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Acid-base status and fructose diphosphatase activity in rats exposed to fluoride and induced periodontitis

This study was conducted to evaluate acid-base status and fructose diphosphatase (FDPase) activity in 40 (4 groups of 10) male Wistar rats. One group of rats was left untreated as control, fed a standard diet, and given distilled water. Periodontitis model induced with 5 mg/kg NH_4Cl (group 1), exposed to sodium fluoride (NaF) at the concentration 5 mg/l (group 2), exposed to NaF (5 mg/l) and supplemented with minerals and vitamins (group 3). At the termination of experimental period (30 days) the pH and pCO_2 value of arterial blood were analysed. Then, the FDPase activity in the hemogenized heart, kidney, liver, mandible, pelvis, and teeth were determined by measuring inorganic phosphate that converts from fructose-1.6-diphosphate and using spectrophotometer at 350 nm. The differences in the acid-base status and FDPase activity in the groups 1 and 2 were statistically significant in comparison with the control and group 3 ($P<0.001$). Increased FDPase activities are associated with acid-base status. The minerals and vitamins supplementation proved to restore acid-base balance, reduce toxicity and establish steady enzyme activity, which has not been previously reported.

Key words: fluoride, ammonium chloride, pH, pCO_2, fructose diphosphatase, minerals and vitamins, rats.

INTRODUCTION

Hydrogen ions concentration in fluids and tissues is one of the strict regulated variables in physiological organism. The maintenance of a stable physiologic systemic pH is critically important to mammals.

The toxic action of fluoride (F) is based on the fact that F ions act as enzymatic poisons that inhibit enzyme activity and, ultimately, interrupt metabolic processes. The most obvious early toxic effects of F in humans are dental and skeletal fluorosis. It is characterized by clinical manifestations in bones and teeth [1]. Moreover, detrimental effects of elevated F intake are also observed in soft tissues [2]. Since this disease is irreversible, by appropriate and timely intervention it's preventable. Therefore, a greater understanding at biochemical and molecular levels of the disease progression is very important.

Metabolism is an extremely complex subject in biochemistry. It is usually consists of sequences of enzymatic steps, the so-called metabolic pathways that interact in a complex way in order to allow an adequate regulation. In each pathway, a principal chemical is modified by chemical reactions and often require dietary minerals for both physiological and biochemical functions, vitamins and other co-factors in order to function properly [3]. In addition, pathways are important to the maintenance of homeostasis within an organism. Thus, fructose diphosphatase (FDPase; EC3.1.3.11) play a key role in gluconeogenesis (GNG) [4].

Therefore, the present study aimed to determine the pH and pCO_2 value of arterial blood, as well for the first time, we conducted to evaluate the FDPase activity in the heart, kidney, liver, mandible, pelvis, and maxillary incisor teeth of rats exposed to ammonium chloride (NH_4Cl), Sodium (Na) F and NaF supplemented with minerals and vitamins.

METHODS

Forty male Wistar rats approximately 42 ± 5 day of age, each weighing 140 ± 3 g on aver-

age were randomly divided into 4 equal groups and maintained for 30 consecutive days. They were placed in a quiet polypropylene cages with stainless still grill tops, as 5 animals per cage, temperature and humidity controlled room (22 ± 2°C and 60 ± 5 %, respectively) in which a 12 h/12 h light/dark cycle was maintained (lights on: 08:00 h), fed a standard pelleted diet, and given distilled water ad libitum. One group of rats was left untreated as control. The experimental group 1 (periodontitis model) were given distilled water, standard diet and treated with NH_4Cl (5 mg/kg), the group 2 were administered standard diet, distilled water containing NaF (5 mg/l) and rats in the group 3 were given standard diet containing minerals (Mg-, Mn-, Zn-sulfate, and Na-citrate) 300 mg/kg and vitamin A, C and D (1/50 adults dose per kg of rats) and distilled water containing NaF (5 mg/l). At the termination of experimental period, under light ether anesthesia, tail arterial blood samples were collected. Then animals were sacrificed and decapitated. The heart, kidney and liver were removed. The mandibles and pelvises were dissected. Then maxillary incisor teeth were extracted.

Biochemical assay. The pH and pCO_2 value in the arterial blood of study subjects were analysed as previously described [5] and the results are expressed as unites (U) and millimeters of mercury (mm Hg) respectively. The FDPase activity in the hard and soft tissues of study subjects was assayed, as previously described [6]. Briefly, the bones and teeth were pulverized to the consistency of a fine powder at the temperature of liquid nitrogen. The heart, kidney, liver, mandible, pelvis, and maxillary incisor teeth were hemogenized. Then, the FDPase activity of 1 g hemogenized tissue was determined by measuring inorganic phosphate that converts from F-1,6-DF to fructose-6-phosphate in incubated environment using spectrophotometer at 350 nm. The results are expressed as nanomoles per gram per second.

The experiments performed in this study have been carried out according to the rules in the Guide for the Care and Use of Laboratory Animals adopted by National Medical Academy of Postgraduate Education (NMAPE), Ministry of Health (Ukraine) and the Declaration of Helsinki. This study was approved by the Ethics Committee of NMAPE. The values were expressed as mean ± SD. To compare the differences in all parameters between experimental and control groups the data were statistically analyzed by student's t-test using SPSS 11.5 statistical package (SPSS, Chicago, IL, USA).

RESULTS

The pH value of arterial blood among the three groups of experimental and control subjects are shown in Figure 1. The differences in the

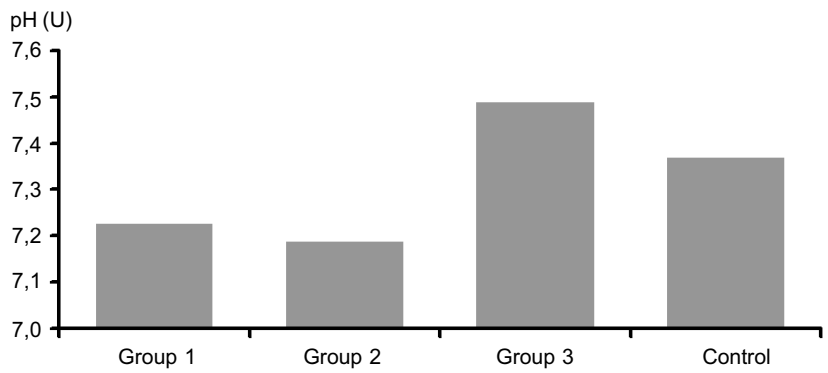

Figure 1. The pH value of arterial blood among 4 groups of rats: NH_4Cl-induced periodontitis model (group 1), NaF-intoxicated (group 2), NaF-intoxicated and supplemented with minerals and vitamins (group 3) and control. The differences between the control and groups 1-2 were significant at ($P<0.001$). The differences between the groups 3 and 1-2 were significant at ($P<0.001$)

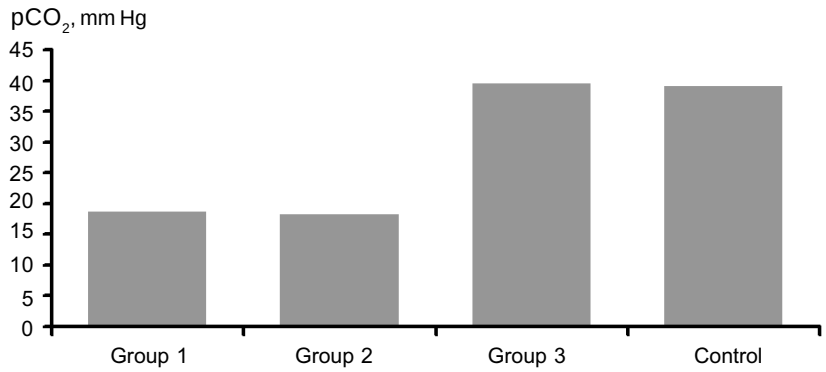

Figure 2. The pCO_2 value of arterial blood among 4 groups of rats: NH_4Cl-induced periodontitis model (group 1), NaF-intoxicated (group 2), NaF-intoxicated and supplemented with minerals and vitamins (group 3) and control. The differences between the control and groups 1-2 were significant at ($P<0.001$). The differences between the groups 3 and 1-2 were significant at ($P<0.001$)

values of pH in the groups 1 and 2 were statistically significant in comparison with the control and group 3 ($P<0.001$). No significant differences in the values of pH among the control and group 3 existed ($P>0.001$). The differences in the values of pH between the groups 1 and 2 were not significant ($P>0.001$).

The pCO_2 value of arterial blood among the three groups of experimental and control subjects are shown in Figure 2. The differences in the values of pCO_2 in the groups 1 and 2 were statistically significant in comparison with the control and group 3 ($P<0.001$). No significant differences in the values of pCO_2 among the control and group 3 existed ($P>0.001$). The differences in the values of pCO_2 between the groups 1 and 2 were not significant ($P>0.001$).

The results of the FDPase activity in the osseous tissues (mandible, pelvis, and teeth) and soft tissues (heart, kidney and liver) among the three groups of rats and control are shown in Table 1 and 2 respectively. The differences in the activities of FDPase in the groups 1 and 2 were statistically significant in comparison with the control and group 3 ($P<0.001$). No significant differences in the FDPase activities among the control and group 3 existed ($P>0.001$). The differences in the activities of FDPase between the groups 1 and 2 were not significant ($P>0.001$).

DISCUSSION

Most common man and animal diseases are accompanied with compensated acid-base balance shift development. During metabolic acidosis, the responses of kidney, intestine and bone may be mediated by changes in 1,25-dihydroxycholecalciferol and parathyroid hormone (PTH), resulting in increased bone resorption [7]. Chronic ingestion of NH_4Cl produces metabolic acidosis in animals and humans have been reported [8-9]. In periodontitis model of this study our findings were in

Table 1. Activity of the fructose diphosphatase (FDPase) in the mandible, pelvis, and maxillary incisor teeth of rats with NH_4Cl-induced periodontitis model (group 1), NaF-intoxicated (group 2), NaF-intoxicated and supplemented with minerals and vitamins (group 3) and control. The values are expressed as mean ± s.d; n = 10 in each group

FDPase (nmol g^{-1} s^{-1})	Group 1	Group2	Group3	Control
Mandible	90.01 ± 0.01*	95.10 ± 0.06*	22.29 ± 0.25	21.50 ± 0.13
Pelvis	88.30 ± 0.11*	94.41 ± 0.09*	24.63 ± 0.28	24.20 ± 0.16
Teeth	67.50 ± 0.31*	73.70 ± 0.16*	16.22 ± 0.32	17.80 ± 0.12

*Significant differences when compared with controls and group 3 ($P<0.001$).

Table 2. Activity of the fructose diphosphatase (FDPase) in the heart, kidney and liver of rats with NH_4Cl-induced periodontitis model (group 1), NaF-intoxicated (group 2), NaF-intoxicated and supplemented with minerals and vitamins (group 3) and control. The values are expressed as mean ± s.d; n = 10 in each group

FDPase (nmol g^{-1} s^{-1})	Group 1	Group2	Group3	Control
Heart	85.40 ± 0.23*	93.40 ± 0.12*	23.08 ± 0.32	24.10 ± 0.18
Kidney	95.60 ± 0.11*	96.70 ± 0.31*	20.57 ± 0.25	23.10 ± 0.17
Liver	91.50 ± 0.42*	91.70 ± 0.31*	21.09 ± 0.32	20.40 ± 0.03

*Significant differences when compared with controls and group 3 (P<0.001).

agreement with their report and indicated that NH_4Cl induced acidosis conditions (Figures 1 and 2).

F comes from fluoridated water, medicines, dental products, pesticides, fertilizers and fuels. Exposures to F can result in dental [10] and skeletal [11] fluorosis. Moreover, chronic fluorosis can severely damage many systems of the human body, but its pathogenesis is poorly understood [12]. F has been shown to inhibit many enzymes such as those involved in the pentose pathway [13], antioxidant defense system [14], and the myosin-ATPase path [15]. In this regard, the current results have demonstrated that the lack of significant differences in the value of pH and PCO_2 among groups 1 and 2 indicates an acidosis condition in the groups 1 and 2 (Figures 1 and 2). But, diagnosis of metabolic acidosis and alkalosis with blood indices doesn't reflect true tissue condition that is connected not only with respiratory and excretion system functioning but with hypercompensation of metabolic processes [5]. Previous studies have shown that alterations in the acid-base balance modify renal gluconeogenesis. Metabolic acidosis stimulates GNG in a variety of preparations by increasing the level of phosphoenolpyruvate carboxykinase mRNA and hence enzyme activity [16]. This study examined the effects of NH_4C1 in periodontitis model "acidification" and NaF "intoxication" on the FDPase activities. We noticed that the FDPase activity was elevated in the groups 1 and 2 (Tables 1 and 2). We suggest, therefore, that metabolic acidosis may have an effect on the activity of FDPase.

Minerals are essential elements that are necessary for body as these are required for the regulation of the body's metabolic functions. In this regard, magnesium (Mg)-linked ATP processes activate hundreds of different enzymes which are involved in diverse functions such as DNA and RNA synthesis, glycolysis, intracellular mineral transport, nerve impulse generation, cell membrane electrical potential, muscle contraction, blood vessel tone, and the regeneration of ATP [17]. Manganese (Mn) has been shown to influence lipid metabolism in several studies using mice and rats as models. Also, it is a co-factor in certain enzymes involved in intermediary metabolism of carbohydrates [18]. High dietary Mn has been shown to be insulinomimetic and to increase glucose uptake by isolated rat adipocytes [19]. There is considerable evidence that GNG and the reabsorption of Na^+ are reciprocally related. Thus, American opossum kidney (OK) cells respond to acidosis with increased glutamine metabolism and ammonium formation [20]. In these cells, acidosis decreases the activity of the Na^+-H^+ exchanger, thereby increasing intracellular H^+ [21]. Studies using isolated proximal tubules have shown that enhanced glutamine metabolism and ammonia production are linked to increased gluconeogenesis [22]. Zinc (Zn) is a known co-factor in some of the metabolic processes of glucose metabolism, including key role in insulin activity [23]. Zn deficiency would negatively impact on the glucose metabolic process [24].

Enzyme activity can be stimulated and potentiated by making the required minerals and

vitamins available to the body thus ensuring that essential chemical reactions are maintained. Vitamin A participates in several biological mechanisms [25]. The antioxidant activity of vitamin A against lipid peroxidation induced by other test chemical in rat tissues in vivo is known [26]. This vitamin also acts as an antioxidant by decreasing peroxidation products, scavenging reaction oxygen species and inhibiting the activation of promutagen [27]. The liver plays a central role in the uptake, storage and is also the oxidation site of vitamin A catabolism and responsible for the regulated release of this vitamin to other tissues [28]. Vitamin C exerts a powerful antioxidant effect on biological water-soluble compartments and represents an outstanding antioxidant in plasma; it reacts directly with superoxide anion (O_2), hydroxyl radical (OH^-) and various lipid hydroperoxides [29]. Both animal [30] and human [31] studies have shown ascorbic acid to be a potent antioxidant, which mediates its antioxidant effect by scavenging free reactive oxygen species. In animals, metabolic acidosis was found to decrease1,25-$(OH)_2$ vitamin D levels [32]. Chronic metabolic acidosis was repeatedly shown to increase 1,25-$(OH)_2$ vitamin D (by stimulation of its production rate) and to concomitantly decrease PTH concentrations in humans [33]. Vitamin D deficiency was shown to result in metabolic acidosis in chicks [34] and chronic 1,25-$(OH)_2$ vitamin D administration results in metabolic alkalosis inthyroparathyroidectomised rats [35]. The current results have demonstrated a steady activity of FDPase by supplementation of selected minerals and vitamins in the group 3 (Tables 1 and 2) that closely associated with the value of pH and pCO_2 (Figures 1 and 2). We suggest, therefore, enzyme activity can be stimulated and potentiated by making the required vitamins and minerals available to the body thus ensuring that essential chemical reactions are maintained. In addition, application of F in periodontal disease must be limited.

In conclusion, although the findings obtained for rats cannot be directly referred to the human body. Therefore, based on the data presented in this study, it is concluded that the biochemical changes observed in the rats exposed to NH_4Cl and F clearly demonstrate that metabolic acidosis in rats elicits increased FDPase activity. The minerals and vitamins supplementation proved to restore acid-base balance, reduce toxicity and establish steady enzyme activity. This novel approach might be a key mechanism for understanding of metabolic acidosis and may provide additional advantages in the pathogenesis of fluorosis.

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КИСЛОТНО-ЩЕЛОЧНЫЙ БАЛАНС И АКТИВНОСТЬ ФРУКТОЗОДИФОСФАТАЗЫ У КРЫС ПОД ВЛИЯНИЕМ ФТОРИДА НАТРИЯ И МОДЕЛИРОВАНИИ ПАРОДОНТИТА

Исследование было проведено с целью определения кислотно-щелочного баланса и активности фруктозодифосфатазы (ФдФаза) у 40 (4 группы по 10 особей) крыс-самцов линии Вистар. Животных контрольной группы удерживали на стандартном рационе питания и дистиллированной воде. У крыс 1-й группы был моделирован пародонтит с помощью введения в рацион питания хлористого аммония (NH_4Cl, 5 мг/кг), животные 2-й группы получали фторид натрия (NaF, 5 мг/л), рацион крыс 3-й группы кроме NaF был дополнен минералами и витаминами. По окончанию 30-суточного экспериментального периода в образцах артериальной крови были проанализированы показатели pH и pCO_2. Активность ФдФазы в гомогенатах миокарда, почек, печени, нижней челюсти,

тазовых костей и зубов определяли путем измерения неорганического фосфата, отщепляемого от фруктозо-1,6-дифосфата, с помощью спектрофотометра (λ=350 нм). Разница показателей кислотно-щелочного баланса и активности ФдФазы в 1-й и 2-й группе была статистически достоверной в сравнении с контрольной и 3-й группами (P<0.001). Увеличение активности ФдФазы связано с кислотно-щелочным балансом. Дополнительное употребление минералов и витаминов способствует восстановлению кислотно-щелочного баланса, уменьшению токсичности, а также нормализации активности фермента, о чем раньше не сообщалось.

Ключевые слова: хлористый амоний, фторид натрия, pH, pCo_2, фруктозодифосфатаза, минералы и витамины, крысы.

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КИСЛОТНО-ЛУЖНИЙ БАЛАНС І АКТИВНІСТЬ ФРУКТОЗОДИФОСФАТАЗИ У ЩУРІВ ПІД ВПЛИВОМ ФТОРИДУ НАТРІЮ ТА МОДЕЛЮВАННЯ ПАРОДОНТИТУ

Дослідження було проведене для визначення кислотно-лужного балансу та активності фруктозодифосфатази (ФдФази) у 40 (4 групи по 10 особин) щурів-самців лінії Вістар. Тварин контрольної групи утримували на стандартному раціоні та дистильованій воді. Щурам 1-ї групи моделювали пародонтит за допомогою введення до раціону хлористого амонію (NH_4Cl; 5мг/кг), тварини 2-ї групи отримували фторид натрію (NaF; 5 мг/л), раціон щурів 3-ї групи окрім NaF був доповнений мінералами та вітамінами. По завершенні 30-добового експериментального періоду у зразках артеріальної крові були проаналізовані показники pH та pCO_2. Активність ФдФази в гомогенатах міокарда, нирок, печінки, нижньої щелепи, тазових кісток і зубів визначали вимірюванням неорганічного фосфату, що відщеплюється від фруктозо-1,6-дифосфату, за допомогою спектрофотометра (λ=350 нм). Різниці показників кислотно-лужного балансу та активності ФдФази в 1-й та 2-й групі були статистично достовірними у порівнянні з контрольною та 3-ю групами (P<0.001). Встановлено, що підвищення активності ФдФази пов'язано з кислотно-лужним балансом. Додаткове вживання мінералів і вітамінів сприяє відновленню кислотно-лужного балансу, зменшенню токсичності, а також нормалізації активності ферменту, про що раніше не повідомлялося.

Ключові слова: хлористий амоній, фторид натрію, pH, pCO_2, фруктозодифосфатаза, мінерали та вітаміни, щури.

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