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Research Article

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The effect of combined N-acetyl cysteine and vitamin C supplementation on blood biomarkers during single bout of exhaustive exercise in wistar rats

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ABSTRACT

Exhausting physical activity can cause harmful effects on the human, due to release of free radicals. This research is focused on effect of combined N-acetyl cysteine (NAC) and vitamin C (VC) supplementation on biomarkers of blood total antioxidant capacity (TAC), malondialdehyde (MDA) and C- reactive protein (CRP) during the exhaustive exercise in Wistar rats. This study was carried out on 32 female Wistar rats, which divided into 4 groups. In First group was given the effervescent tablets (NAC 600 mg, dissolved in water) four hours before the experiment by gavages. Second group received VC tablets (500 mg) and for third, a combination of VC and NAC (VICNAC), and fourth, was taken as the control group. Three stages of Blood samples were taken 1hour before starting, immediately after the exhaustive exercise, and after one hour at rest, respectively. After separating the serum samples; they were immediately stored at -80° C until analysis. The MDA, CRP and TAC concentrations were respectively measured using spectrophotometric and ELISA methods. A Significant reduction was observed in the concentration of MDA immediately after the exhaustive exercise in NAC group, in compared with the control (P ≤0.05); as well as CRP level in VICNAC group (P ≤0.05). There was no change in the TAC blood concentration during the study except in the NAC group one hour after exercise, which was significantly decreased in compared with the control groups (P≤0.05). In Conclusion, The results demonstrated that oral administration of NAC at least four hours before an exhaustive exercise can significantly decrease the harmful effects of oxidative stress in rats.

Keywords: N-acetylcystein, Vitamin C, Malondialdehyde, Total antioxidant capacity, C- reactive protein, Exhaustive exercise.

INTRODUCTION

Stress can cause physical exercise temporarily impairs the body Homeostasis [1], and affected directly on skeletal muscles and most of the organs the exertion during exercise [2]. Studies show that exercise can induce structural damage to muscle cells [3], inflammation and metabolic products such as lactate [4] and reactive oxygen species (ROS) [5].

Production of reactive oxygen species (ROS) increase the free radicals during irregular and long-term activities that can give rise to fatigue, inflammation and tissue damage [6], and then the appearance of different types of cytokine pro inflammatory and inflammation, such as IL-1, IL-6, IFN-gamma, TNF-α and reactive protein (CRP) in the blood [7].

Further confirmations suggests that amplified ROS induced by acute exercise may cause an imbalance between oxidative mediates and antioxidant systems, muscle fat accumulation, protein oxidation and the development of oxidative stress [8, 9].

As crucial role of free radicals in the oxidation of fats, proteins and damage to DNA, it is not unexpected that are presented a number of antioxidant defense mechanisms in body against the effects of these free radicals. In general, antioxidants have the great capacity to react with free radicals and minimize their actions, so delaying or preventing the oxidative stress [10].

Many athletes believe that regular food does not meet the severity of exercise-induced stress [11].

N-Acetyl cysteine (NAC), the form of acetylated amino-L-cysteine, is an excellent resource for sulfide group, that transformed to metabolites which capable the stimulating the synthesis of glutathione, and the elimination of free radicals in body [12, 13].

Cysteine can conserve the level of glutathione, a major antioxidant in intracellular antioxidant system. Intake the NAC can raise the body's protective enzymes and consequently reduce some of the cellular damages [11]. It can reduce fatigue [10, 14] and effect on the sub maximal contractions [15, 16]. Furthermore, NAC can moderate the oxidant indices of the blood without any tiredness [17].

Vitamin C is a water soluble antioxidant that protects the body against damages induced the free radicals such as hydroxyl and superoxide anions. In addition the enzyme cofactor, it plays a role in production of collagen, carnitine, as well as reduction of mitochondrial vitamin E, lipoic acid, and GSH [18]. Laboratory researches revealed that the ascorbate can trap to 24% of the oxygen free radicals in the plasma. Reactive protein C (CRP) is a protein that released from the liver in response to numerous injuries such as surgery, tissue damage and inflammation as well as exercises. This protein can act as indicator of a systemic inflammatory disease. It is an inverse association between CRP and regular physical exercise [7, 20]. It was shown that those with low cardio-respiratory fitness had higher levels of CRP and red cell distribution. It was a significant association between elevated CRP levels and red cell with low cardio-respiratory fitness [21].

In This study the effect of combined N-acetyl cysteine (NAC) and vitamin C supplementation on biomarkers of blood total antioxidant capacity (TAC), malondialdehyde (MDA) and C- reactive protein (CRP) during single bout of exhaustive exercise in Wistar rats was performed.

EXPERIMENTAL SECTION

Chemicals

The NAC and vitamin C were respectively purchased from *Zambon* pharmaceutical, and *Lpks Farma*, Switzerland; other chemicals were purchased from Merck (Germany).

Animals

In this study 32 female Wistar rats (250- 300 g weight, and 8- 10 weeks age) were used. All animals were kept in a climate controlled environment on a 12h light/ 12h dark cycle. Food and water were obtainable during all experimental process and all efforts were made to minimize suffering. All animal work was approved in accordance with the National Ethical Guidelines for Animal Research in Iran (2005), under Project code of 73021404911001 which was approved by the Animal Care and Use Committee of Bushehr University of Medical Sciences, Iran.

Experimental Groups

The study for examine the combined effects of NAC and vitamin C based on antioxidant and inflammatory markers were carried out in four groups of rats: First group effervescent tablets were given (600 mg of NAC dissolved in water) four hours before the experiment by gavages. Second group received tablets of Vitamin C (500 mg dissolved in water) and the third group received a combination of vitamin C (500 mg) and NAC (600 mg) (VICNAC), and fourth, was taken as the control group. Three times of Blood samples were taken 1 hour before starting, immediately after the exhaustive exercise, and after one hour at rest, respectively. After separating the serum samples; they were immediately stored at -80° C until analysis.

Exhaustive Exercise Protocol

The protocol was performed using swimming exercise. This method has more advantages than the treadmill, because swimming is a natural ability that mice are widely used [22, 23]. The result of this test was attained using swimming the rats in a deep water pool (length 75, width 70 and depth of 53 cm and the water temperature between

33- 36 ºC), until exhaustion fatigue and suffocation sense in inspected rats, according to Veskoukis et al., (2008) [24].

Blood Biochemical Parameters

For the in vitro dependent antioxidant parameters of malondialdehyde (MDA), total antioxidant capacity (TAC), and reactive protein-C (CRP), with ranging from 1-3 hours before test and one hour after the test samples were immediately taken the blood samples from eyes of mice by capillary tube. After separating the serum samples; they were immediately stored at -80° C until analysis.

The MDA was measured by spectrophotometric method. The TAC and CRP were also measured by ELISA technique with biovision and DRG Assay kits, respectively.

A 600 mg sulfur amino acid of NAC from Zambon pharmaceutical, and Supplement of vitamin C (500 mg) from Lpks Farma, Switzerland as effervescent tablets were prescribed orally, using a gastric tube through the mouth and took.

Statistical Methods

The data obtained from the Study were analyzed by using the SPSS software Version 18. The degree of normality was measured by Kolmogrov-smirnov. For significances differences between data, were used the ANOVA test, as well as the post-hoc test of Tukey test (α =0/05) for comparison between means.

RESULTS AND DISCUSSION

Concentration of CRP

The difference in CRP concentrations between experimental groups of N- acetyl cysteine, vitamin C and VICNAC in compare to the control group at different times of pre exercise, exercise and post exercise was shown in Figure (1).

As shown in Figure. 1, comparable to results of VC, the concentrations of CRP in NAC group, in three confirmed times were decreased in compared with the control group, but not significant.

The mean concentrations of CRP in VICNAC group were decreased in before and one hour after exhaustive exercise times, but not significant; while these results were revealed the significant falling (P< 0/05) in immediately after exhaustive exercise, than the control group.

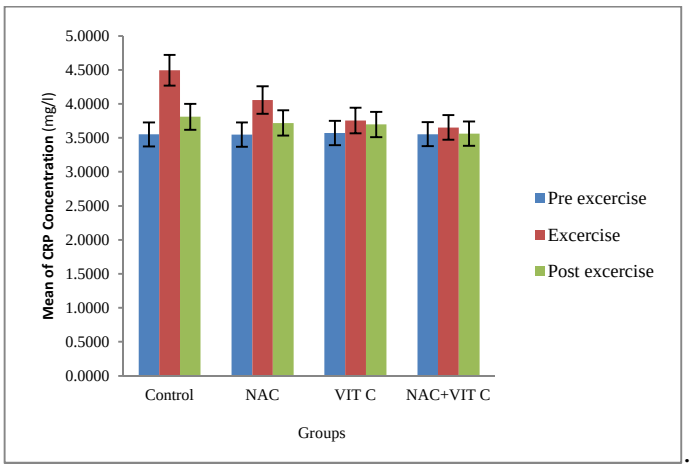

Fig. 1: The difference in CRP concentrations between experimental groups (N - acetyl cysteine, vitamin C. and NAC + VC) and the control group at different times of pre exercise, exercise and post exercise

Concentration of TAC

It was shown the mean concentrations of TAC for three experimental groups of N- acetyl cysteine, vitamin C, VICNAC and their control group in different intervals of pre exercise, exercise and post exercise(after 1h.) in Figure (2).

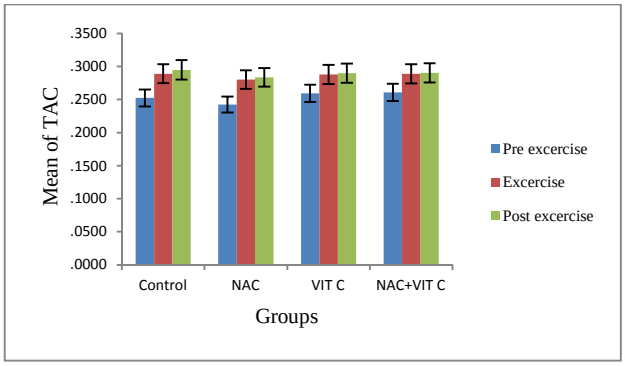

Fig. 2: The difference in TAC concentrations among three experimental groups of N- acetyl cysteine, vitamin C. and NAC + VC with the control group, every one, at different times of pre exercise, exercise and post exercise

According to Figure (2), the mean concentrations of TAC in pre exercise, exercise and post exercise times (vitamin C) were respectively higher, lower and lower than the control group. Their differences were no significances.

The Concentration of TAC in VICNAC group, pre exercise and post exercise (after 1h.) times were respectively higher and lower than the control group. The mean of this amount, was the same in immediately after exhaustive exercise, and the control groups.

Correspondingly, the mean concentrations of TAC in NAC receiving group in three pre exercise, exercise and post exercise times had a values lower than the control groups. This change in one hour after exhaustive exercise time was significant (P< 0.006) (Figure 2).

Concentration of MDA

The difference in MDA concentrations between three experimental groups of NAC, vitamin C, and VICNAC in compare to the control group at not the same times of pre exercise, exercise and post exercise was shown in Figure (3).

The MDA concentration in VIC group was increased in pre exercise group, with no significant difference than control group. Furthermore, in both phases of immediately and one hour after exhaustive exercise, had an insignificant decreasing in their MDA concentrations.

This value, in VICNAC group, was increased in before and one hour after exhaustive exercise times than the control group but there was no significant difference; however, in the time of immediately after exhaustive exercise, it was an insignificant decreasing in MDA mean concentration. According to our results, the concentration of lipid peroxidation end product of the malondialdehyde (MDA), in receiving NAC group was increased in pre exercise group, but not significant than control group. This value had an insignificant increasing in one hour after exhaustive exercise time than the control group. Comparably, it indicated a significant decline, in immediately after extensive exercise (P< 0/014).

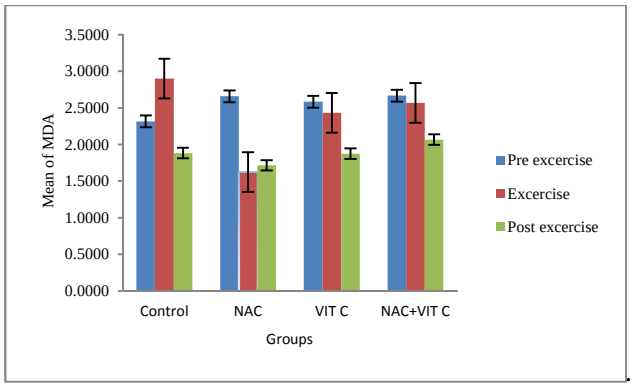

Fig. 3: The difference in MDA concentrations between experimental groups (N- acetyl cysteine, vitamin C. and NAC + VC) and the control group at different times of pre exercise, exercise and post exercise

Table (1) revealed the mean ± SE of serum concentration associated with MDA, TAC and CRP among three groups of NAC , vitamin C, VICNAC and the control group at different intervals of Pre exercise, immediately after exhaustive exercise and Post exercise.

Table 1: the mean ± SE of serum concentration associated with MDA, TAC and CRP among three groups of NAC , vitamin C, VICNAC and the control group

Groups	The research process	MDA	CRP	TAC
Control	Pre exercise	2.31±0.14	3.55±0.12	0.14±0.07
	Exercise	2.9±0.071	4.49±0.52	0.28±0.02
	Post exercise	1.88±0.26	3.81±0.24	0.29±0.03
NAC	Pre exercise	2.49± 0.15	3.54±0.24	0.24±0.01
	Exercise	1.62±0.27*	4.05±0.13	0.28±0.01
	Post exercise	1.71±0.53	3.72±0.14	0.28±0.05*
VC	Pre exercise	2.39±0.31	3.57±0.18	0.25±0.05
	Exercise	2.34±0.65	3.75±0.18	0.28±0.04
	Post exercise	1.87±0.14	3.69±0.15	0.28±0.04
VIC NAC	Pre exercise	2.48±0.22	3.55±0.15	0.26±0.02
	Exercise	2.56±0.72	3.65±0.15*	0.28±0.06
	Post exercise	2.06±0.08	3.56±0.28	0.29±0.05
* P<0/05				

During the substantial exercise, whole body oxygen consumption rises up to 20 times, and ROS are generated in excess [14].

Nowadays, Exercise supporters, researchers and academics have become interested to find any resources to help minimize the harmful effects of oxidative stress that are usually associated with extreme exercise [19].

However, its history come back to the distant past; At first, Hugh (1900) in his ergo graphic studies in muscular fatigue and soreness described that exercise induced muscle damage is not a fatigue phenomenon but the consequence of mechanical overload followed by structural and functional muscular changes [25].

Nutritional antioxidants are non-enzymatic compounds, whichever, lipid-soluble compounds like vitamin E, β-carotene, co-enzyme Q10, or the water-soluble compounds like vitamin C, glutathione, and uric acid. They can scavenge ROS into less reactive molecules or inhibit their change into more highly reactive forms, having intra and extracellular sites of action [26].

According to some previous studies, a decline in the volume of oxidative stress can increase health and performance. Thompson et al., in (2001) reported the beneficial effect of prolonged vitamin C supplements on antioxidant defense and recovery from demanding exercise [27].

In a similar study, Khassaf et al., (2003) revealed the beneficial effects of vitamin C supplements on antioxidant defense and stress proteins in human lymphocytes and skeletal muscle [28], as well as a protective effect of acute dose of ascorbic acid against ROS production after extensive exercise in study of Ashton et al. in 1998, that the oxygen radicals in human serum was detected by Electrons spin resonance (ESR) spectroscopic method [29].

Correspondingly, Medved et al (2004) indicated that a decrease in lipid peroxidation following a session of exhaustive exercise with orally N- acetylcysteine supplementation was occurred [17].

Even though, the vitamin C supplementation prior to exercise bouts has met with conflicting results. Study of Connolly et al., (2006) with aim of the effects of 8 days (3 days prior to an exercise bout, and for 5 days after) of vitamin C supplementation on elbow flexor delayed onset muscle soreness (DOMS) to 8 days in compare with placebo ingestion revealed that a vitamin C supplementation protocol of 3 x 1000 mg/day for 8 days is ineffective in protecting against selected markers of DOMS [30].

Latest study by the Lodhi et al (2012) was made known that unaccompanied vitamin C, cannot reduce the MDA concentration. Likewise, the consumption of one gram of vitamin C in two hours prior to the exercise, does not significant change the level of lipid peroxidation [31], which agrees with the our results.

In study of Yan et al., (2008), an increase in malondialdehyde level after acute aerobic exercise on a treadmill in overweight women, was shown [32].

The mean concentrations of CRP in VICNAC group were decreased in before and one hour after exhaustive exercise times, but not significant;

The findings of current study in NAC and VC supplementations, confirm the results of Davison and Gleeson (2007) on the relationship between physical activities with no significant changes in CRP concentrations in VC supplementation [33]. However, the mean concentrations of CRP in VICNAC group in our study, was shown a significant decreasing (P< 0/05) in immediately after exhaustive exercise, than the control group.

Interestingly, the findings of Childs et al., (2001) were shown that use vitamin C and NAC simultaneously during exhaustive exercise increases the oxidative stress and ultimately cause "acute inflammation of muscles [34]. Increasing the free metal, fast absorbing the complement and activation of peroxide-producing cells can be reason the greater than before levels of oxidative stress and cell damage in patients receiving complement [34].

Current study was carried out the special effects of supplementation of NAC , vitamin C and VICNAC on reduction of the oxidation and inflammation induced by exhaustive exercise in rats. It was shown the respectable effects of NAC , vitamin C, VICNAC in all stages of our experimental study, however, according to table (1), the significant decrease was observed in the concentration of MDA, immediately after the exhaustive exercise in the group consumed NAC in compared with the control ($P \leq 0.05$); as well as CRP level in VICNAC group ($P \leq 0.05$). There was no significant change in the TAC blood concentration during the study except in the NAC group one hour after exhaustive exercise which was significantly decreased in compared with the control groups ($P \leq 0.05$).

As these controversies, it was suggested that Consumption of these supplements four hours prior to exhaustive exercise increases the production of glutathione and increases the antioxidant ability of the cells, which leads to decreased production of reactive oxygen species and the neutralization of their side effects [12].

CONCLUSION

According to our study, the concentration of TAC in the receiving NAC group had a significant decline in single hour after extensive exercise, as well as the concentration of MDA in indicated group, immediately after extensive exercise. Thoroughly, it can be concluded that the intake of the N– acetyl cysteine as antioxidant supplement, especially before exercise can significantly reduce the oxidative stress induce exhaustive exercises.

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