*Biomed. Papers 148(2), 131–133 (2004)*



# THE ANTIOXIDANT ACETYLCYSTEINE REDUCES OXIDATIVE STRESS BY DECREASING LEVEL OF AOPPS

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*Received: Nevember 7, 2004; Accepted: December 5, 2004*

*Key words: Antioxidant therapy/N-acetylcysteine/NAC/AOPP/ACB*

Oxidative stress and especially its connection with many diseases has been discussed much recently. Among markers of oxidative stress there appear new and quite specific ones called advanced oxidation protein products (AOPPs). We tried to influence the level of AOPPs by an antioxidant therapy with N-acetylcysteine. Fourteen individuals with many cardiovascular risk factors were examined. All these patients were administered acetylcysteine (NAC) 600 mg/day orally during 20 days. Before starting the therapy we determined AOPP, albumin cobalt binding (ACB), glucose, creatinine, urea, ALT, AST, cholesterol, LDL, HDL and triglycerides values in peripheral venous blood in all individuals. After finishing our intervention we determined AOPP, ACB and glucose level again. Our results show a statistically significant decrease in AOPP levels after 20-day N-acetylcysteine therapy (medians, initially 82.2, at study end 74.3 umol/l, p = 0.039). We demonstrate a significant decrease in AOPP levels after 20-day N-acetylcysteine therapy in dose 600 mg/day.

## INTRODUCTION

Oxidative stress is defined as a disruption of the equilibrium between pro- and antioxidant systems. Generation of free oxygen radicals can be very useful for the organism, particularly in the processes connected with the immune system[1].

On the other hand, oxidative stress plays a very important role in pathology of many diseases, especially its connection with atherosclerosis has been proved and discussed[2].

As atherosclerosis is the basis of many diseases, we can also say that oxidative stress is the basis of many health problems, especially those called "civilisation diseases".

Also its connection with chronic renal failure and hemodialysis has recently been a subject of intensive research and discussion[3–5].

There are many markers of oxidative stress. First, we can detect free radicals themselves. This process is quite difficult, because they are not stable. Then we can measure antioxidant systems, which is also not common. The best way to measure the intensity of oxidative stress is detection of oxidation products. For example we can measure lipid peroxidation products, advanced glycation end products (AGEs).

Currently advanced oxidation protein products (AOPPs) receive much concentration and discussion. This marker shows oxidation damage to proteins and AOPPs also act as inflammatory mediators[6].

We have found no reference in the scientific literature on how to influence the level of AOPPs by antioxidant therapy. The aim of our work was to validate the effects of antioxidant therapy with N-acetylcysteine on AOPP levels.

## MATERIAL AND METHODS

We examined 14 probands (9 male, 5 female, 56.7 ± 12 years) with many cardiovascular risk factors (all individuals should be their risk of cardiovascular complications > 20 % within 10 years according to Framingham algorithm and the risk of fatal coronary events > 5 % within 10 years according to Cardioscore).

All patients were administered acetylcysteine 600 mg/day orally over 20 days. Before starting the therapy we determined AOPPs (ELISA Marc5+Max, Witko-Sarsat method[7, 8]), albumin cobalt binding (ACB) (COBAS MIRA+, Barr-Or method), glucose (ADVIA 1650, GOD-POD method), creatinine (ADVIA 1650, Jaffe method), urea (ADVIA 1650, enzyme method), ALT (ADVIA 1650, IFCC method), AST (ADVIA 1650, IFCC method), cholesterol (ADVIA 1650, enzyme method), LDL direct (ADVIA 1650, method with direct elimination), HDL (ADVIA 1650, method with direct elimination) and triglycerides (ADVIA 1650, enzyme) values in peripheral venous blood in all individuals.

After finishing our intervention we determined AOPPs, ACB and glucose values again.

All data were statistically evaluated using software MedCalc (Frank Schoonjans). For comparing the level of AOPPs before and after our intervention, we used the Wilcoxon paired test. We used Spearman correlation test to show any correlation between the other data.

## RESULTS

We confirmed that acetylcysteine in a dose of 600 mg per day over a 20-day therapy significantly reduces the level of AOPP (Table 1, Fig. 1). AOPPs values were characteristic with non Gaussian distribution.

No changes in glucose and ACD levels were discovered before or after finishing our intervention.

No correlations between AOPP, ACB and other examined parameters mentioned above were found.

## DISCUSSION

Several recent papers point out the role of oxidative stress in the acceleration of atherosclerosis and the potential effects of some antioxidant therapies.

Stress is associated with the origin of a large number of free radicals that may impair cells and tissues. Free radicals are atoms or groups of atoms with an unpaired electron. They display the same ability, a high reactivity, so that during a millionth of second (but also during several hours) they are able to bind to lipoproteins, nucleic acids, proteins and enzymes. Free radicals comprise oxygen reactive forms (e.g. hydroxyl radical OH), superoxide and peroxide ions. Binding of free radicals to nitrogen oxide (NO) in the endothelium leads in its lower availability and subsequently in reduced vasodilatation abilities, thus accounting for the origin of coronary ischemia.

An important role in the origin of oxidative stress is assigned to xantinoxidase, NADPH oxidase, high concentration of oxidised LDL cholesterol and delayed flow in the coronary artery. Several factors involved in the origin of oxidative stress are combined. Important enough is the level of anti-oxidative protection of the organism.

Recent papers suggest that AOPP is not only a marker of oxidative stress, but also acts as an inflammatory mediator. A detailed investigation of AOPP is expected to provide valuable information about the origin and progression of atherosclerosis.

Kaneda et al. published the results obtained by measurement of AOPP in patients with CAD, and proved that their AOPP values were significantly higher than those in individuals without signs of CAD. This paper indicates that AOPP concentrations are associated with the extent of involved coronary arteries. However, their association with clinical state of patients remains unclear[9]. The results of our another study suggest that patients with stable angina pectoris, i.e. patients with a favourable prognosis, had no significantly higher AOPP concentrations compared to the control group.

It was discovered that NAC therapy has beneficial effects against the ischemic/reperfusion injury and may have more pronounced protective effects on the liver and kidneys[10–12]. NAC is also effective in replenishing depleted glutathione stress in oxidative stress. Adjunctive use of NAC was associated with improved glutathione homeostasis, improved bile output and ATP regeneration, and increased survival in pigs[13]. In humans, however, parallel findings were not detected.

Our results are quite single and seem to be very interesting because in recent papers there is no mention about any possibility of reducing the level of AOPPs *in vivo*.

It can be said that the results of our study show, for the first time, acetylcysteine being a potential drug reducing oxidative stress by lowering the level of AOPPs.

We admit that our study is handicapped by a small number of probands. We take this fact as a challenge and we would like to solve this problem in our future study.

**Table 1.** AOPP values (in μmol $l^{-1}$) before and after N-acetylcysteine therapy. The p-value of Wilcoxon paired test was 0.039. SD indicates standard deviation.

| | Average | SD | Median | p |
|---|---|---|---|---|
| AOPP before | 117.2 | 68.9 | 82.2 | |
| AOPP after | 72.4 | 22.0 | 74.3 | 0.039 |

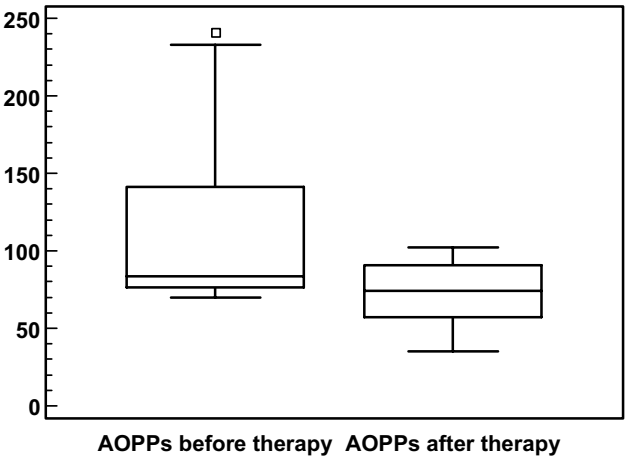


**Fig. 1.** Box and whiskers plot of AOPPs values before and after N-acetylcysteine therapy.

## REFERENCES

1. Štípek S, Borovanský J, Čejková J, Homolka J, Klener P, Lukáš M, Špičák J, Tesař V, Zeman M, Zima T, Žák A. (2000) Antioxidants and free radicals in health and disease. (in Czech) Praha: Grada Publishing, 2000.
2. Nguyen-Khoa T, Massy ZA, Witko-Sarsat V, Canteloup S, Kebede M, Lacour B, Drüeke T, Descamps-Latscha B. (1999) Oxidized Low-Density Lipoprotein Induced Macrophage Respiratory Burst via Its Protein Moiety: A Novel Pathway in Atherogebesis? Biochem Biophys Res Comm *263*, 804–809.

3. Descamps-Latscha B, Witko-Sarsat V. (2001) Importance of oxidatively modified proteins in chronic renal failure. Kidney Int *59*, 108–113.
4. Descamps-Latscha B, Drüeke T, Witko-Sarsat V. (2001) Dialysis-Induced Oxidative Stress: Biological Aspects, Clinical Consequences, and Therapy. Semin Dial *14*, 193–199.
5. Witko-Sarsat V, Gausson V, Descamps-Latscha B. (2003) Are advanced oxidation protein products potential uremic toxins? Kidney Int *63*, 11–14.
6. Witko-Sarsat V, Friedlander M, Nguyen-Khoa T, Capeillère-Blandin C, Nguyen AT, Canteloup S, Dayer J-M, Jungers P, Drüeke T, Descamps-Latscha B. (1998) Advanced Oxidation Protein Products as Novel Mediators of Inflammation and Monocyte Activation in Chronic Renal Failure. J Immunol *161*, 2524–253.
7. Witko-Sarsat V et al. (1996) AOPP as a novel marker of oxidative stress in uremia. Kidney Int *49*, 1304–1313.
8. Witko-Sarsat V, Gausson V, Nguyen A-T, Touam M, Drüeke T, Santangelo F, Descamps-Latscha B. (2003) AOPP-induced activation of human neutrophil and monocyte oxidation metabolism: A potential target for N-acetylcysteine treatment in dialysis patients. Kidney Int *64*, 82–91.
9. Kaneda H, Taguchi J, Ogasawara K, Aitawa T, Ohno M. (2002) Increased level of advanced oxidation protein products in patients with coronary artery disease. Atherosclerosis *162*, 221–225.
10. Sener G, Tosun O, Sehirli AO, Kacmaz A, Arbak S, Ersoy Y, Ayanoglu-Dulger G. (2003) Melatonin and N-acetylcysteine have beneficial effects during hepatic ischemia and reperfusion. Life Sci *72*, 2707–2718 .
11. Demir S, Inal-Erden M. (1998) Pentoxifylline and N-acetylcysteine in hepatic ischemia/reperfusion injury. Clin Chim Acta *275*, 127–35.
12. Sehirli AO, Sener G, Satiroglu H, Ayanoglu-Dulger G. (2003) Protective effect of N-acetylcysteine on renal ischemia/reperfusion injury in the rat. J Nephrol *16*, 75–80.
13. Fukuzawa K, Emre S, Senyuz O, Acarli K, Schwartz ME, Miller CM. (1995) N-acetylcysteine ameliorates reperfusion injury after warm hepatic ischemia. Transplantation *59*, 6–9.