Asian Journal of Chemistry Vol. 20, No. 4 (2008), 3182-3188

Effects of Sodium Bicarbonate Induced Blood pH Change on Oxidant Status and Thromboplastic Activity of the Kidney and Lung

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Oral ingestion of sodium bicarbonate is used to neutralize the acid in digestion, to decrease dental plaque and to heal the bladder infections. Excessive bicarbonate ingestion places individuals at risk for a variety of metabolic dearrangements. Our aim was to determine if sodium bicarbonate induced blood pH change has any effects on oxidant status and thromboplastic activity of lung and kidney. In the present study, the effects of sodium bicarbonate induced blood pH change on oxidant status and thromboplastic activity of lung and kidney tissues were investigated. The results revealed that sodium bicarbonate administration significantly increased blood pH and bicarbonate level, decreased chloride level and pO_2 (partial oxygen pressure) and did not significantly change sodium, potassium concentration and pCO_2 (partial carbon dioxide pressure). Kidney thromboplastic activity significantly decreased following sodium bicarbonate administration, which shows the impaired hemostatic balance in the kidney tissue. Lung thromboplastic activity did not significantly changed. Sodium bicarbonate administration also did not cause any oxidative damage both on kidney and lung. It seems likely that eight weeks oral sodium bicarbonate administration caused mild alkalosis and did not change oxidative status both on kidney and lung tissues but it impaired the hemostatic balance at kidney which can cause uncontrolled bleeding after any kidney damage.

Key Words: Sodium bicarbonate, Thromboplastic activity, Lipid peroxidation, Glutathione, Lung, Kidney.

INTRODUCTION

Sodium bicarbonate is well known agent that historically has been used for a variety of medical conditions such as stomach upset[1], kidney disorders[2], urine alkalization[3], minimization uric acid crystallization during gout treatment[4], metabolic acidosis[5], some drug intoxication[6], decreasing dental

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plaque acidity[7]. It is also available as a nonprescription drug alone (sodium bicarbonate tablets) or in combination with other nonprescription drugs for short-term treatment of various conditions to treat fever and mild to moderate pain[8]. The most obvious side effects of sodium bicarbonate are the fluid and sodium load. Bicarbonate loading, whether exogenous or endogenous, is rarely a sole cause of significant persistent metabolic alkalosis because the normal kidney is so efficient at excreting bicarbonate. Such transient metabolic alkalosis states may occur during and immediately after an oral or intravenous infusion of $NaHCO_3$ or base equivalent, *e.g.*, citrate in transfused blood or fresh frozen plasma[9]. The generation of metabolic alkalosis occurs with the loss of acid, the gain of alkali or the contraction of the extracellular fluid compartment with a consequent change in bicarbonate concentration[10].

The kidneys have an enormous capacity to excrete excess bicarbonate generated and to restore normal acid-base balance[10,11]. As a compensatory mechanism, increasing of blood pH leads hypoventilation with a rise in arterial carbon dioxide tension which diminishes the change in pH that would otherwise occur[12]. Both lung and kidney are the main organs in compensation of increased blood pH. Short term, a decrease in respiratory rate leads to an increase in serum carbon dioxide levels. This lowers the pH towards normal, partially compensating for the additional alkali present in the blood. The slow process of eliminating bicarbonate through the kidney then begins. Hydrogen ions are transported from the filtered urine back into plasma, with sodium ions and bicarbonate left behind. Alkaline sodium bicarbonate is thus eliminated[13,14].

Alkaline pH may acts like a sink to draw protons away from the mitochondria, which normally maintains a proton gradient through respiration. Thus, under alkaline conditions, the mitochondria compensate for the loss of protons by increasing respiration which in turn will also produce more superoxide radicals[15]. As the effects of alkali administration both on the oxidant status and thromboplastic activity of the lung and kidney tissues are unknown. This study investigated the effects of sodium bicarbonate induced metabolic alkalosis on oxidant system and thromboplastic activity for the evaluation of generalized tissue damage in lung and kidney tissues.

EXPERIMENTAL

Wistar Albino rats were housed in a room at a constant temperature with 12 h light/dark cycles and fed standard pellet chow. The study was approved by the Marmara University (Istanbul, Turkey), School of Medicine, Animal Care and Use Committee.

Experimental design: 14 Wistar Albino rats (200-250 g) were divided into two groups as follows: control group (n = 7): Fed with standard pellet

chow and water *ad libitum*; experimental group: Fed with standard pellet chow and water *ad libitum* supplemented[16] with 0.25 M $NaHCO_3$. The experiments were conducted for 8 weeks. At the end of the experiment blood samples were taken from the cut tips of the tails into capillary tubes containing heparin and an "iron flea" for stirring under urethane anesthesia (1.25 g/kg i.p.). Lung and kidney tissues were taken after heart removal.

Blood gases, pH and electrolyte analysis: Blood samples were used for the measurement of pH, bicarbonate (HCO_3^-), oxygen (pO_2) and carbon dioxide (pCO_2) partial pressures , Na^+, K^+, Cl^- with a blood gas analyzer (Corning 168 pH/Blood Gas Analyzer, Corning Medical, U.S.A.).

Lipid peroxidation (LPO), glutathione (GSH) and total protein assays: Lung and kidney tissue samples were homogenized in 10 volumes of physiological saline solution. Aliquots of the homogenate were used to measure the LPO and GSH levels. The LPO levels were assayed by monitoring thiobarbituric acid reactive substance formation by the method of Ledwozwy *et al.*[17]. Glutathione, a key intracellular antioxidant, was measured by the method of Beutler[18]. Total protein levels were measured by the method of Lowry *et al.*[19].

Thromboplastic activity test: The thromboplastic activities of lung and kidney tissues were evaluated according to Quick's one-stage method using normal plasma[20]. This was performed by mixing 0.1 mL tissue homogenate with 0.1 mL of 0.02 M $CaCl_2$; the clotting reaction was started by the addition of 0.1 mL of plasma. All reagents were brought to the reaction temperature (37 ºC) before mixing. The thromboplastic activity was expressed as seconds. Shortened clot formation time showed an increased thromboplastic activity.

Statistical analysis: A Unistat 5.0 TM (UNISTAT Ltd, London W9 3DY, UK) statistical computer program was used to evaluate the results. Student's t test was used. A value of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

As shown in Table-1, blood pH and bicarbonate (HCO_3^-) concentration is significantly increased and chloride (Cl^-) concentration is significantly decreased in experimental group when compared with the control group ($p < 0.05$, Table-1). Sodium and potassium level, pCO_2 and pO_2 did not significantly change (Table-1). Although pCO_2 increase was not significant in experimental group when compared to the control group, the ratio of increase is consistent with HCO_3^- increase. Body weights of the rats also did not significantly change at the end of the experiment (data not shown). In kidney and lung tissues LPO, GSH, total protein levels did not significantly change with the $NaHCO_3$ administration (Tables 2 and 3).

TABLE-1
pH, BLOOD GASES AND ELECTROLYTIC LEVELS OF CONTROL AND EXPERIMENTAL GROUP RATS SUBMITTED TO 0.25 M OF $NaHCO_3$ IN DRINKING WATER FOR 8 WEEKS

Blood parameters	Control group (n = 7)		Experimental group (n = 7)	
	Mean	SD	Mean	SD
pH	7.30	0.06	7.37*	0.05
HCO_3 (mmol/L)	18.43	2.30	20.89*	1.55
pCO_2 (mm-Hg)	39.01	7.69	41.4	5.74
pO_2 (mm-Hg)	62.77	11.18	52.03	7.86
Na^+ (mmol/L)	139.99	1.51	140.59	1.81
K^+ (mmol/L)	3.84	0.55	3.48	1.12
Cl^- (mmol/L)	101.67	2.41	97.67**	2.11

*$p < 0.05$, **$p < 0.01$ compared with control group.

TABLE-2
MEAN LEVELS OF KIDNEY GLUTATHIONE, LIPID PEROXIDATION, TOTAL PROTEIN LEVELS AND THROMBOPLASTIC ACTIVITY IN CONTROL AND EXPERIMENTAL GROUPS

Kidney	Control group (n = 7)		Experimental group (n = 7)	
	Mean	SD	Mean	SD
GSH (nmol GSH/mg protein)	6.54	3.88	7.57	2.88
LPO (nmol MDA/mg protein)	0.93	0.34	1.07	0.24
Total Protein (mg protein/mL homogenate)	10.12	1.81	9.66	0.70
Thromboplastic activity (sec)	33.42	4.31	39.14*	3.71

*$p < 0.05$ compared with control group.

TABLE-3
MEAN LEVELS OF LUNG GLUTATHIONE, LIPID PEROXIDATION, TOTAL PROTEIN LEVELS AND THROMBOPLASTIC ACTIVITY IN CONTROL AND EXPERIMENTAL GROUPS

Lung	Control group (n = 7)		Experimental group (n = 7)	
	Mean	SD	Mean	SD
GSH (nmol GSH/mg protein)	3.40	1.73	1.97	1.19
LPO (nmol MDA/mg protein)	0.56	0.07	0.57	0.06
Total Protein (mg protein/mL homogenate)	12.19	1.12	12.53	0.87
Thromboplastic activity (sec)	20.00	1.73	21.71	1.88

In experimental group, kidney thromboplastic activity showed a significant decrease when compared with the control group ($p < 0.05$, Table-2). The lung thromboplastic activity did not show a significant change compared with the control group (Table-3).

Acute changes in blood pH induce powerful regulatory effects at the level of the cell, organ and organism[21]. As the mechanism responsible for local and systemic acid base balance are incompletely understood, this study was observed to assess the effects of sodium bicarbonate induced alkalosis on the oxidant status and thromboplastic activities of the kidney and lung tissues.

Metabolic alkalosis is recognized by increases in both arterial blood pH-alkalemia- and plasma bicarbonate concentration. The increase in arterial blood pH promptly, normally and predictably depress ventilation resulting in increased pCO_2 and the buffering of the alkalemia. The pCO_2 increases about 0.5 to 0.7 mm Hg for every 1.0 mM increase in plasma HCO_3^- concentration[22,23]. In the present study, 8 weeks administration of sodium bicarbonate to rats was observed to increase HCO_3^- and pCO_2 consistent with the literature. pCO_2 increase was not significant when compared to the control group but the ratio of increase is consistent with the increase of HCO_3^-. We also observed a significant decrease in chloride concentration in blood during experimentally induced metabolic alkalosis. This observation was in agreement with Cambier *et al.*[24] who experimentally induced metabolic alkalosis. In the present study decreased Cl^- level also showed the type of metabolic alkalosis which was hypochloremic.

Cells cultured under alkaline conditions were found to exhibit elevated levels of mitochondrial membrane potential, reactive oxygen species and calcium which was accompanied by mitochondrial damage, DNA fragmentation and cell death. Although it is well known that alkaline conditions are cytotoxic, the mechanisms leading to alkaline-induced cytotoxicity are unknown. Alkaline pH may act like a sink to draw protons away from the mitochondria, which normally maintains a proton gradient through respiration. Thus, under alkaline conditions, the mitochondria compensate for the loss of protons by increasing respiration which in turn will also produce more superoxide radicals[15].

As kidney and lung tissues are responsible for the compensation of the acid-base balance changes, it is beneficial to follow the free radical induced changes in these tissues at any pH change. As a free radical generating system, lipid peroxidation has been suggested to be closely related with the tissue damage. In the present study, sodium bicarbonate induced alkalosis was not significantly change LPO levels both in kidney and lung tissues. The present results show that metabolic alkalosis does not change LPO in kidney and lung tissues, implying that sodium bicarbonate intake does not cause any tissue damage.

The concentration of intracellular GSH is essential for the protection of thiol and other nucleophilic groups in proteins from the toxic metabolites[25]. Therefore it is key determinant for the detection of the effects of sodium bicarbonate on tissues. Thomas and Stones[26] suggested that despite the widespread use of oral bicarbonate, little documented toxicity has occurred and the emergency medicine literature contains no reports of toxicity caused by the ingestion of baking soda. Risks of acute and chronic oral bicarbonate ingestion include metabolic alkalosis, hypernatremia, hypertension, gastric rupture, hyporeninemia, hypokalemia, hypochloremia, intravascular volume depletion and urinary alkalination. Abrupt cessation of chronic excessive bicarbonate ingestion may result in hyperkalemia, hypoaldosteronism, volume contraction and disruption of calcium and phosphorus metabolism[9,23]. In the present study, 8 weeks sodium bicarbonate administration did not significantly change the lung and kidney GSH stores. This finding also implicates that sodium bicarbonate induced increased blood pH did not change oxidant status as evidenced with the unchanged LPO and GSH parameters in lung and kidney tissues.

Thromboplastin, also known as tissue factor, Factor III, is an important coagulation factor that initiates extrinsic blood coagulation with FVII. It is not actively found in the blood but as a component of the cell membranes[27-29]. In addition to the complex role in coagulation, tissue factor acts as a signalling receptor and has non-haemostatic actions. Tissue factor is involved in the pathophysiology of sistemic inflammatory disorders, coagulopathies, atherosclerotic disease, tumour angiogenesis and metastasis[30,31]. It has been shown that some body cells, tissues and fluids have thromboplastic activity[31-35]. Increased thromboplastic activity in tissue samples contributes to low thromboplastin levels and bleeding tendency of the tissues. It is observed that decreased thromboplastic activity in kidney, but this decrease has not been seen in lung. Thromboplastin is not a stable protein[36]. Its activity can easily be changed by the changed membrane composition, heating or changing pH. In alkalosis pH of the blood and the medium changes, this may impair the tissue integrity or may effect the structure of membrane proteins. It is yet not clear which mechanisms regulate the kidney or lung thromboplastic activity in metabolic alkalosis. In the present study, decreased thromboplastic activity of the kidney refers the decreased protection of this vital organ from bleeding. However, decreased thromboplastic activity in kidney tissue following sodium bicarbonate administration can be contributed to the changed membrane composition by pH change.

As a conclusion, the present data show that oral administration of sodium bicarbonate for 8 weeks induced metabolic alkalosis and did not increase oxidant stress both in kidney and lung as evidenced with LPO and GSH but decreased the thromboplastic activity of the kidney.

REFERENCES

1. L.J. Fitzgibbons and E.R. Snoey, *J. Emerg. Med.*, **17**, 57 (1999).
2. P. Roderick, N.S. Willis, S. Blakeley, C. Jones and C. Tomson, *Cochrane Database Syst. Rev.*, **24**, CD001890 (2007).
3. E. Fjellstedt, T. Denneberg, J.O. Jeppssonn and H.G. Tiselius, *Urol. Res.*, **29**, 295 (2001).
4. S.K. Sharma and R. Indudhara, *Urol. Int.*, **48**, 81 (1992).
5. C.C. Szeto, T.Y. Wong, K.M. Chow, C.B. Leung and P.K. Li, *J. Am. Soc. Nephrol.*, **14**, 2119 (2003).
6. S.M. Bradberry, H.K. Thannacody, B.E. Watt, S.E. Thomas and J.A. Vale, *Toxicol. Rev.*, **24**, 195 (2005).
7. C.J. Kleber, M.S. Putt, J.L. Milleman, K.R. Davidson and H.M. Proskin, *Compend. Contin. Educ. Dent.*, **22**, 4 (2001).
8. A. Turan, D. Memis, B. Karamanlioglu, N. Sut and Z. Pamukcu, *Anaesth. Intensive Care*, **31**, 277 (2003).
9. J.H. Galla, D.N. Bonduris, P.W. Sanders and R.G. Luke, *J. Clin. Invest.*, **74**, 2002 (1984).
10. J. McNamara and L.I. Worthley, *Crit. Care Resusc.*, **3**, 188 (2001).
11. C.A. Wagner, J. Kovacikova, P.A. Stehberger, C. Winter, C. Benabbas and N. Mohebbi, *Nephron. Physiol.*, **103**, 1 (2006).
12. N.E. Madia and H.J. Androgue, *Nephron. Physiol.*, **93**, 61 (2003).
13. H.J. Adrogue and N.E. Madias, *N. Engl. J. Med.*, **338**, 107 (1998).
14. S. Sabatini, *Kidney Int.*, **49**, 906 (1996).
15. H.J. Majima, T.D. Oberley, K. Furukawa, M.P. Mattson, H.C. Yen, L.I. Szweda and D.K. Clair, *J. Biol. Chem.*, **273**, 8217 (1988).
16. T. Backman and M.A. Lamas, *Arch. Oral Biol.*, **42**, 299 (1997).
17. A. Ledwozyw, J. Michalak, A. Stephien and A. Kodziolka, *Clin. Chim. Acta*, **155**, 275 (1986).
18. E. Beutler, Glutathione: Red Cell Metabolism a Manual Biochemical Methods. Newyork: Grune and Stratton, pp. 112-114 (1975).
19. O.H. Lowry, W.I. Rosenbrough, A.L. Forr and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
20. G.I.C. Ingram and M. Hills, *Thromb. Haemostas.*, **36**, 237 (1976).
21. J.A. Kellum, *Crit. Care*, **4**, 6 (2000).
22. A. Khanna and N.A. Kurtman, *J. Nephrol.*, **19**, S86 (2006).
23. H.J. Galla, *J. Am. Soc. Nephrol.*, **11**, 369 (2000).
24. C. Cambier, T. Clerbaux, H. Amory, B. Detry, S. Florquin, V. Marville, A. Frans, A.P. Gustin, *Vet. Res.*, **33**, 697 (2002).
25. G.M. Habib, Z.Z. Shi and M.W. Lieberman, *Free Radic. Biol. Med.*, **42**, 191 (2007).
26. S.H. Thomas and C.K. Stone, *Am. J. Emerg. Med.*, **12**, 57 (1994).
27. E. Bachlie, *Br. J. Haematol.*, **110**, 248 (2000).
28. B.A. Lwaleed, *Saudi Med. J.*, **23**, 135 (2002).
29. L.C. Petersen, P.O. Freskgard and M. Ezban, *Trends Cardiovasc. Med.*, **10**, 47 (2000).
30. F.R. Rickles, S. Patierno and P.M. Fernandez, *Chest*, **124**, 58 (2003).
31. A. Ila, *Biull. Eksp. Biol. Med.*, **83**, 664 (1977) (In Russian).
32. B.A. Lwaleed, J.L. Francis and M. Chrisholm, *Ann. Saudi Med.*, **20**, 197 (2000).
33. J.A. Tutuarima, E.A.H. Hische, L. Trotsenburg and H.J. Helm, *Clin. Chem.*, **31**, 99 (1985).
34. G.H. Utter, J.T. Owings, R.C. Jacoby, R.C. Gosselin and T.G. Paglieroni, *J. Trauma*, **52**, 1071 (2002).
35. A. Yarat, T. Tunali, R. Pisiriciler, S. Akyuz, A. Ipbuker and N. Emekli, *Clin. Oral Investig.*, **8**, 36 (2004).
36. K.G. Mann, D. Gaffney and E.G. Bovill, William's Hematology, E. Beutler, M.A. Lichtman, B.S. Coller, T.J. Kipps, USA: McGraw Hill Book Company, pp. 1206-1226 (1995).

(*Received*: 26 September 2007; *Accepted*: 19 January 2008) AJC-6231