Effect of experimentally induced metabolic acidosis on aortic endothelial permeability and serum nitric oxide concentration in normal and high-cholesterol fed rabbits

Majid Khazaei[1], Mehdi Nematbakhsh[2]

[1]Department of Physiology, Isfahan University of Medical Sciences, Iran
[2]Kidney Basic Sciences Research Center, Isfahan University of Medical Sciences, Iran

Submitted: 9 December 2010
Accepted: 22 January 2011

Arch Med Sci 2012; 8, 4: 719-723
DOI: 10.5114/aoms.2012.30296

Corresponding author:
Assoc. Prof. Majid Khazaei MD, PhD
Department of Physiology
Isfahan University of Medical Sciences
Isfahan, Iran
Phone: 00983117922407
E-mail: Khazaei@med.mui.ac.ir

Abstract

Introduction: Metabolic acidosis is present in end stage renal disease. There is a link between enhanced endothelial permeability and accelerated atherosclerosis. In this study, we investigated the effect of experimentally induced metabolic acidosis on aortic endothelial permeability and serum nitric oxide (NO) concentration in normal and high-cholesterol fed rabbits.

Material and methods: Twenty-four male rabbits were divided into four groups: normal, hypercholesterolemic, acidemic, and hypercholesterolemic plus acidemic. Acidosis and hypercholesterolemia were induced by drinking water containing ammonium chloride (NH_4Cl), and cholesterol-rich animal chow (1%), respectively. After 6 weeks, blood samples were taken and endothelial permeability was measured using the Evans blue dye injection method.

Results: Hypercholesterolemic animals had higher aortic endothelial permeability compared with normal groups (16.18 ±0.91 µg EB/g tissue vs. 12.89 ±0.66 µg EB/g tissue, $p < 0.05$). Acidosis significantly increased endothelial permeability in the normal group (17.10 ±0.56 µg/g tissue vs. 12.89 ± 0.66 µg/g tissue; $p < 0.05$) but did not further increase endothelial permeability in hypercholesterolemic animals (16.18 ±0.91 µg EB/g tissue vs. 17.29 ±0.46 µg EB/g tissue; $p > 0.05$). Serum total cholesterol, low density lipoprotein (LDL) and NO concentrations in hypercholesterolemic animals were significantly higher than the normal group and acidosis could not change them either in the normal or in the high-cholesterol diet group.

Conclusions: Alterations of serum lipids and NO are not the main mechanism for accelerated atherosclerosis during metabolic acidosis. Acidosis increases aortic endothelial permeability at least in a normal diet which may be a possible mechanism for progression of atherosclerosis processes in end-stage renal disease.

Key words: acidosis, endothelium, permeability, nitric oxide.

Introduction

Cardiovascular events are the main cause of death in patients with end stage renal disease [1, 2]. Renovascular atherosclerotic diseases are responsible for one-third of deaths in end stage renal disease [3]. Atherosclerosis is an inflammatory disease which is accompanied by increased mortality and morbidity [4]. One of the major hypothetical changes in endothelial function during atherogenesis is the increase of endothelial

permeability to atherogenic lipoproteins such as oxidized low density lipoprotein (LDL) [5].

Autopsy [6] and clinical studies [7] demonstrated that atherosclerotic plaque in coronary arteries of patients under dialysis is higher than in the normal population. Several possible mechanisms have been suggested for accelerated atherosclerosis in chronic renal failure including increased lipoprotein (a), higher oxidative stress, endothelial dysfunction, hyperhomocysteinemia and elevated tumor necrosis factor-α [3, 8-10], However, the exact mechanism is not exactly determined.

Metabolic acidosis is present in end stage renal disease, which begins as early as when the creatinine clearance decreases below around 30 ml/min. Studies indicated that acidosis increases oxidation of LDL, which is involved in development of the atherosclerotic lesion [9, 11].

Nitric oxide is not only an important marker for endothelial function, but also has several key functions in vascular homeostasis [12, 13]. The objective of this study was to evaluate the effect of experimentally induced metabolic acidosis on aortic endothelial permeability and serum NO concentrations in normal and high-cholesterol fed rabbits.

Material and methods

Animals

Twenty-four male rabbits weighing 1.7-2 kg were purchased from the Pasteur institute of Iran. The animals were kept in an animal room with a 12 h light/dark cycle with temperature 22-25°C. All animals had free access to standard chow and drinking water according to the experimental protocol. All experimental procedures were designed and performed in accordance with the guidelines of the animal care committee of Isfahan University of Medical Sciences.

Experimental protocol

After one week habituation, arterial blood samples were taken for pH, bicarbonate and base excess measurements. Sera were stored at –70°C for further analysis of serum lipids and NO levels. Then, the animals were divided into normal and hypercholesterolemic groups and each group was divided into acidemic and non-acidemic groups. Experimental groups were as follows:

- group 1 (n = 6): hypercholesterolemic + drinking water containing ammonium chloride (NH_4Cl);
- group 2 (n = 6): hypercholesterolemic + drinking water;
- group 3 (n = 6): normal diet + drinking water containing NH_4Cl.
- group 4 (n = 6): normal diet + drinking water.

Hypercholesterolemic groups received a cholesterol-rich diet (1%), prepared by adding 1 g of pure cholesterol (Merck, Germany) in 4 ml of olive oil to 0.1 kg of commercial rabbit chow [14, 15]. Metabolic acidosis was induced by adding 0.75% NH_4Cl (Sigma) in drinking water ad libitum as previously described [16]. Body weight was measured during the experiment.

Measurement of endothelial permeability

After 6 weeks, the animals were anesthetized by intraperitoneal injection of ketamine (100 mg/kg). Left femoral artery and vein were cannulated using a polyethylene catheter (PE-50) and blood samples were taken. Endothelial permeability was measured using the Evans blue (EB) dye injection method [17]. This technique is based on the principle that EB dye binds to the intravascular albumin. Briefly, EB (Merck, Germany) diluted in normal saline (20 mg/ml) was administered through the right femoral vein catheter. After 3 h, allowing circulation of the dye, the animals were sacrificed. Heart and aorta (from base of heart to renal arteries) were isolated and cleaned from surrounding connective tissues and weighed, immediately. Then, the tissues were placed in formamide solution for 24 h at room temperature for EB dye extraction. The extracted amount of EB was determined by a spectrophotometer at 620 nm wavelength. The results were plotted on a standard curve of EB in 0.2 to 10 μg/ml formamide using regression analysis to find the relationship between EB concentration and optical density. Concentration of EB in these tissues was expressed as μg EB/g tissue.

Serum NO measurement

Serum NO concentrations were determined by evaluation of its metabolite (nitrite) by the Griess

Table I. Results of pH, bicarbonate and base excess at the end of the experiment

Variable	Normal diet	Normal diet + acidemic	Hypercholesterolemic	Hypercholesterolemic + acidemic
pH	7.48 ±0.00	7.30 ±0.04*	7.44 ±0.01	7.31 ±0.02**
HCO_3^- [meq/l]	21.24 ±0.75	16 ±1.71*	23.10 ±0.92	14.66 ±1.04**
Base excess	0.1 ±0.67	–8.05 ±2.1*	0.5 ±0.85	–9.01 ±1.06**

*$p < 0.05$ when compared with normal diet group, **$p < 0.05$ when compared with hypercholesterolemic group*

reagent method (Promega Corp, U.S.A, Cat#G2930) using available reagents. Briefly, serum samples were added to the wells (96-well enzymatic assay plate). Sulfanilamide solution was added to all experimental samples, and after incubation, N-1-naphthylethylenediamine dihydrochloride solution was added. Then, absorbance was measured by a microreader at the wavelength of 450 nm. Samples' NO concentrations were determined in comparison to a nitrite standard reference curve. The limit detection was 2.5 μM nitrite.

Statistical analysis

Data are reported as mean value ± SEM. SPSS version 16 was used for data analysis. Comparison of data between groups was performed using ANOVA. Paired data were analyzed by paired *t*-test. Value of *p* less than 0.05 were considered statistically significant.

Results

Serum pH, bicarbonate and base excess

Serum pH, bicarbonate and base excess before and after experiments are shown in Table I. In groups 1 and 3 who received NH4Cl, serum pH, bicarbonate and base excess were significantly lower than other groups ($p < 0.05$).

Serum lipids profile

At the end of the experiment, hypercholesterolemic animals who received cholesterol 1% had significantly higher serum cholesterol and LDL levels compared with normal diet groups ($p < 0.05$) (Table II). Acidosis increased the serum cholesterol level in normal and hypercholesterolemic animals, although it was not statistically significant ($p > 0.05$).

Endothelial permeability

Aortic endothelial permeability (expressed as quantitative extravasation of EB) in high-cholesterol fed animals was significantly higher than in those in the normal diet group (16.18 ±0.91 μg EB/g tissue vs. 12.89 ±0.66 μg EB/g tissue; respectively). Acidosis increased endothelial permeability in the normal diet group ($p < 0.05$) but did not further increase endothelial permeability in the hypercholesterolemic group (Figure 1).

Serum NO concentration

Figure 2 illustrates that serum NO levels in hypercholesterolemic animals were significantly higher than the normal group ($p < 0.05$). Acidosis could not change serum NO concentrations either in normal or in hypercholesterolemic groups (Figure 2).

Table II. Serum lipid profile in all experimental groups before (pre) and at the end of the experiment (post)

Time	Pre				Post		
Lipid profile [mg/dl]	Normal diet	Normal diet + acidemic	Hypercholesterolemic	Hypercholesterolemic + acidemic	Normal diet	Normal diet + acidemic	Hypercholesterolemic	Hypercholesterolemic + acidemic
Cholesterol	72.90 ±6.27	70 ±5.69	74.90 ±7.23	79.80 ±5.01	97 ±17.57	111.17 ±18.59	507.70 ±28.31*	569.67 ±26.01*
Triglyceride	126.56 ±12.66	123.10 ±11.03	112 ±13.76	113.60 ±11.65	189.2 ±31.89	202.1 ±75.61	200.13 ±27.85	296.67 ±54.92
HDL	12 ±2.1	12.60 ±2.46	13.70 ±2.81	14.80 ±1.66	16.86 ±5.27	22 ±6.18	218.1 ±19.26*	257.56 ±16.58*
LDL	32.78 ±3.26	35.67 ±3.76	38.80 ±4.48	40.44 ±3	36.77±3.36	38.6 ±4.87	254.68 ±9.26*	252.78 ±12.29*

**p < 0.05 when compared with normal diet groups*

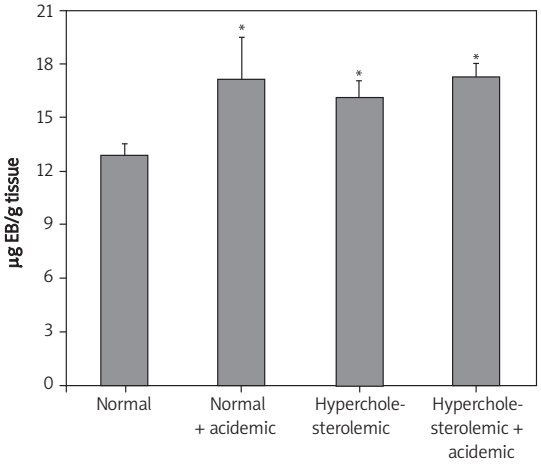

Figure 1. Aortic endothelial permeability in all groups
**p < 0.05 when compared with normal group*

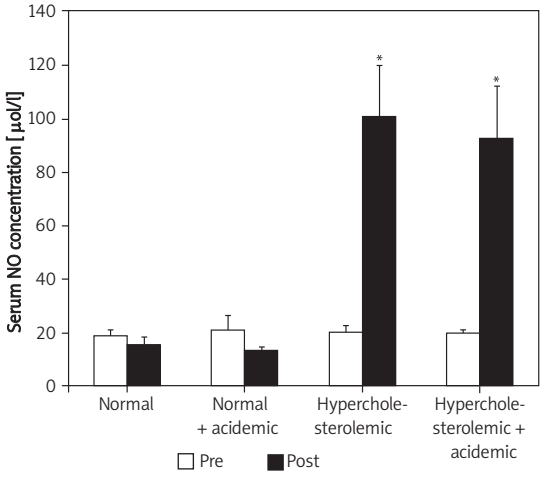

Figure 2. Serum NO concentration before and after experiment
**p < 0.05 when compared with normal group*

Discussion

Incidence of cardiovascular disease in patients with end-stage renal disease is 20 times higher than the normal population and is responsible for 40-50% of all deaths in these patients [3]. Atherosclerotic plaque is found in 30% of patients with chronic renal disease [3]. Although several mechanisms have been suggested for accelerated atherogenesis in renal insufficiency, the exact mechanism of atherosclerosis is not completely determined.

Acidosis is one of the features of end stage renal disease. There are few studies on the role of acidosis in atherogenesis. For the first time, Tavor *et al*. indicated an association between acidosis and atherosclerosis [16]. In the present study, we investigated the effect of metabolic acidosis on endothelial permeability as well as serum NO and lipid concentrations in normal and high-cholesterol fed animals. We found that hypercholesterolemic groups had higher serum cholesterol and LDL levels and acidosis could not significantly change these values. Therefore, it seems that alteration of lipid profile is not responsible for accelerated atherosclerosis in metabolic acidosis.

We also investigated the effect of acidosis on endothelial permeability. It is established that there is a link between endothelial permeability, retention of oxidized LDL in the intima of arteries and progression of atherosclerosis [5]. The present study demonstrated that hypercholesterolemic animals had higher aortic endothelial permeability compared with normal diet groups. In agreement with our observation, LaMack *et al.* indicated that endothelial permeability to albumin in hypercholesterolemic pigs was higher than in those on a normal diet [18]. Our results indicate that acidosis increased aortic endothelial permeability in animals with a normal diet, but did not further increase endothelial permeability in the hypercholesterolemic group. Previous studies demonstrated that acidosis, by itself, can predispose to oxidation of LDL, which has a key role in progression of atherosclerosis plaque [9, 11, 19, 20]. Acidosis is associated with reduced synthesis of Apo A through enhanced DNA binding activity of a repressor of apolipoprotein A gene [19]. Furthermore, acidosis increases interaction of arterial glucosaminoglycan with plasma LDL [16].

The present study also demonstrates that hypercholesterolemic animals had a higher serum NO level compared with normal-diet animals, which supports the results of previous studies [21-23]. This may be a protective response of endothelial cells in the early stage of atherosclerosis [21]. Moreover, NO synthase is increased in atherosclerotic blood walls [23].

In conclusion, it seems that alterations of serum lipid profile and NO concentration are not the main mechanisms for accelerated atherosclerosis in metabolic acidosis. However, acidosis increases aortic endothelial permeability, which may be a possible mechanism for accelerated atherosclerosis. Further studies are needed to understand the complete mechanism.

Acknowledgments

This study was supported by a grant from Isfahan University of Medical Sciences (grant number: 186071).

References

1. Barylski M, Banach M, Mikhailidis DP, Pawlicki L, Kowalski J. Decreased kidney function as a risk factor for cardiovascular events in subjects with metabolic syndrome – a pilot study. Arch Med Sci 2008; 4: 417-23.

2. Valderrabano F, Jones EH, Mallick NP. Report on management of renal failure in Europe, XXIV, 1993. Nephrol Dial Transplant 1995; 10 Suppl 5: 1-25.
3. Amann K, Tyralla K, Gross ML, Eifert T, Adamczak M, Ritz E. Special characteristics of atherosclerosis in chronic renal failure. Clin Nephrol 2003; 60 Suppl 1: S13-21.
4. Lusis AJ. Atherosclerosis. Nature 2000; 407: 233-41.
5. Ross R. Atherosclerosis: an inflammatory disease. N Engl J Med 1999; 340: 115-26.
6. Collins AJ. Cardiovascular mortality in end-stage renal disease. Am J Med Sci 2003; 325: 163-7.
7. Bax L, van der GY, Rabelink AJ, Algra A, Beutler JJ, Mali WP. Influence of atherosclerosis on age-related changes in renal size and function. Eur J Clin Invest 2003; 33: 34-40.
8. Kalantar-Zadeh K, Stenvinkel P, Pillon L, Kopple JD. Inflammation and nutrition in renal insufficiency. Adv Ren Replace Ther 2003; 10: 155-69.
9. Lamb DJ, Leake DS. Iron released from transferrin at acidic pH can catalyse the oxidation of low density lipoprotein. FEBS Lett 1994; 352: 15-8.
10. Schwarz U, Buzello M, Ritz E, et al. Morphology of coronary atherosclerotic lesions in patients with end-stage renal failure. Nephrol Dial Transplant 2000; 15: 218-23.
11. Morgan J, Leake DS. Acidic pH increases the oxidation of LDL by macrophages. FEBS Lett 1993; 333: 275-9.
12. Sadeghi-Hashjin G, Abuhosseini M, Asri-Rezaei S. Role of nitric oxide in the plasma lipid profile in the rabbits. Arch Med Sci 2009; 5: 308-12.
13. Bian K, Doursout MF, Murad F. Vascular system: role of nitric oxide in cardiovascular diseases. J Clin Hypertens (Greenwich) 2008; 10: 304-10.
14. Zhao SP, Yang J, Li J, Dong SZ, Wu ZH. Effect of niacin on LXRalpha and PPARgamma expression and HDL-induced cholesterol efflux in adipocytes of hypercholesterolemic rabbits. Int J Cardiol 2008; 124: 172-8.
15. Pakala R, Dilcher C, Baffour R, et al. Peroxisome proliferator-activated receptor gamma ligand pioglitazone alters neointimal composition in a balloon-denuded and radiated hypercholesterolemic rabbit. J Cardiovasc Pharmacol 2006; 48: 299-305.
16. Tovar AM, Pecly IM, Rangel EP, Melo-Filho NM, Mourao PA, Leite M Jr. Experimentally induced metabolic acidosis in rabbits modulates the interaction of aortic glycosaminoglycan with plasma low-density lipoprotein – an interesting observation about the association of acidosis and atherosclerosis. Atherosclerosis 2007; 192: 33-9.
17. St Pierre P, Bouffard L, Maheux P. Rosiglitazone increases extravasation of macromolecules and endothelial nitric oxide synthase in skeletal muscles of the fructose-fed rat model. Biochem Pharmacol 2004; 67: 1997-2004.
18. LaMack JA, Himburg HA, Friedman MH. Effect of hypercholesterolemia on transendothelial EBD-albumin permeability and lipid accumulation in porcine iliac arteries. Atherosclerosis 2006; 184: 255-63.
19. Haas MJ, Reinacher D, Li JP, Wong NC, Mooradian AD. Regulation of apoA1 gene expression with acidosis: requirement for a transcriptional repressor. J Mol Endocrinol 2001; 27: 43-57.
20. Leake DS. Does an acidic pH explain why low density lipoprotein is oxidised in atherosclerotic lesions? Atherosclerosis 1997; 129: 149-57.
21. Ferlito S, Gallina M, Catassi S, Bisicchia A, Di Salvo MM. Nitrite plasma levels in normolipemic and hypercholesterolemic patients with peripheral occlusive arteriopathy. Panminerva Med 1999; 41: 307-9.
22. Minor RL Jr, Myers PR, Guerra R Jr, Bates JN, Harrison DG. Diet-induced atherosclerosis increases the release of nitrogen oxides from rabbit aorta. J Clin Invest 1990; 86: 2109-16.
23. Wilcox JN, Subramanian RR, Sundell CL, et al. Expression of multiple isoforms of nitric oxide synthase in normal and atherosclerotic vessels. Arterioscler Thromb Vasc Biol 1997; 17: 2479-88.