Original Paper

One-Year Conservative Care Using Sodium Bicarbonate Supplementation Is Associated with a Decrease in Electronegative LDL in Chronic Kidney Disease Patients: A Pilot Study

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Keywords

Chronic kidney disease · Alkali therapy · Electronegative LDL · Cardiovascular diseases

Abstract

Background: Chronic kidney disease (CKD) patients develop metabolic acidosis when approaching stages 3 and 4, a period in which accelerated atherogenesis may ensue. Studies in vitro show that low pH may increase low-density lipoprotein (LDL) oxidation, suggesting a role for chronic metabolic acidosis in atherosclerosis. The present study attempted to evaluate the effects of conservative care using oral sodium bicarbonate (NaHCO_3) supplementation on the electronegative LDL [LDL(-)], a minimally oxidized LDL, plasma levels in CKD patients. **Methods:** Thirty-one CKD patients were followed by a multidisciplinary team during 15 months of care in which 1.0 mmol/kg/day oral NaHCO_3 supplementation was first given in the third month. Blood samples were collected 3 months before the initiation of oral NaHCO_3 supplementation (T1), at the time of the beginning of supplementation (T2), and thereafter, each 4 months (T3, T4 and T5) until month 15 of care. Blood parameters and LDL(-) were measured from these collections. **Results:** After 12 months of conservative care, creatinine clearance (MDRD) was kept stable, and serum bicarbonate (HCO_3^-) increased from 20.5 ± 2.9 to 22.6 ± 1.1 mM ($p < 0.003$). LDL(-) plasma levels declined from 4.5 ± 3.3 to 2.1 ± 0.9 U/L ($p < 0.007$) after reaching mean serum HCO_3^- levels of 22.6 ± 1.1 mM. **Conclusions:** Conservative care using oral NaHCO_3 supplementation was able to stabilize renal function and decrease serum

levels of LDL(–), a modified proatherogenic lipoprotein, only when mean serum HCO_3^- levels approached 22 mM. This study constitutes evidence that alkali therapy, in addition to its beneficial effect on renal disease progression, might serve as a preventive strategy to attenuate atherogenesis in CKD patients.

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Introduction

A high prevalence of cardiovascular disease and atherosclerosis is well documented in chronic kidney disease (CKD) patients, and they constitute the main causes of morbidity and mortality in this population. Several nontraditional factors have been implicated, such as low-grade systemic inflammation and oxidative stress, facilitating low-density lipoprotein (LDL) oxidation and accelerated atherogenesis of CKD patients. In this context, an increased proportion of electronegative LDL [LDL(–)], a minimally oxidized lipoprotein, has been detected in CKD patients on dialysis, as compared with healthy individuals [1]. It is noteworthy that this lipoprotein has also been documented in patients at high cardiovascular risk as in familial hypercholesterolemia [2], hypertriglyceridemia [3, 4], diabetes, [5–11], as well as in renal patients [12–14]. The LDL modification, which confers negative charge to the molecule, has been demonstrated to be atherogenic [15]. In fact, the study by De Castelarnau et al. [16] clearly demonstrated the effect of LDL(–) on the expression of IL-8 and MCP-1 in endothelial cells in culture, suggesting a potential role for LDL(–) in the development of atherosclerosis.

Some studies have shown that low pH increases LDL oxidation. In an in vitro study, Morgan and Leake [17] observed that low pH was able to increase the oxidation of LDL in macrophages. The rate of oxidation of LDL by iron ions was also greatly increased even at mildly acidic pH [18]. It is quite possible that atherosclerotic lesions have an acidic extracellular pH, particularly in the vicinity of macrophages, and the observation that LDL oxidation by macrophages is increased at acidic pH may therefore help to explain why atherosclerotic lesions are apparently one of the very few sites in the body where LDL oxidation occurs [17].

Metabolic acidosis is a well-known feature of CKD, which has several consequences including aggravation of cardiovascular failure, protein breakdown, insulin resistance, and bone disease. Alkali therapy is suggested when serum bicarbonate (HCO_3^-) concentration is less than 22 mM, and studies showed that this therapy attenuates the progression of renal failure [19, 20]. In the present study, we attempted to test the hypothesis that metabolic acidosis may be related to increased levels of LDL(–), and that the use of oral sodium bicarbonate (NaHCO_3) supplementation could be related to the decrease in LDL(–) in CKD patients under regular conservative management.

Subjects and Methods

Subjects and Study Design

This longitudinal study enrolled 98 CKD patients on conservative treatment (stages 2–4), and 31 patients completed the study. The number of patients throughout the study is shown in the flowchart (Fig. 1). Inclusion criteria were age >18 and <80 years and serum HCO_3^- levels below 22 mM. Patients with autoimmune diseases, cancer, AIDS, inflammatory diseases, using catabolizing drugs or alkali treatment and the presence of any dialysis access were excluded. Seven patients were taking calcium channel blockers, 5 angiotensin converting enzyme (ACE inhibitors), and 19 the combination of both. None of the patients used alpha- or beta-blockers. All patients were using furosemide. There were no changes in drug protocol as all patients remained stable and responded well to the medical treatment throughout the period. All patients were

followed for 15 months, and oral NaHCO_3 supplementation (1.0 mmol/kg/day) was initiated after the third month in order to reach serum HCO_3^- levels of 22 mM or higher. Daily dose was calculated for each individual according to body weight measured at the time of initiation. Considering that no patient presented loss or weight gain over 2.0 kg for the 12 months of supplementation, the initial dosage of NaHCO_3 was maintained. Low protein diet (0.6–0.8 g/kg/day) was prescribed for all patients. There was no interruption of NaHCO_3 supplementation by none of the 31 patients.

Biochemical Analyses

Blood samples were collected at the time of initiation (T1) and 3 months later (T2), which was the starting point for the oral NaHCO_3 supplementation, and thereafter each 4 months until 1 year of NaHCO_3 supplementation (T3, T4, and T5). Blood samples were drawn from each subject in the morning after fasting overnight. The blood was drawn with ethylenediaminetetraacetic acid 1.0 mg/mL, and the plasma was immediately separated and stored at -80°C into tubes containing butylated hydroxytoluene 100 μM , aprotinin 10.0 $\mu\text{g/mL}$, benzamidine 10.0 μM , and phenylmethylsulfonyl fluoride 5.0 μM , until analysis. The following serum biochemical parameters were measured using routine enzymatic methods: blood urea nitrogen (BUN), creatinine, glucose, potassium, hemoglobin, hematocrit, uric acid, cholesterol, triglycerides, LDL, HDL, and albumin. Blood pressure measurements for each participant were taken during the follow-up. Serum HCO_3^- was measured by venous blood gas analysis. The values of creatinine clearance (CrCl) were measured by the MDRD equation [21].

LDL(–) Isolation and Plasma Measurement

LDL(–) was obtained as described in Faulin et al. [22]. Briefly, the LDL fraction was separated by ultracentrifugation of human blood plasma from volunteers. LDL(–) was isolated from LDL by FPLC (BioLogic Duo Flow, Bio-Rad Laboratories Inc., Hercules, CA, USA) using an ion exchange column (Sephacrose UNO Q-12; Bio Rad) and eluted with a gradient constituted by 20 mM TRIS, pH 7.4 (pump A) and 20 mM TRIS plus 1 M NaCl pH 7.4 (pump B). The eluent was monitored by UV at 280 nm, and the LDL(–) collected peak was visualized by agarose gel electrophoresis. This purified LDL(–) subfraction was used as standard in the ELISA for LDL(–).

Ninety-six-well flat-bottomed polystyrene plates (Costar; Corning Inc., NY, USA) were coated with 0.5 $\mu\text{g/well}$ of anti-LDL(–) 1A3H2 monoclonal antibody in 0.05 M carbonate-bicarbonate buffer, pH 9.6, at 4°C overnight. The plates were washed 3 times with PBS, pH 7.4, containing 0.05% Tween 20, and blocked by adding PBS containing 2% nonfat dry milk and 0.01% Tween 20 for 1.5 h at 37°C , then washed as described above. Plasma diluted in PBS containing 1% nonfat milk and 0.01% Tween 20 was added to the plates and incubated for 1.5 h at 37°C . The plates were washed and incubated with 0.5 $\mu\text{g/well}$ of anti-LDL(–) 2C7D5F10 monoclonal antibody conjugated to biotin for 1 h at 37°C . After washing, streptavidin-conjugated horseradish peroxidase (Invitrogen Corp., Carlsbad, CA, USA) was added and incubated for 1 h at 37°C . After washing, plates were incubated with o-phenylenediamine and diluted in citrate phosphate buffer pH 5.3, at 37°C for 15 min. The reaction was stopped by adding 2 M sulfuric acid, and the absorbance at 492 nm was measured by spectrophotometry using a plate reader (Synergy™ Mx; Biotek Instruments Inc., Winooski, VT, USA).

Statistical Analysis

One-way ANOVA repeated measures, followed by the Tukey test, were used to analyze differences among the periods of collections. Values were expressed as mean $\pm$ standard deviation, and statistical significance was considered when $p < 0.05$.

Results

Thirty-one patients from stages 2–4 completed 1 year of oral NaHCO_3 supplementation (21 men, 59 ± 12.7 years, BMI: 28.2 ± 5.7 , CrCl: $38.7 \pm 19.3.7$ mL/min). The demographic data are depicted in Table 1. We assumed that they all received and ingested the prescribed daily dose of NaHCO_3 . No patient had significant changes in blood pressure, or complained of anything related to taste.

Table 1. Demographic data

Characteristic	Value
Patients	31
Age, years	59 ± 12.7
Gender, M/F	21/10
Ethnicity, W/B	28/3
Etiology of CKD	
Diabetes/ins-dependent	16 (52)/11 (69)
Hypertension	13 (42)
Other	2 (6)
Current smoker	2 (6.25)
BMI	28.2 ± 5.7

Values are presented as n (%) or mean ± SD. CKD, chronic kidney disease; BMI, body mass index; ins-dependent, insulin dependent.

Table 2. Blood data from the collection periods

Parameters	T1	T2	T3	T4	T5
BUN, mg/dL	41.8 ± 17.5	34.9 ± 11.5	36.2 ± 13.3	38.3 ± 12.9	32.4 ± 7.7*
Creatinine, mg/dL	2.3 ± 0.7	2.3 ± 0.8	2.3 ± 0.9	2.0 ± 0.6	2.0 ± 0.6
CrCl, MDRD, mL/min	38.7 ± 19.3	40.1 ± 19.4	40.6 ± 20.1	42.6 ± 16.0	46.3 ± 19.2
Glucose, mg/dL	114.2 ± 43.8	106.8 ± 27.8	106.5 ± 32.9	110.6 ± 27	105.2 ± 22.1
Potassium, mEq/L	4.9 ± 0.5	4.7 ± 0.6	4.7 ± 0.6	4.5 ± 0.4	4.6 ± 0.6
Hemoglobin, g/dL	11.7 ± 2.8	12.6 ± 1.6	12.6 ± 1.6	12.8 ± 1.3	12.8 ± 1.4
Hematocrit, %	37.6 ± 5.1	35.3 ± 11.1	36.9 ± 5.2	39.6 ± 4.9	38.6 ± 5.4
HCO ₃ [–] , mM	18.9 ± 1.5	20.5 ± 2.9	21.6 ± 1.9	23.1 ± 1.8	22.6 ± 1.1**
Uric acid, mg/dL	7.2 ± 1.8	6.6 ± 1.0	6.6 ± 1.0	6.8 ± 0.9	6.4 ± 0.8
Cholesterol, mg/dL	195.2 ± 61.9	209.9 ± 67.1	198 ± 62.5	200.1 ± 54.2	200.1 ± 52.2
Triglycerides, mg/dL	158.7 ± 77.6	150.5 ± 44.6	161.2 ± 61.2	134.9 ± 26.8	139.1 ± 32.9
LDL, mg/dL	135.3 ± 66.1	155.1 ± 77.6	140.1 ± 66.9	145.9 ± 55.9	136.7 ± 53.4
HDL, mg/dL	31.8 ± 8.0	38.5 ± 16.1	37.6 ± 16.4	36.6 ± 12.8	47.2 ± 10.3
Albumin, g/dL	4.3 ± 0.6	4.3 ± 0.5	4.3 ± 0.8	4.3 ± 0.6	4.3 ± 0.6
LDL(–), U/L	3.6 ± 3.1	4.5 ± 3.3	5.5 ± 4.5	3.4 ± 3.4	2.1 ± 0.9**

* $p < 0.05$; ** $p < 0.01$ compared to T1.

The biochemical parameters are shown in Table 2. There was no significant change in concentration of BUN and creatinine after 1 year of treatment. In addition, after alkali therapy, serum HCO₃[–] levels were increased from 20.5 ± 2.9 to 22.6 ± 1.1 mM (Fig. 1). There was no significant change of CrCl (40.1 ± 19.4 to 46.3 ± 19.2 mL/min/1.73 m², Fig. 2). Figure 3 shows the significant reduction of LDL(–) levels from 4.5 ± 3.3 to 2.1 ± 0.9 U/L ($p < 0.007$), remaining unchanged in the first 3 months (T1 and T2), reaching the highest levels at T3 and decreasing thereafter.

There were no significant differences in the changes of lipid profile, uric acid, albumin, glucose, potassium, hematocrit, or hemoglobin. No significant correlation was found between LDL(–) and other biochemical parameters.

Fig. 1. Flowchart of patients throughout the study. CKD, chronic kidney disease; NaHCO_3 , sodium bicarbonate.

Fig. 2. Effects of oral sodium bicarbonate (NaHCO_3) supplementation on serum bicarbonate (HCO_3^-) levels.

Discussion

Metabolic acidosis, as defined by HCO_3^- levels of 22 mm or less, can be generally observed as soon as renal function approaches levels around 30 mL/min [23]. Recent studies have addressed the beneficial effect of alkali therapy on the attenuation of progressive renal disease and on nutritional status [19, 20, 24]. However, a beneficial effect of treatment and prevention of metabolic acidosis on the risk factors of cardiovascular disease has not been consistently addressed. The relationship between acidosis and atherosclerosis was suggested by a previous study from our laboratory, in which rabbits submitted to acid diet showed more pronounced atherosclerotic lesions, with higher aortic subendothelial glycosaminoglycan-LDL interaction, when compared to rabbits with normal blood pH [25]. In fact, in 1993, Morgan and Leake [17] observed that LDL oxidation is enhanced in low pH, and additional in vitro studies suggested that acidosis in the microenvironment of the vessels could be related to the increase in LDL oxidation [26]. More recently, Hassan et al. [27] documented the effect of acidosis on lipid peroxidation in liver preparation.

Metabolic acidosis also seems to contribute to the activation of innate immunity. The study by Rajamäki et al. [28] stressed the potential role of the acidic environment to activate

Fig. 3. Effects of oral sodium bicarbonate (NaHCO_3) supplementation on serum creatinine clearance (CrCl).

Fig. 4. Effects of oral sodium bicarbonate (NaHCO_3) supplementation on electronegative low-density lipoprotein [LDL(–)] levels.

NLRP3 inflammasome in human macrophages. More recently, a beneficial effect of alkali therapy on inflammation was suggested by the study by Ori et al. [29], in which IL-10 secretion from mononuclear cells decreased after 1 month of NaHCO_3 supplementation in predialysis CKD stage 4 and 5 patients. The involvement of innate and adaptive immunological response on atherosclerosis and cardiovascular disease has been elegantly reviewed by Hansson et al. [30].

High levels of modified LDL(–) in hemodialysis patients have been initially documented by Ziounzenkova and Sevanian [12], with important relevance for accelerated atherogenesis in this population [31]. We have previously shown that LDL(–) is highly elevated in hemodialysis patients and moderately elevated in peritoneal dialysis patients, when compared to healthy individuals [1]. In the present study, NaHCO_3 supplementation, used as part of a conservative care, was able to significantly increase mean serum HCO_3^- levels (Fig. 2), while renal function, as measured by MDRD calculation of eGFR, was kept stable during the entire time of observation (Fig. 3). These results support the recent lines of evidence from previous trials using alkali supplementation in CKD patients, in order to preserve renal function [19, 20]. Finally, the results of studies on LDL(–) showed a decrease in the mean levels at the end of the study period, when compared to the mean value at the starting point of NaHCO_3 supplementation (Fig. 4). It is noteworthy that an intriguing ascending slope of LDL(–) levels was observed at T3, which is at the fourth month of NaHCO_3 supplementation, although target mean serum HCO_3^- levels of 22.0 mM or above were not reached at that point. In fact, decreasing LDL(–) levels cannot be simply assigned to increased serum HCO_3^- levels and, despite previous

studies relating low pH to a pro-oxidative environment, these results certainly deserve further investigation.

In conclusion, in a group of CKD stage 2–4 patients, the use of oral NaHCO₃ supplementation for the correction of metabolic acidosis was associated with a decrease in serum levels of LDL(–), a minimally oxidized LDL. These findings constitute evidence for a beneficial effect of alkali therapy to prevent LDL oxidation, which has important implications for atherogenesis in CKD patients.

Statement of Ethics

All the patients were asked to sign informed consent, and the study protocol was reviewed and approved by the Ethics Committee of the Federal University of Rio de Janeiro.

Disclosure Statement

The authors declare no conflicts of interest.

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