Correlation Between The Erythrocyte Sedimentation Rate and Blood Nitric Oxide Levels in Rabbits?

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Summary

This study was designed to determine whether elevated or decreased blood nitric oxide (NOx) values following injections of L-arginine and N^{∞}-nitroarginine methylester (L-NAME) are positively correlated with the erythrocyte sedimentation rate (ESR) in healthy rabbits. The study involved 21 New Zealand rabbits of both sexes, aged between 10 and 15 months old. They were divided into 3 equal groups. The control group received 1 ml isotonic saline per rabbit, and Groups I and II received 100 mg/kg L-NAME and 250 mg/kg L-arginine intraperitoneally, respectively. After two hours blood samples were obtained and ESR (30, 60, and 120 minutes) and NOx were estimated. There were no significant differences between the ESR values at all times; however significant correlation between the NOx and ESR existed in Group II at 2 hours after injection. In conclusion, there was a positive correlation between high blood NOx and ESR values, but detailed studies are needed to disclose this correlation.

Introduction

The erythrocyte sedimentation rate (ESR) is a simple and inexpensive laboratory test measuring the distance that erythrocytes have fallen in a vertical column under the influence of gravity (*Brigden, 1999*). ESR is the most widely used indicator by rheumatologists to aid the assessment of disease activity in rheumatoid arthritis (RA) and other inflammatory disorders (*Sarban et al., 2005; Wolfe, 1997*) and inflammatory bowel disease in children (*Barnes et al, 2004*).

Nitric oxide (NO), a major messenger molecule in many pathophysiological functions, is synthesized from L-arginine by a family of isoformic enzymes (eNOS, nNOS and iNOS) known as nitric oxide synthase (NOS). Because NO has a very short half-life and is highly labile, plasma concentration of nitrate and nitrite (NOx) are often used as a marker of NO production and NOS enzyme activity (*Le Melledo et al., 2004*).

In recent years, elevated blood NOx values were determined in RA (*Beri et al., 2004; Sarban et al, 2005; Yki-Jarvinen et al., 2003*) ankylosing spondylitis (*Ersoy et al., 2002*) and active spondyloarthropathies (*Stichtenoth et al., 1995*). It is well known that ESR increases in rheumatical diseases and can be used as an indirect marker of inflammation. However the data about the correlation between the ESR and NOx are controversial. Stichtenon *et al.* (*1995*) and Ersoy *et al.* (*2002*) found a correlation between the ESR and serum nitrate level in spondyloarthropathies, but Choi (*2003*) did not see such a correlation in RA patients. Researchers found correlation between the ESR and NOx but did not explain any details or mechanism about this relationship and to the best of our knowledge this remains unknown. As some inflammatory markers released in rheumatoid diseases affect ESR, the purpose of the present study was to

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determine the relationship between ESR and NOx levels in healthy animals.

Materials & Methods

The study involved 21 New Zealand Rabbits (Laboratory Animal Unit of The University of Kafkas, Kars, Turkey) of both sexes, aged between 10 and 15 months. They were fed a special pelleted rabbit diet (Bayramoglu Yem AS, Erzurum, Turkey) *ad libitum* and divided into 3 equal groups. The study animals were kept in cages (four rabbits per cage; 70 deep x 70 wide x 50 high cm) and at room temperature (22-25ºC) with a 12:12h light:dark cycle. The mean body weight of the control group (CG, n=7), N^{∞}-nitroarginine methylester (L-NAME) injected group (Group I, n=7) and L-arginine injected group (Group II, n=7) was 2.9 ± 0.2 kg, 3.3 ± 0.2 kg, and 3.1 ± 0.2 kg, respectively. The Laboratory Animal Care and Use Committee of the Faculty of Veterinary Medicine approved all experimental protocols.

The control group received 1 ml isotonic saline per rabbit. Groups I and II were given a single dose of 100 mg/kg L-NAME and 250 mg/kg L-arginine per rabbit, respectively. All injections were performed intraperitoneally.

Blood samples were taken from the marginal ear vein 2 hours after injection into plain and sodium citrate treated tubes for determination of NOx using a colorimetric (*Miranda et al., 2001*) and ESR at 30, 60 and 120 minutes using the Westergreen method (*Konuk, 1981*), respectively.

For the comparison of groups, one-way ANOVA (Tukey's *t*-test) and Pearson's correlation test were used (MINITAB statistical package, Version 11.2, 1996, USA). Data are represented as mean ± SEM (Standard Error of Mean).

Results & Discussion

The NOx level decreased in the L-NAME injected group (not significant compared with control), and increased in the L-Arginine injected group ($p < 0.001$) when compared with the control and Group I. There were no significant differences among the ESR values at all times in all groups. ESR and NOx values are presented in Table 1. The significant cor-

Table 1. The 30, 60 and 120 minute ESR and NOx values of isotonic saline (control), L-NAME (Group I), and L-arginine (Group II) injected groups two hours after injections. [(ESR: erhythrocyte sedimentation rate (mm/min), NOx: total nitrate and nitrite values in blood (µM)].

	Two hours after injection		
Groups/ Values	Control (n=7)	Group I (n=7)	Group II (n=7)
ESR (mm/30 min)	12 ± 1,9	16 ± 2,8	13 ± 1,9
ESR (mm/60 min)	26 ± 3,2	35 ± 4,3	27 ± 2,1
ESR (mm/120 min)	47 ± 4,1	55 ± 5,2	47 ± 2,7
NOx	35 ± 0,8[a]	32 ± 1,4[a]	44 ± 1,4[c]

Values with different superscript on the same line are different ($p \leq 0.001$).

relation between NOx and ESR values at 30 min ($r = 0.87$, $p < 0.05$), 60 min ($r = 0.80$, $p < 0.05$) and 120 min ($r = 0.79$, $p < 0.05$) were estimated in Group II (Figure 1-3; $p < 0.05$).

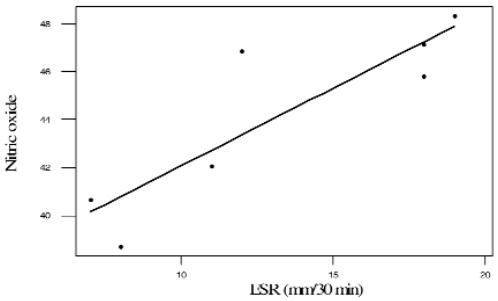

Figure 1. The correlation between the NOx levels and 30 min ESR values in Group II ($r = 0.87$, $p < 0.05$).

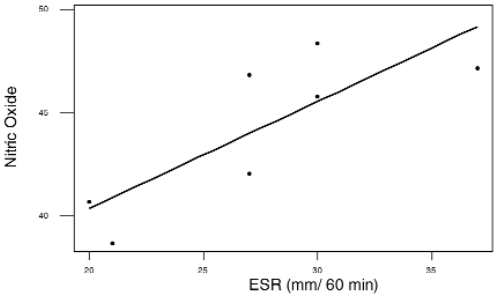

Figure 2. The correlation between the NOx levels and 60 min ESR values in Group II ($r = 0.80$, $p < 0.05$).

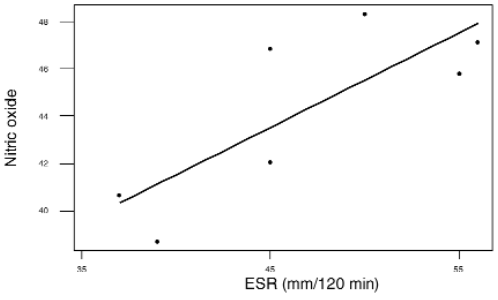

Figure 3. The correlation between the NOx levels and 120 min ESR values in Group II ($r = 0.79$, $p < 0.05$).

There is growing evidence that NO is involved in immune regulation, inflammation and autoimmunity and arthritis (*Stefanovic-Racic et al., 1993*). Some investigators have demonstrated high NOx production in patients with RA or Osteoarthritis (*Farrel et al., 1992; Ueki et al., 1996*) juvenile-rheumatoid arthritis in childhood (*Beri et al., 2004*), and in animals with experimentally induced arthritis (*Stichtenoth and Frolich, 1998*).

The ESR values are important diagnostic criteria for the diseases listed above and they are extensively used by rheumatologists in RA and other inflammatory disorders. However, the levels of the ESR and NO increased together in the same kind of diseases and some scientists have focused on the correlation. But the data about the correlation between the ESR and NOx are controversial (*Ersoy et al., 2002; Stefanovic-Racic et al., 1993; Stichtenoth et al., 1995*).

In the present study, NOx levels were significantly higher in the animals at the second hour from L-arginine application; however no significant difference was observed in terms of ESR among control and experimental group animals. In addition, there was a positive and significant correlation between high blood NOx and ESR values in Group II.

In conclusion, high blood NOx concentration may be correlated with ESR values and detailed, long term and molecular studies are needed to investigate this correlation.

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