Original Article

CLINICAL ROLE OF DIETARY THIAMINE ON REGULATION OF RENAL RESPONSE TO METABOLIC ACIDOSIS IN ADULT RATS

PAPEL CLÍNICO DE TIAMINA NA DIETA SOBRE A REGULAÇÃO DA RESPOSTA RENAL DE ACIDOSE METABÓLICA EM RATOS ADULTOS

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ABSTRACT: Kidney plays a central role in maintaining the composition of body fluids by regulating water, NaCl, acid base, and solute reabsorption and excretion, respectively. The study was done to investigate the physiological role of thiamine in regulation of renal response to metabolic acidosis induced by NH_4Cl in adult male rats. For this experiment, fifty rats were used. They were divided into five groups. Control rats received basal diet; rats fed on basal diet mixed with NH_4Cl (4g NH_4Cl/100g diet) to induce severe metabolic acidosis, rats fed on basal supplemented diet with thiamine (600 mg/kg diet), and rats fed on basal supplemented diet with thiamine before and after induction of metabolic acidosis by NH_4Cl for 14 days. The results showed that the plasma levels of chloride, urea, and creatinine were significantly elevated in metabolic acidosis induced by NH_4Cl. Thiamine supplementation at high dose before or after induction improved the chloride values. Feeding diets supplemented with thiamine modulated the plasma sodium and bicarbonate values. Supplementation with vitamin B1 as preventive agent significantly restored these changes to near control value and when used as curative agent improved plasma creatinine and urea levels. Urinary pH and potassium levels were decreased significantly in metabolic acidotic rats when compared to all experimental groups. Urinary ammonia and aldosterone levels were decreased by thiamine supplementation as protective agent. Supplementation with vitamin B1 as preventive and curative agents, restored the affected parameters and regulate the response of kidney to metabolic acidosis induced by ammonium chloride.

KEYWORDS: Kidneys. Vitamin B1 Supplementation. Ammonium chloride. Acidotic rats.

INTRODUCTION

Metabolic acidosis (MA) refers to the condition where the acid base balance of the body is disrupted due to an increased production of acid or the reduced excretion and decreased production of bicarbonates (JONES; WALTER, 2007).

Sometimes, metabolic acidosis can occur even without the excessive production of acids, when the kidney fails to excrete them through urine, which can be a symptom of renal failure (NOWIK et al., 2010). The kidney plays a central role in maintaining the composition of body fluids by regulating water, NaCl, acid base, and solute reabsorption and excretion, respectively (QUENTIN et al., 2004).

The standard protocol to induce metabolic acidosis in experimental animals involves application of ammonium chloride (NH_4Cl), which is supplied most frequently in diet (INGRID; ARCANGELO, 2014). Metabolic acidosis induced by NH_4Cl has been associated with decreased reabsorption of NaCl and water in the proximal tubule of rat (FAROQUI et al., 2006). Thiamine is especially important in glucose metabolism. It acts as a cofactor for enzymes that break down glucose for energy production (Figure 10.3). Additionally, thiamine plays a role in the synthesis of ribose from glucose and is therefore required for RNA, DNA, and ATP synthesis. The brain and heart are most affected by a deficiency in thiamine (SHRADHA et al., 2007).

Thiamine is an essential cofactor for aerobic metabolism and facilitates the entry of pyruvate into the tricarboxylic acid cycle (ABDOULAYE, 2008). Deficiency of thiamine impairs pyruvate utilization and thus results in a rise in serum lactate level (HUNG et al., 2001). Thiamine is the precursor of thiamine pyrophosphate (TPP), a coenzyme in the cleavage of carbon-to-carbon bonds and the oxidative decarboxylation of keto acids of the citric-acid cycle (PARVESH et al., 2004). As a result, thiamine is essential for producing energy from glucose. Thiamine is an important co-factor required for multiple enzymes involved in carbohydrate metabolism (DERRICK, 2006). To maintain acid-base homeostasis the kidney increases its excretion of the acid load, and any failure on the part of the kidney to excrete the acid will lead to metabolic acidosis (APPEL; DOWNS, 2008).

Thiamin serves as a cofactor for multiple enzymes involved in critical metabolic reactions which relate to energy metabolism . Because it bridges the glycolytic and the pentose phosphate

Received: 21/04/15
Accepted: 20/01/16

metabolic pathway, thiamin is also critical for creating chemical reducing power in cells . Therefore, thiamin is thought to play an important role in reducing cellular oxidative stress . Thus, low intracellular levels of thiamin lead to impairment in energy metabolism and a propensity for oxidative stress, which are a common finding in kidney desease. At the clinical level, thiamin deficiency leads to a variety of abnormalities that include neurologic (neuropathy, Wernicke-Korsakoff syndrome) and cardiovascular (e.g. peripheral vasodilatation, biventricular myocardial failure, edema and potentially acute fulminant cardiovascular collapse) disorders (HAZELL and BUTTERWORTH,2009).

The study was done to investigate the physiological role of thiamine in regulation of renal response to metabolic acidosis induced by NH_4Cl in adult male rats.

MATERIAL AND METHODS

Material

Thiamine (Vitamin B1) and ammonium chloride (NH_4Cl) were purchased from EL-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt.

Experimental animals

Male Albino rats (Sprague-Dawely) (~160g of bodyweight) were divided in five groups (10 rats/ group). were used. The animals were 6 weeks old at the beginning of the experiment. They were supplied from breeding unit of the Egyptian Organization for Biological Products and Vaccines (Helwan breeding farm, Cairo, Egypt). Animals housed individually in metabolic stainless steel cages with wire mesh bottoms and maintained at temperature 25°C±5°C, humidity 50% ± 5% and light dark cycle held constant 12/12 h. During the experiment, basal diet according to AIN and water were provided ad libitum.

Groups were classified as follows:

G1: Normal control untreated rats [Control group];

G2: Rats were fed on basal diet mixed with NH_4Cl for 14 days (4g NH_4Cl/100g diet) to induce severe metabolic acidosis;

G3: Rats were fed on basal diet supplemented with high dose of thiamine for 14 days (600 mg/kg diet);

G4: Rats were fed on basal diet supplemented with high dose of thiamine (600 mg/kg diet); for 14 days before and 14 days after mixed with NH_4Cl as a protective agent against metabolic acidosis;

G5: Rats were fed on basal diet mixed with NH_4Cl for 14 days before and 14 days after supplemented with high dose of thiamine (600 mg/kg diet); as a curative agent against metabolic acidosis.

Plasma Analysis

At the end of the experiment (6 weeks), rats were fasted for 12 hrs. Animals were fasted overnight then scarified under ether anesthesia and blood samples were collected from hepatic portal vein in centrifuge tubes. The tubes centrifuged at 600 x g for 30 minutes at 25°C to provide plasma needed for the biochemical analysis. Plasma samples were taken and kept in dry clean plastic and stored at −20°C till used for the different analysis Fresh plasma was tested for content of bicarbonate (BELDING; JAMES, 1954). Plasma chloride was determined according to the colorimetric method (SCHOENFELD; LEWELLEN, 1964), urea (PATTON; CROUCH, 1977), creatinine (BARTELS et al., 1972), albumin (DOUMAS, 1971), and sodium (HENRY, 1974). Anion gap was calculated by the equation: Anion gap (AG) = ($[Na^+]$) - ($[Cl^-]$ + $[HCO_3^-]$) (JEFFREY; NICOLAOS, 2007).

Urine Analysis

All animals were kept in individual metabolic cages, and 24h urine samples were collected at the end of experimental period. The preparation of samples was carried out immediately after urine collection and analyzed for urinary parameters. Urine samples were acidified with 10% hydrochloric acid to block the growth of bacteria and molds and stored below 4°C for subsequent analysis, then urinary pH was measured using a pH meter (Beckman coulter). Urine was also analyzed for calcium (TIETZ, 1970), phosphate (DREWES, 1972) potassium (HENRY, 1974) and ammonia (GIPS et al., 1970). Urinary aldosterone in urine was measured with a radioimmunoassay method according to Philip et al. (1979).

Statistical Analysis

The data were statistically analyzed by SPSS version 10.0 statistical packages. Data were presented as the means ± SD, statistical differences between groups were performed using t-test. Differences were considered significant when $p<0.01$.

RESULTS

Effect of NH_4Cl and thiamine (Vitamin B1) supplementation on plasma sodium, bicarbonate, chloride, and anion gap values

The concentration of plasma chloride was significantly ($p<0.01$) elevated in metabolic acidosis induced by NH_4Cl group at 14 days when compared to the control group. Addition of thiamine at high dose before or after induction improved the chloride values when compared with MA rats (G2). Induction of metabolic acidotic state by NH_4Cl resulted in significant reduction of bicarbonate levels as compared to control rats. Feeding diets supplemented with thiamine (G4 and G5) were modulate the plasma sodium and bicarbonate values than that MA rats (G2) fed diets mixed with NH_4Cl alone without any thiamine treatments. Plasma values of anion gap was reduced in the metabolic acidotic rats when compared to control group and then gradually improved by thiamine supplementation (Table 1).

Table 1. Effect of different treatments on plasma sodium, bicarbonate, chloride, and anion gap

Grupos	**Sodium (Na^+)** (mmol/L)	**Bicarbonate (HCO_3^-)** (mmol/L)	**Chloride (Cl^-)** (mmol/L)	**Anion Gap (AG)** (mmol/L)
Basal Control	147.8±11.6	30.5±2.8	104.8±8.1a	12.5±2.6
Basal + NH_4Cl	135.6±6.6	17.8±3.6	113.7±3.2b	4.10±0.8
Thiamine Supplemented	145.3±9.3	29.7±3.0	100.1±4.6	15.6±1.7
Thiamine as protective agent	141.6±6.4	22.9±5.8	107.3±9.3	11.4±0.75
Thiamine as curative agent	139.2±12.1	23.3±5.5	103.1±3.2	12.8±2.5

Values are means ± SD, n = 10 animais por grupo, Letras diferentes na coluna significant difference at $p<0.01$.

Effect of NH_4Cl and thiamine (Vitamin B1) supplementation on plasma creatinine, urea and albumin levels

Plasma creatinine and urea concentrations were significantly higher in MA group and gradually decreased in other groups by adding thiamine at high level. Supplementation with vitamin B1 as preventive agent in G4 significantly modulated these changes and restored it to near control value. Also, feeding of thiamine as curative agent after metabolic acidosis induction improved plasma creatinine and urea levels as compared to G2 (Table 2). Plasma albumin concentration was reduced significantly ($p<0.01$) in the metabolic acidotic rats (MA) when compared to control group.

Table 2. Effect of different treatments on plasma creatinine, urea, and albumin levels.

Grupos	**Creatinine** (mg/dL)	**Urea** (mg/dL)	**Albumin** (g/dL)
Basal Control	1.03±0.08a	33.5±2.8a	5.1±1.1b
Basal + NH_4Cl	1.60±0.12b	60.9±4.4b	2.4±0.8a
Thiamine Supplemented	0.95±0.06	31.5±2.5a	4.8±0.9
Thiamine as protective agent	1.25±0.09c	39.2±6.7a	2.6±0.5
Thiamine as curative agent	1.32±0.09c	45.2±3.1a	2.7±0.9

Values are means ± SD, n = 10 Significant difference at $p<0.01$

Effect of NH_4Cl and thiamine (Vitamin B1) supplementation on urinary pH, ammonia, and aldosterone values

Urinary pH value was decreased significantly ($p<0.01$) in metabolic acidotic (G2) when compared to all experimental groups. The results of the study showed that the urinary ammonia excretion was increased significantly ($p<0.01$) in NH_4Cl induced group (G2) and gradually decreased by thiamine supplementation as preventive agent. Also, the urinary aldosterone hormone concentration was increased in metabolic acidosis induced group (G2) when compared to control group.

Supplementations with vitamin B1 before induction prevented these changes and regulate renal response against any changes induced by NH_4Cl load. Addition of thiamine at high dose mixed with basal diet alone without metabolic acidosis induction maintains theses urinary parameters as control rats (Table 3).

Table 3. Effect of different treatments on urinary pH, ammonia, and aldosterone values.

Grupos	**Urine pH**	**Ammonia (NH_3)** (µmol/24h)	**Aldosterone** (nmol/24h)
Basal Control	7.1±0.1b	15.4±1.6a	0.99±0.06
Basal + NH_4Cl	6.8±0.2a	45.2±3.9b	2.21±0.35
Thiamine Supplemented	7.2±0.1b	14.2±2.3a	0.86±0.15
Thiamine as protective agent	7.0±0.2b	19.8±1.8a	1.25±0.09
Thiamine as curative agent	7.0±0.2b	28.3±2.7a	1.98±0.07

Values are means ± SD, n = 10 Significant difference at p< 0.01

Effect of NH_4Cl and thiamine (Vitamin B1) supplementation on urinary potassium, calcium, and phosphate levels

NH_4Cl feeding with basal diet resulted in a significant increase in urinary calcium and phosphate excretion as compared to control (G1). Feeding of thiamine before or after metabolic acidosis induction had no improvement effect on urinary calcium or phosphate levels as compared with control group. Whereas, supplementation of thiamine before metabolic acidosis induction improved urinary potassium concentrations as compared with MA rats (Table 4). There are no significant differences in the value of urinary potassium, calcium and phosphate in rats supplemented with thiamine after induction as curative agent as compared to G1 and G3 groups (Table 4).

Table 4. Effect of different treatments on urinary potassium, calcium, and phosphate levels

Grupos	**Potassium (K^+)** (mg/24h)	**Calcium (Ca^{+2})** (mg/24h)	**Phosphate (Pi)** (mg/24h)
Basal Control	28.2±1.9b	0.322±0.04	0.150±0.03
Basal + NH_4Cl	25.1±3.5	0.638±0.08	3.55±0.45
Thiamine Supplemented	27.6±1.2b	0.352±0.02	0.091±0.001
Thiamine as protective agent	29.1±1.7	0.611±0.03	2.34±0.02
Thiamine as curative agent	25.3±4.2b	0.623±0.05	3.10±0.1

Values are means ± SD, n = 10 Significant difference at p< 0.01

DISCUSSION

Urinary excretion of ammonia is essential for maintenance of normal acid-base homeostasis in several species including rat, and it was demonstrated that an increase in urinary ammonia is a typical response to metabolic acidosis (TOM; ANDREW, 2012). The data of studied in rats made acidotic by oral feeding of ammonium chloride show that after NH_4Cl, the animals already have a severe metabolic acidosis, and in response to this, there is increased urinary excretion of ammonia. The rise in urinary ammonia at acidotic rats induced by NH_4Cl indicates an increase in renal ammonia production (NOWIK et al., 2010).

Results of the study showed that, animals fed on diets supplemented with high dose of thiamine were less acidotic compared to the group receiving NH_4Cl in diet without any treatment. The results found higher urea excretion in metabolic acidotic rats (MA) compared to the control group. Nowik et al. (2010) Rats on the acid diet tended to receive a higher NH_4Cl load than the respective thiamine supplemented groups and therefore an overall increased nitrogen load. Moreover, the higher urinary urea excretion might also reflect increased catabolism of proteins (MARIA et al., 2012). The excretion of chloride was lower in rats receiving thiamine as protective agent before metabolic acidosis induction by NH_4Cl. Since urinary chloride excretion might reflect net chloride intake and ammonium absorption, it might indicate a lower absorbed NH_4Cl load in metabolic acidotic rats receiving NH_4Cl mixed with basal diet without thiamine supplementation (Table 1). However, interpretation of urinary chloride excretion is difficult since it may not directly correlate with intestinal chloride or even ammonium absorption.

The results of the study showed that the urinary aldosterone was elevated in metabolic acidotic rats. Gabriel e Geetha (2014) observaram que a metabolic acidosis state activates the renin-angiotensin-aldosterone system and stimulates synthesis of angiotensin II and secretion of aldosterone. Induction of MA followed by feeding thiamine supplemented diet resulted in an elevation

of urine aldosterone excretion as compared with control. Elevated aldosterone levels were not reflected by changes in plasma sodium and urinary potassium excretion. This might be explained by direct effects of acidosis on aldosterone targets such as potassium channel or sodium channel. Urinary calcium excretion was increased in all acidotic rats. Hypercalciuria is thought to develop at least in part due to inhibition of calcium channel in the distal convoluted tubule and connecting tubule (WATTS, 2005).The results of the study showed that the value of plasma bicarbonate, albumin, sodium, and anion gap were reduced in metabolic acidotic rats as compared with other experimental groups. Anion gap represents unmeasured anions in plasma. Thiamin plays a fundamental role in cellular metabolism and energy production, principally via thiamin pyrophosphate, which serves as a coenzyme required for normal cellular function, growth and development . Furthermore, thiamin plays a role in blunting reactive oxygen species generation via its capacity to bridge the glycolytic and the pentose phosphate metabolic pathways . Insufficient amounts of dietary thiamin can lead to a variety of clinical consequences that include neurologic and cardiovascular dysfunctions (KUMAR, 2010).

The unmeasured anions include–anionic proteins, phosphate, sulphate and organic anions (FIGGE et al., 1998). An increase in AG is due to increase in unmeasured anions and less commonly due to decrease in unmeasured cations (Ca^{+2}, Mg^{+2} and K^{+}). The results of the study showed that the urinary calcium was increased significantly in metabolic acidotic rats and the supplementation of thiamine had no protective or curative effect as compared with control group (Table 4). On the other hand, urinary potassium concentration reduced by feeding NH_4Cl in G1 rats. Addition of thiamine modulates renal response, and resulted in an elevation of urinary potassium levels as protective agent. The AG may decrease with a decrease in anionic albumin, either due to decreased albumin concentration or alkalosis which alters albumin charge (FIGGE et al., 1998). Supplementation with vitamin B1 as preventive agent in G4 significantly modulated these changes and restored it to near control value. Also, feeding of thiamine as curative agent after metabolic acidosis induction improved plasma creatinine and urea levels as compared to MA group (Table 2). It was concluded that, thiamine had a regulatory role in maintaining renal acid base balance. High thiamine diet improved the renal function and response to metabolic acidosis induced byNH_4Cl in adult male rats.

CONCLUSION

Supplementation with vitamin B1 as preventive and curative agents, restored the affected parameters and regulate the response of kidney to metabolic acidosis induced by ammonium chloride.

ACKNOWLEDGEMENT

I wish to express my gratitude to King Abdulaziz University, Faculty of Science for supporting to publish my research work, Biochemistry and Nutrition department of girls' college, Ain Shams University for providing me an opportunity to do this work.

RESUMO: O rim desempenha um papel central na manutenção da composição de fluidos corporais através do controlo de água, NaCl, ácido-base, e reabsorção do soluto e excreção, respectivamente. O estudo foi realizado para investigar o papel fisiológico de tiamina na regulação da resposta renal à acidose metabólica induzida por NH4Cl em ratos machos adultos. Para esta experiência, foram utilizados cinquenta ratos. Eles foram divididos em cinco grupos. Os ratos de controlo receberam dieta basal; os ratos alimentados com dieta basal misturado com NH 4 Cl (4 g NH 4 Cl / 100 g de dieta) para induzir uma grande taxa de acidose metabólica , os ratos alimentados com dieta suplementada basal com tiamina (600 mg / kg de ração), e ratos alimentados com dieta suplementada basal com tiamina antes e após a indução de acidose metabólica por NH4Cl durante 14 dias. Os resultados mostraram que os níveis plasmáticos de cloreto, ureia e creatinina foram significativamente elevados em acidose metabólica induzida por NH4Cl. A suplementação de tiamina em doses elevadas antes ou após a indução aumentou os valores de cloreto. O fornecimento de dietas suplementadas com valores de tiamina modulado o sódio plasmático e bicarbonato. A suplementação com vitamina B1 como agente preventivo restaurou significativamente estas alterações para aproximar o valor de controle e, quando utilizado como agente curativo melhorou os níveis de creatinina e uréia plasmática. Os níveis de pH e de potássio na urina foram reduzidos significativamente em ratos acidóticos metabólicos, quando comparado com todos os grupos experimentais. os níveis de amônia e de aldosterona urinário foram reduzidos pela suplementação de tiamina como agente de proteção. A suplementação com vitamina B1 como agentes preventivos e curativos, restaurou os parâmetros afetados e regulou a resposta do rim a acidose metabólica induzida por cloreto de amónio.

PALAVRAS-CHAVE: Rins. Vitamina A. Suplementação B1. Cloreto de amónio. Ratos acidóticos.

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