, and essential for cellular proliferation (Pakala, 2003). These compounds are also the regulators of the synthesis process of triglycerides, proteins (especially glycoproteins) and nucleic acids (Jamdar, 1979). In patients with proliferative diseases such as cancer, intracellular level of polyamines extends to millimolar concentrations (Pakala, 2003; Jamdar, 1979). In addition, polyamines act as an inhibitor of platelet responses, platelet aggregation, and hemagglutination activity of endogenous platelet lecithin (Pakala, 2003). On the other hand, polyamines have an increasing impact on the secretion and synthesis of plasminogen activator and decreasing impact on the secretion of plasminogen inhibitor (Pakala, 2003). Polyamines protect mitochondrial functions, membrane permeability and act as reactive oxygen species (ROS) scavenger (Toninello et al., 2004).

Regarding the other effects of biogenic polyamines on the metabolic processes, it can be mentioned that *in vitro* studies have shown that polyamines increase phosphatase activity ($Ca^{2+}$ independent phosphatase). Moreover, spermine increases phosphatase activity in the presence of $Mg^{2+}$ due to decreasing the $K_m$ value (Damuni et al., 1984; Rahmatullah and Roche, 1988). The stimulatory effect of polyamines on pyruvate dehydrogenase phosphatase activity may be related to the insulin stimulation of pyruvate dehydrogenase complex activity in the adipose tissue (Lilley et al., 1992).

In the present study, the effects of different doses of spermidine in the range of nanomoles on the simulated type B acidosis (by administration of phenformin) induced in rat were analyzed.

## 2. Materials and methods

### 2.1. Chemicals

Phenformin hydrochloride and spermidine hydrochloride were purchased from Sigma–Aldrich. Male Sprague dawley rats were obtained 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 (40–70%) 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. Administration was started at 4 weeks of age with approximately 250–300 g body weight.

### 2.2. Induction of lactic acidosis by phenformin

Five male Sprague dawley rats weighting 250–300 g were selected to attain the condition of acidosis induction. This group was administrated to repeated gavage doses of phenformin hydrochloride up to 6 days. Phenformin hydrochloride was freshly prepared in doubly distilled water ($ddH_2O$) and the dosage level was 400 mg/kg/day. The dose volumes were calculated based on the latest body weight of animals (~4 mL/kg). In order to measure the lactate levels in the animals' blood, blood samples were collected after anesthetizing the animals by ketamine 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 program), the blood samples were taken. Then, the first phenformin dose was administrated. After one day, blood samples were taken (to analyze the first day acidosis level) followed by administration of the next phenformin dose, to follow up the acidosis induction in the next day. This procedure was repeated for the second, third and sixth days. Based on the obtained results, a single dosage of phenformin of 400 mg/kg/day induced the accepted value of lactate for acidosis condition after 24 h. Therefore, phenformin was administrated and the investigation of treatment effect of spermidine was performed after 24 h.

### 2.3. Treatment with spermidine hydrochloride

In order to investigate the treatment of acidosis by spermidine hydrochloride, the animals weighting 250–300 g were randomly separated in 10 groups of 10 animals each. They were anesthetized with ketamine and diazepam and the blood samples were taken by cardiac puncture and collected in sodium fluoride sample tubes for lactate assay tests (before acidosis induction). Then, a single gavage dose of phenformin hydrochloride (400 mg/kg/day, 4 mL/kg) was administrated to animals. Immediately after this step, spermidine was injected through the saphenus vain with different doses (0–20 nmol dissolved in 250 μL $ddH_2O$). After 24 h, blood samples were obtained by cardiac puncture and collected in sodium fluoride sample tubes for lactate assay tests (24 h after acidosis induction). A control group of animals consisting of 10 male rats was tested similarly, but this group was fed with $ddH_2O$ rather than phenformin.

### 2.4. Blood lactic acid assay

Blood samples were stored in an ice bath. The plasma was then separated by centrifugation. Lactate 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 (⩾1000 U/L) + lactate oxidase (⩾600 U/L) + ascorbate oxidase (⩾10,000 U/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:

$$\text{l-lactate concentration (mg/dL)} = [\text{Abs}_{\text{sample}}/\text{Abs}_{\text{standard}}] \times \text{Standard concentration(mg/dL)}$$