

The levels of nitric oxide in megaloblastic anemia

Megaloblastik anemide nitrik oksit düzeyleri

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Abstract

Objective: The purpose of this study was to investigate the relationship between nitric oxide degradation products (nitrate and nitrite) levels and megaloblastic anemia which is treated with cyalocobalamin.

Materials and Methods: A total of 30 patients with megaloblastic anemia (16 Male, 14 Female) were included in the study. Cyanocobalamin was administered (1.000 µg/day intramuscularly) until the reticulocyte crisis occurred to the normal range. The control group consisted of 30 healthy subjects (15 Male, 15 Female). Nitric oxide levels were measured before treatment and compared with the values obtained during peak reticulocyte count.

Results: Plasma direct nitrite, total nitrite and nitrate levels were 24,86±3,87, 60.56±7,01 and 36,02±5,24 in before treatment versus 15,48±3,05, 38,92±6,44 and 22,77±6,04 µmol/dl in after treatment, respectively. Plasma direct nitrite, total nitrite and nitrate levels were significantly lower in after treatment compared with the before treatment (p<0.001).

Conclusion: Nitric oxide levels are seen to increase in megaloblastic anemia. This study suggested that abnormalities in the nitric oxide levels in megaloblastic anemia are restored by vitamin B12 replacement therapy.

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Key words: Megaloblastic anemia, vitamin B12, nitric oxide

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Özet

Amaç: Bu çalışmada siyanokobalamin ile tedavi edilen megaloblastik anemi ile nitrik asit degranülasyon ürünleri olan nitrat ve nitrit arasındaki ilişki araştırıldı.

Yöntem ve Gereçler: Çalışmaya 16'sı erkek, 14'ü kadın olan toplam 30 megaloblastik anemili hasta alındı. Hastalara retikü losit krizi görülene kadar 1.000 µg/gün dozunda siyanokobalamin intramüsküler olarak uygulandı. Kontrol grubu tamamen sağlıklı 15'i erkek, 15'i kadın toplam 30 kişiden oluşturuldu. Nitrik oksit düzeyleri tedavi öncesinde ölçüldü ve retikülosit sayısının en yüksek seviyesinde tekrar ölçülerek karşılaştırıldı.

Bulgular: Tedavi öncesindeki ortalama plazma direk nitrit seviyesi µmol/dl olarak 24,86±3,87, total nitrit 60,56±7,01 ve nitrat 36,02±5,24 bulundu. Buna karşılık kontrol grubunda sırasıyla 15,48±3,05, 38,92±4,42 ve 22,77±6,04 bulundu. Tedavi sonrası

plazma direk nitrit, total nitrit ve nitrat seviyesinin tedavi öncesine göre istatistiksel olarak anlamlı derecede azaldığı tespit edildi ($p < 0.001$).

Sonuç: Nitrik oksit düzeyleri megaloblastik anemide artmaktadır. Bu çalışma megaloblastik anemide nitrik oksit düzeylerindeki anormalliklerin B12 replasman tedavisi ile düzeldiğini ortaya koymaktadır. (*Turk J Hematol 2009; 26: 197-200*)

Anahtar kelimeler: Megaloblastik anemi, vitamin B12, nitrik oksit

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Introduction

Megaloblastic anemia is characterized by megaloblastic erythropoiesis and is secondary to decreased activity of methionine synthase, one of two mammalian enzymes that requires vitamin B12 (cobalamin) as a cofactor. Methionine synthase catalyzes the transfer of the methyl group of 5-methyltetrahydrofolate to homocysteine via a methylcobalamin intermediate with cycling of cobalamin between the +1 valency state cobalamin and the +3 valency state cobalamin [1,2]. Methyltetrahydrofolate is the major intracellular storage form of folates, and its synthesis from 5,10-methylene tetrahydrofolate is essentially irreversible in vivo [2,3]. Thus, decreased methionine synthase activity leads to trapping of intracellular folates as 5-methyltetrahydrofolate, and the megaloblastic anemia of vitamin B12 deficiency is virtually indistinguishable from the megaloblastosis of folate deficiency [4]. Nitric oxide (NO) is produced by most cell types and regulates a diverse array of biological functions [5]. NO has been reported to inhibit methionine synthase activity in vitro [6-8], it might be expected to bind to the cobalt in cobalamin because (i) NO binds tightly to the iron in heme; (ii) ferrous heme and cbl (III) are isoelectronic; and (iii) in both heme and cobalamin, the metal ion is coordinated to four in-plane nitrogen atoms of a tetrapyrrole ring and has two out-of-plane ligands [2]. In literature published that NO inhibits methionine synthase activity in vivo and that NO produced by three different pharmacological agents or produced physiologically by rat C6 glioma cells inhibits carbon flow through the folate pathway [4].

In the light of above mentioned information, NO inhibits methionine synthase and direct cause of ineffective erythropoiesis. So, Nitric oxide leads megaloblastic anemia. The onset of anemia due to B₁₂ or folate deficiency begins the production of nitric oxide then a vicious cycle of anemia sets in due to inhibition of methionine synthase. There have been no studies in humans regarding the effects of nitric oxide on patients with megaloblastic anemia. Given the lack of studies regarding the relations between serum levels of nitrate and nitrite in patients with megaloblastic anemia, the aim of this study was to explore these relations and restoration effect of cyanocobalamin in adult with megaloblastic anemia.

Material and Methods

This study was conducted in Turgut Özal Medical Center, Department of Hematology, between January 2005 and December 2006. Thirty patients (16 male and 14 female, age 17-75, average 55 years) with megaloblastic anemia were enrolled in the study. Patients with acute or chronic infections,

proven chronic inflammatory diseases, heart diseases and other anemia with patients were not included in the study. Informed consent was obtained at the beginning of the study from all participants, both the megaloblastic anemia patients and the healthy control subjects. Diagnostic criteria of the patients are summarized in Table 1. All patients showed low serum levels of vitamin B12 (the average value and the normal range were 85 and 200-900 pg/ml, respectively). Diagnosis was based on the medical history, macroovalocytosis in peripheral blood, megaloblastic changes in bone marrow, low serum levels of vitamin B12, increased serum LDH and indirect bilirubin levels, and grade 4 atrophic gastritis in endoscopic biopsy. NO levels were measured before treatment and compared with the values obtained during peak reticulocyte count (average seventh day). Cyanocobalamin was administered (1.000 µg/day intramuscularly) until the reticulocyte crisis occurred and serum vitamin B12 levels returned to the normal range. The control group consisted of 30 healthy subjects (15 M, 15 F, average age: 28 years), NO levels in the blood samples were measured from pro and post cyanocobalamin treatment and statistical significance was evaluated.

Assay for Nitric Oxide

Plasma nitrite/nitrate levels were measured with the Griess reaction using a spectrophotometer at 545 nm. Nitrite (0.1M sodium nitrite in water) has been mixed with sulfanilamide solution (1% sulfanilamide in 5% phosphoric acid) first, followed immediately by addition of NED solution (0.1% N-1-naphthylethylenediamine dihydrochloride in water). The absorbance has been measured within 30 minutes.

Statistical Analysis

Statistical analysis was done by SPSS (Statistical Program for Social Sciences, version 15.0). Significance of differences was evaluated with independent and paired Student's t test; $p < 0.05$ was regarded as statistically significant.

Results

The average hemoglobin level during diagnostic period 7,3 g/dl, leukocyte count 4,1/103/µl, platelet count 137,4/103/ml, MCV 115,6 /fl, vitamin B12 83,8 pg/ml and folic acid 7,8 ng/ml detected (Table 1).

Plasma direct nitrite, total nitrite and nitrate levels were 24,86±3,87, 60,56±7,01 and 36,02±5,24 in before treatment versus 15,48±3,05, 38,92±6,44 and 22,77±6,04 µmol/dl in after treatment, respectively. Serum nitrite and nitrate levels were significantly higher in the before treatment than in the after treatment and control group ($p < 0.001$). Almost seven days later than cyanocobalamin treatment, the nitric oxide levels returned to normal and reached nearly the same levels as that of the control group (Table 2).

Table 1. Some blood parameters of patients in initial diagnosis

Parameters	Min.	Max.	Mean	SD	Normal range
Hemoglobin, g/dl	3.4	10.7	7.3	1.9	11-16
Hematocrit, %	8.9	31.2	21.1	5.9	34-45
Mean corpuscular volume, fl	99	136	115.6	12.67	80-95
Platelet count, x103/ml	9	497	137.4	108.3	150-450
Leukocyte count, x103/ μ l	1.3	10.9	4.1	2.26	4-11
LDH, U/l	564	12773	4640	325.4	200-350
Potassium, mmol/l	3.4	4.7	4.4	0.32	3.5-5.5
Uric acid, μ mol/l	123.6	563.8	342.1	91.6	154-422
Unconjugated bilirubin, μ mol/l	0.5	17.4	6.4	3.1	0-10
Conjugated bilirubin, μ mol/l	0.29	5.9	1.6	1.4	0-4
Vitamin B12, pg/ml	42	167	83.8	82.5	200-900
Folic acid, ng/ml	1.7	21.6	7.8	5.76	2.7-16.1

Table 2. Nitric oxide levels of megaloblastic anemia patients and controls

Parameters	Megaloblastic anemia patients (n=30)		Control group (n=30)	p value
	Treatment			
	Before	After		
Direct nitrite (μ mol/dl)	24.8 \pm 3.8	15.4 \pm 3	16.6 \pm 4.5	p< 0.001*
Total nitrite (μ mol/dl)	60.5 \pm 7	38.9 \pm 4.4	35.4 \pm 5.1	p<0.001*
Nitrate (μ mol/dl)	36.0 \pm 5.2	22.7 \pm 6	20.1 \pm 3.2	p<0.001*

*Statistically significant

Discussion

NO plays a critical role in many different physiological processes including blood pressure regulation, platelet aggregation, neurotransmission, and macrophage cytotoxicity [5]. Many of NO's effects (e.g. blood pressure regulation and platelet aggregation) are mediated via NO binding to the iron in the heme prosthetic group of guanylate cyclase, which markedly activates the enzyme and thereby increases the intracellular concentration of the second messenger cGMP [9,10]. NO has a remarkably high affinity for ferrous heme with a binding constant on the order of 10^{12} to 10^{14} M⁻¹, and NO also binds to ferric heme. Iron and cobalt are transition metals adjacent in the periodic table, and the porphyrin ring of heme and the corrin ring of cobalamin are both substituted tetrapyrrole rings [11]. Thus, it is not surprising that NO binds to the cobalt in cobalamin. In the light of above studies, In megaloblastic anemia, serum levels of vitamin B12 decrease. The lack of vitamin B12 has been thought to be the main factor in this decrease, but another potential factor is nitric oxide, which has been shown to affect cobalamin metabolism in vitro and in vivo. Nitric oxide is an inhibitor of erythropoiesis. Cytokine-induced NO is known to decrease human erythropoiesis, and NO is likely an important mediator of the anemia of chronic disease in humans. Also, NO inhibits methionine synthase and direct cause of ineffective erythropoiesis. Therefore, Nitric oxide leads megaloblastic anemia [12]. In megaloblastic anemia, reductions

in cobalamin synthesis are known to be associated with low levels of intracellular cobalamin, but nitrate and nitrite levels have not been previously implicated. In this study we found that patients with megaloblastic anemia, as defined by low levels of serum vitamin B12, had higher serum levels of nitrate and nitrite than did normal controls. NO levels are known to have increased in anemia. In published studies, NO levels in anemia with iron deficiency [13] and aplastic anemia [14] found increased. On the other hand, In another studies, NO bioavailability in thalassemia [15] and sickle cell anemia [16] reported decreased. In this study, NO levels were measured averagely one week later than the diagnosis, not a tangible improvement is anticipated within this duration. That the anemia increased the NO levels which hence deepened the megaloblastic anemia can be derived from this study. After treatment of cobalamin(average seventh day), the levels of nitric oxide returned normally. We observed that cyanocobalamin administration had restored the increased levels of nitric oxide which was the main abnormality. Our hypothesis is that when B12 vitamin is applied on the patients with megaloblastic anemia, there is a likelihood of decreased activity of inhibition caused by NO to methionine synthase which may contribute the NO level's decrease and return to normal at the end of the treatment. We, however, were unable to disclose this molecularly. In the future, to get a truer picture of the subject, more detailed molecular studies in higher numbers of patients are needed.

In conclusion, we suggest that nitric oxide is associated with the serum level of vitamin B12 in patients with megaloblastic anemia. The replacement of vitamin B12 in patients with megaloblastic anemia restored, at least in the early phase of treatment, the significant increase in the levels of nitric oxide. This study appears to be the first to investigate a relation between nitric oxide and vitamin B12 levels in a clinical setting.

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