The effect of anti-tumor necrosis factor (TNF)-alpha therapy with etanercept on endothelial functions in patients with rheumatoid arthritis

Romatoid artrit'li hastalarda etanercept ile yapılan anti-tümör nekroz faktör (TNF)-alfa tedavisinin endotel fonksiyonları üzerine etkisi

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ABSTRACT

Objective: To investigate the effects of tumor necrosis factor (TNF)-α antagonism with etanercept (ENC) on endothelial functions in patients with active rheumatoid arthritis (RA).

Methods: A total of 21 patients with RA were enrolled in this prospective study. Eleven of them (8 women, 3 men mean age 47.0±10.1 years) with high disease activity despite the conventional treatment were assigned to Group 1 and were given ENC treatment twice a week (25 mg SC injection) for 12 weeks. Ten patients with RA (8 women, 2 men mean age 55.0±6.4 years) under conventional methotrexate and prednisone therapy were assigned as Control group (Group 2). Endothelium-dependent and -independent vasodilator responses of the brachial artery were assessed by high-resolution ultrasound. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were also measured at baseline and at the post treatment period. Mann-Whitney U and Wilcoxon tests were used to compare the data and correlation analysis was performed using Pearson correlation test.

Results: Endothelium-dependent vasodilatation improved from 5.2±0.8% to 7.9±1.3% (p=0.04) in ENC group, while no significant change was observed in the control group (from 6.6±1.1% to 7.0±1.8% p=0.67). No significant changes were found in endothelium-independent vasodilatation and baseline brachial artery diameters in both groups. A significant reduction in ESR and CRP were observed in patients receiving ENC (from 16.2±6.8 to 9.2± 5.1 mm/h, p=0.003 and from 14.68±3.4 to 9.25± 3.7 mg/L, p=0.003, respectively).

Conclusion: Treatment with ENC for 12 weeks significantly improved endothelial function in patients with active RA compared to those under conventional therapy. The findings of the present study support the hypothesis that the use of TNF-α blockers in patients with active RA may reduce the high incidence of cardiovascular complications. *(Anadolu Kardiyol Derg 2010; 10: 98-103)*

Key words: Endothelial function, tumor necrosis factor-alpha, etanercept, rheumatoid arthritis

ÖZET

Amaç: Aktif romatoid artriti (RA) olan hastalarda, bir tümör nekroz faktör (TNF)-α antagonisti olan etanerseptin (ENC) endotel fonksiyonları üzerindeki etkinliğini araştırmaktı.

Yöntemler: Bu prospektif çalışmaya toplam 21 RA'lı hasta dahil edildi. Bu hastalardan konvansiyonel metotreksat ve prednisolon tedavisine karşın yüksek hastalık aktivitesi devam eden 11 hastaya (8 kadın, 3 erkek ortalama yaş 47.0+10.1 yıl) haftada 2 kez, 25 mg/SC, 12 hafta süreyle ENC tedavisi başlandı (Grup 1). Konvansiyonel tedavi altında olan 10 hasta ise (8 kadın, 2 erkek ortalama yaş 55.0+6.4 yıl) kontrol grubunu oluşturdu (Grup 2). Brakiyal arterin endotel bağımlı ve bağımsız vazodilatasyon yanıtları yüksek rezolüsyonlu ultrason ile değerlendirildi. Eritrosit sedimantasyon hızı (ESH) ve C-reaktif protein (CRP) değerleri bazal ve tedavi sonrası dönemlerde ölçüldü. Verilerin karşılaştırılmasında Mann-Whitney U ve Wilcoxon testleri, korelasyonlar için Pearson korelasyon testi kullanılmıştır.

Bulgular: Endotel bağımlı vazodilatasyon ENC grubunda %5.2±0.8'den %7.9±1.3'e yükselirken (p=0.04) kontrol grubunda anlamlı değişiklik saptanmadı (%6.6±1.1'den %7.0±1.8'e p=0.67). Bazal çaplarda ve endotel bağımsız vazodilatasyonda her iki grupta da anlamlı değişiklik gözlenmedi. Etanersept grubunda tedavi sonunda ESR ve CRP değerlerinde anlamlı azalma gözlendi (sırasıyla 16.2±6.8 mm/saat'ten 9.2± 5.1 mm/saat'e, p=0.003 ve 14.68±3.4 mg/L'den 9.25± 3.7 mg/L'ye, p=0.003).

Sonuç: Çalışmamızda 12 hafta süreyle uygulanan ENC tedavisinin aktif RA'sı olan hastalarda konvansiyonel tedaviye oranla endotel fonksiyonları anlamlı derecede iyileştirdiği gözlenmiştir. Bu bulgumuz aktif RA'lı hastalarda yüksek oranda gözlenen kardiyovasküler komplikasyonların azaltılması amacıyla TNF- α blokajı yapan ajanların kullanımının faydalı olabileceği yönündeki görüşleri desteklemektedir. *(Anadolu Kardiyol Derg 2010; 10: 98-103)*

Anahtar kelimeler: Endotelyal fonksiyon, tümör nekroz faktör-alfa, etanersept, romatoid artrit

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Introduction

Cardiovascular mortality has been shown to be increased up to a 5-fold in patients with rheumatoid arthritis (RA) (1, 2). Although the exact mechanisms of the increased incidence of atherosclerotic vascular disease in RA patients are not fully understood, many similarities were shown to be present between the pathogenesis of inflammatory and immunologic response in RA and atherosclerotic vascular disease (3). The common findings include involvement of cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6, increased concentrations of C-reactive protein (CRP) and fibrinogen, increased local expression of adhesion molecules, endothelin-1, neoangiogenesis and collagen degradation via activation of macrophages and mast cells (4, 5). These similarities suggest that inflammatory mechanisms responsible of synovial lesions in RA patients might also be involved in endothelial dysfunction and facilitate the development of atherosclerotic vascular disease (6). Moreover, many investigators have shown that different degree of endothelial dysfunction was present in both newly diagnosed and long active RA patients (7-10).

Endothelial dysfunction is well recognized as a key process in atherosclerosis and was shown to independently predict cardiovascular events (11, 12). Ultrasonographic determination of vasodilatation after post-occlusion reactive hyperemia, which is called as flow-mediated vasodilatation (FMV) had been shown to a good predictor for evaluating endothelial functions in humans (13). TNF-α inhibitors have recently been demonstrated to be very effective in controlling signs and symptoms in approximately two thirds of patients with RA (14, 15). In recent years, the effects of different types of TNF-α inhibitors on endothelial functions were investigated in a limited number of studies and controversial results were published (16-22).

The present study was conducted to evaluate the effects of etanercept, a TNF-α inhibitor, on endothelial functions measured with brachial ultrasonography as endothelial- dependent and –independent vasodilatation and compare them with the RA patients receiving conventional disease-modifying antirheumatic drug (DMARD) treatment. Changes in erythrocyte sedimentation rate (ESR), CRP and disease activity score (DAS) were also considered in both groups of patients.

Methods

Patient's characteristics

In this prospective study, a total of 21 patients with diagnosis of RA established according to the criteria of the American College of Rheumatology (23) having DAS-28 score higher than 3.2 and receiving at least two antirheumatic drugs including methotrexate (MTX) at an optimum dose and subjects without previous treatment with anti-TNF-α agents were enrolled in the study.

Eleven of 21 patients (eight women, three men and mean age 47.0±10.1 years, disease duration 5.5±3.0 years) with RA with DAS-28 score higher than 5.1 despite treatment with MTX (≥15mg - <25mg/week), sulphasalazine (≥2gr- 3gr/day) and a dose of methylprednisolone (≥8mg - <16mg/day) for at least three months were included in Group 1 (ENC group) and were given only ENC twice a week (25 mg SC injection) for 12 weeks. In this group of patients all DMARD therapy were stopped and all had a MTX-free period of time not less than three months before starting ENC treatment. Medication other than MTX was discontinued one week prior to study enrollment and only four of the patients included in ENC group continued to use non-steroidal anti-inflammatory drugs. Ten RA patients (8 women, 2 men mean age 55.0±6.4 years, disease duration 6.5±2.8 years) matching for age, gender, height and weight with the ENC group under conventional DMARD served as control subjects (Group 2). All subjects underwent routine clinical examination and were screened for conventional cardiovascular risk factors. Exclusion criteria were age <18 years, uncontrolled hypertension (>160/90 mmHg), diabetes mellitus, valve disease, renal or cardiac insufficiency, previous cardiovascular or cerebrovascular events and total cholesterol (>240 mg/dL). All patients were recruited from the outpatient rheumatology clinic of our hospital. Informed consent was obtained from all patients and controls. The study was approved by the local institutional research committee.

Study protocol

Etanercept was injected subcutaneously 2x25mg/week for twelve weeks. All patients had to be nonsmokers or ex-smokers for at least 5 years. Nonsteroidal anti-inflammatory drugs were stopped at least 5 days prior to each experimental session. Erythrocyte sedimentation rate and CRP were also measured at baseline and at the post treatment period. The disease activity score was obtained from 28-joint counts as described (24) and was assessed just before and after the study period of twelve weeks in all patients.

Biochemical analyses were performed by enzymatic methods on analyzer (Integra Roche Diagnostics Corporation, Indianapolis, IN, USA) with commercial reagents. CRP levels were measured by nephelometry analyzer (Dade Behring Incorporation, Deerfield, IL, USA).

Assessment of endothelial function

Patients were studied in the morning, between 9.00-10.00 h in a quiet room with an ambient temperature of 20-24^{o}C, after an overnight fasting and avoidance of alcohol, caffeine and cigarette smoking the previous night. Each patient stopped all long-acting vasoactive medication at least 24 h. The subjects laid at rest for at least 10 min before a first resting screen was recorded. Endothelium-dependent (flow-mediated) and endothelial-independent (glyceryl-trinitrate (GTN) induced) vasodilatation (0.4mg sublingual, Nitrolingual sprey, Pohl-Boskamp) of the brachial artery were assessed by high-resolution ultrasound (7.5 MHz lineer array transducer, Siemens Sonoline Electra) by a expert physician (PB) blinded to the patient data at baseline and after 12-weeks of treatment period according to previously established and validated method (25). Briefly, the arterial diam-

eter was measured at a fix distance from an anatomical marker such as a bifurcation and calculated as the mean value of 2 measurements at the end of the diastole, concurrent with the onset of the QRS complex. The average diameter of the artery then was calculated over three cardiac cycles. After taking the baseline measurements, increased flow was induced by inflation of a brachial cuff placed to proximal limb to a pressure of 50 mmHg above systolic pressure for 5 minutes. After release, the arterial diameter was recorded every 15 seconds for 3 minutes. Post-test arterial diameter measurements were made 60 seconds after cuff deflation. Fifteen minutes was allowed for vessel recovery before GTN application and the same procedure was repeated after GTN application. To evaluate the reproducibility of imaging and to assess intra-observer variability, additional measurements of randomly selected 10 patients' images were performed on 2 different days. The correlation coefficients for intraobserver variability were; r=0.92 for baseline, r=0.94 for reactive hyperemia and r=0.91 for NTG induced vasodilatation.

Statistical analysis

SPSS version 11.0 (Chicago, IL, USA) was used for statistical analysis. Data are expressed as mean ± standard deviation (SD). Baseline characteristics and differences between pre and post treatment period were compared with a Mann-Whitney U or Wilcoxon test, as appropriate. Correlation analyses for intraobserver variability were performed by using Pearson correlation test. Measurements of flow-mediated dilatation and nitroglycerin-induced vasodilatation represent the maximal increase in brachial diastolic artery diameter and are expressed as percent change from baseline. Percentage changes in vasodilator response were calculated as [(post-treatment value-pre-treatment value)/ pre-treatment value x 100]. P values <0.05 were considered to be significant.

Results

The baseline demographic and clinical characteristics of the patients were similar in both groups (p>0.05) (Table 1).

Both endothelium-dependent and –independent vasodilatation remained unchanged in control group (p>0.05) (Table 2 and Fig. 1). In ENC group endothelium-dependent dilatation improved significantly from 5.2±0.8% to 7.9±1.3% after 12 weeks of treatment (p=0.04). Endothelium-independent vasodilatation induced by GTN remained unchanged in Etanercept group (p=0.37) (Table 2 and Fig. 2). Brachial artery diameters at baseline and after 12 weeks period did not change significantly in both groups (p=0.20 and p=0.67, respectively).

Both ESR and CRP were found to be decreased in ENC group (from 16.2±6.8 to 9.2±5.1 mm/h, p=0.003 and from 14.68±3.4 to 9.25±3.7 mg/L, p=0.003, respectively), while no significant changes were observed in control group (p=0.35 and p=0.28, respectively). Disease activity score-28 (DAS-28) improved significantly from 6.1±0.3 to 3.6±0.5 (p=0.002) in ENC group, while mild but not significant improvements have been achieved in control group (from 4.8±0.2 to 4.6±0.3, p=0.13).

Table 1. The baseline demographic and clinical characteristics of both groups of patients

Variables	Control group (n=10)	ENC Group (n=11)	p*
Age, years	55.0±6.4 56 (47-61)	47.0±10.1 49 (38-59)	0.12
Male/Female	8/2	8/3	0.83
Disease duration, years	6.5±2.8 6.6 (3.2-10.5)	5.5±3.0 5.8 (2.9-8.9)	0.54
DAS-28	4.8±0.2 4.6 (4.3-5.0)	6.1±0.3 6.3 (5.2-9.2)	0.003
BMI, kg/m^2	29.0±4.5 31 (24-34)	28.0±3.5 30 (25-33)	0.6
CRP, mg/L	16.5±5.8 17.2 (10.9-23.5)	14.7±3.4 14.3 (10.6-19.5)	0.15
ESR, mm/h	13.4±5.1 13.8 (7-22)	16.2±6.8 18.1 (9-26)	0.32
MAP, mmHg	88.0±5.2 87 (80-94)	91.0±4.7 92 (83-96)	0.72
Cholesterol, mg/dl	194.5±26.3 190.3 (154-236)	186.5±31.1 183.0 (149-238)	0.41
Medication, %			
NSAIDs	30	36	
Prednisone	40	0	
Methotrexate	100	0	
Folic acid	80	0	
Sulphasalazine	80	0	
Leflunomide	30	0	

Data are represented as mean±standard deviation, median (min-max) values and percentages

*Mann-Whitney U test

BMI - body mass index, CRP - C-reactive protein, DAS - 28- disease activity score, ENC - etanercept, ESR - erythrocyte sedimentation rate, MAP - mean arterial pressure, NSAIDs - nonsteroidal anti-inflammatory drugs

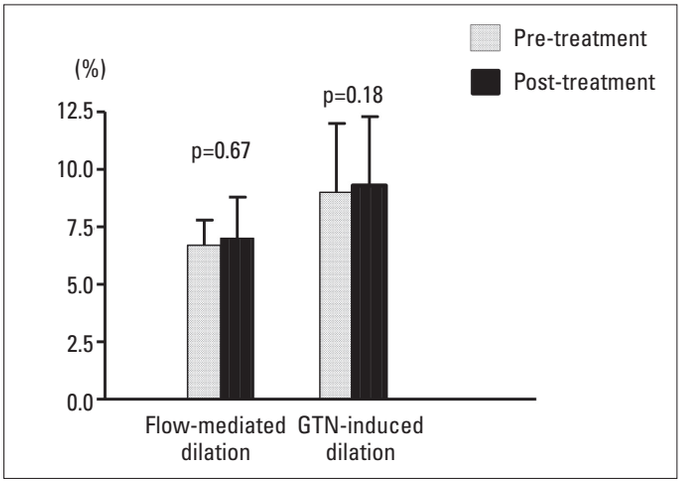

Figure 1. Flow-mediated (endothelial-dependent) and glycerol trinitrate (GTN) induced (endothelial-independent) vasodilatation in patients receiving conventional disease-modifying antirheumatic drug treatment (mean± standard deviation)

Table 2. Baseline diameters and endothelium-dependent (flow-mediated) and -independent (GTN-induced) vasodilator responses of the brachial artery of both groups

Groups	Variables	Baseline	After 12-week treatment	p*
Control Group	Brachial artery diameter, mm	3.7±0.48 3.7 (3.1-4.2)	3.77±0.63 3.8 (3.0-4.4)	0.67
	Flow-mediated dilatation, %	6.67±1.1 6.61 (4.3-7.8)	7.06±1.8 7.1 (4.1-8.3)	0.67
	GTN-induced dilatation, %	9.28±2.57 9.36 (5.3-14.8)	9.97±2.9 8.61 (5.1-13.5)	0.18
ENC Group	Brachial artery diameter, mm	3.28±0.68 3.12 (2.9-4.1)	3.40±0.58 3.32 (3.0-4.6)	0.2
	Flow-mediated dilatation, %	5.2±0.8 5.4 (4.9-7.3)	7.9±1.3 8.2 (7.1-12.9)	0.04
	GTN-induced dilatation, %	8.2±3.36 8.1 (4.1-14.7)	8.7±1.60 8.7 (5.0-11.6)	0.37

Data are represented as mean±standard deviation and median (min-max) values
*Wilcoxon test
ENC - etanercept, GTN - glyceryl-trinitrate

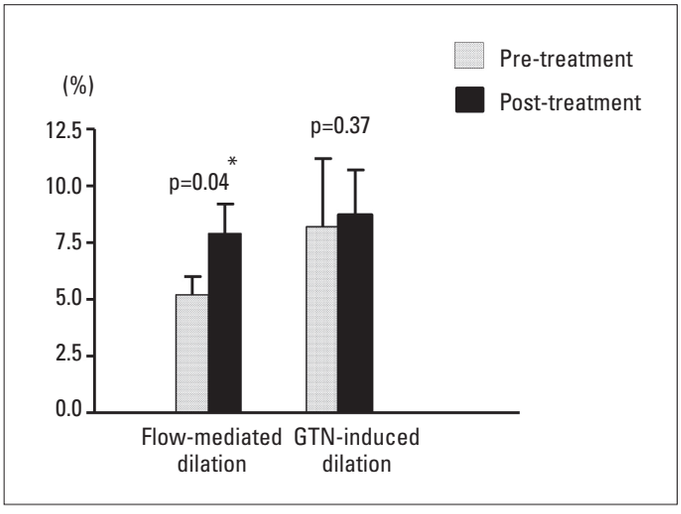

Figure 2. Flow-mediated (endothelial-dependent) and glycerol trinitrate (GTN) induced (endothelial-independent) vasodilatation in patients receiving etanercept therapy additional to conventional disease-modifying antirheumatic drug treatment (mean±standard deviation)

Discussion

In this prospective study which was conducted to evaluate the effects of etanercept, a TNF-α inhibitor, on endothelial functions in patients with active RA, it was found that treatment with ENC for 12 weeks significantly improved endothelial function compared to those under conventional therapy. A significant reduction in ESR and CRP was also observed in patients receiving ENC.

Rheumatoid arthritis is associated with accelerated atherosclerosis and increased cardiovascular morbidity and mortality. The increased risk of cardiovascular events is independent of traditional atherosclerotic risk factors in this group of patients (1). Therefore, search for other predictors of cardiovascular disease seems to be necessary. Vascular endothelial dysfunction, which is increasingly recognized as a key process in atherosclerosis may be a plausible explanation for the high incidence of cardiovascular diseases in RA patients. Reduced arterial elasticity occurring independent of traditional risk factors has already been reported in RA patients (26). Inflammatory markers were higher in patients with RA when compared with healthy controls and inversely correlated with small- artery elasticity and large-artery elasticity values (26). Since anti-TNF-α treatment reduces disease activity in RA patients and accepted as a useful new therapeutic option in RA (14, 15), it was assumed that TNF-α blockers may also improve arterial elasticity and as a result vascular functions. With this regard, in several studies, the validity of this hypothesis was investigated with different type of TNF-α blockers such as infliximab (17-19, 21) and ENC (16, 20, 22).

Hürlimann et al. (17), have shown that infliximab treatment for a duration of 12 weeks significantly improved endothelial function in RA patients. In an other study, Gonzales et al. (18) reported an effective but transient improvement on endothelial function in RA patients treated with infliximab for at least 1 year. These investigators considered that long term use of this drug may reduce the high incidence of cardiovascular complications in RA, however the search for other TNF-α antagonists with longer effect on endothelial function should be considered. In a very recent study performed by Bosello et al. (19) also revealed very similar results as in the study of Gonzales et al. (18). Cardillo et al. (21) evaluated the effects of infliximab on the endothelial functions in early RA patients (duration of symptoms <12 months) using plethysmography and concluded that infliximab infusion for 60 minute duration restore endothelium-dependent vasodilator capacity. In all of these studies, in contrast to ours, MTX was continued as a DMARD therapy along with the anti-TNF-α treatment which may also interfere with the endothelial response. The impact of MTX on endothelial functions has been controver-

sially discussed in the literature. Landewe et al. (27) reported an increased mortality risk in patients with cardiovascular comorbidity receiving MTX compared to matched patients using other DMARDs. They explained their finding by MTX-induced hyperhomocysteinemia, which in turn would have increased the cardiovascular risk. On the contrary, Choi et al. (28) reported improved cardiovascular mortality in RA patients treated with MTX. In the present study, in order to document the net effect of the drug on endothelial function, only the patients whom MTX treatment were stopped for the reason of ineffectively or intolerance to drug were enrolled in ENC group.

Amongst the studies, which evaluated the effects of ENC on endothelial functions in long-term RA patients, Hansel et al. (16) concluded that switching the therapy from MTX to ENC in 8 RA patients has no beneficial effect on endothelial function. Their results are in contradiction to our findings. The reason for this discrepancy may be attributed to the differences in study design. In their study, authors started etanercept injections (with a dose of 2x25 mg/week for only 2 weeks) one week after the final dose of MTX and measured forearm blood flow by a plethysmography 12-24 hours after the fourth subcutaneously injected dose of etanercept. They concluded that specific blockade of TNF-α has no superior effect on endothelial functions compared to conventional MTX therapy when both agents are tested at equal levels of inflammatory activity. We believe that the lack of efficacy of etanercept on endothelial functions in Hansel et al's study -unlike our results- is due to two main reasons. First, 21 days may not be sufficient for complete elimination of MTX when switching from MTX to etanercept. Second, a period of ENC therapy with a dose of 2x25mg/week for only 2 weeks may also not be sufficient h to achieve the optimum effect on endothelial functions for ENC. Etanercept was effective in reducing disease activity as well as improving endothelial functions in our study. Our patients with etanercept were active and previously had been treated with MTX but had to be discontinued due to gastrointestinal side effects such as nausea, vomiting and anorexia. Patients in the etanercept group in our study, all had a MTX-free period not less then three months before etanercept which is enough for wash-out. In the second study, Van Doornum et al. (22) could not be able to show any significant effect in arterial stiffness in long-term RA patients after 6-weeks of treatment with ENC. Relatively small number of patients, short treatment period, difference in measurement techniques and continuation of MTX treatment in the study of Van Doornum et al. (22) may explain the differences between their and our results. In the third study, Bilsborough et al. (20) evaluated the effect of ENC on endothelial function in six RA patients after 36 weeks of treatment. Their methodology and results were similar to ours except the continuity of MTX treatment in their study. They concluded that additional anti-TNF-α treatment to conventional therapy reduced inflammatory symptoms and improved endothelial function.

Study limitations

The present study has two main limitations. First of them is the relatively low number of patients included in the study. Second limitation is the difference in DAS-28 scores between the two groups. Because of the inclusion criteria, the subjects in ENC group had a higher DAS-28 score compared to controls, which indicates a higher inflammation in this group. Thus, it may be assumed that the anti-inflammatory and vasodilatory effect of ENC might be more prominent in this group of patients. Therefore, we think that the effect of ENC treatment in RA patients with lower DAS-28 scores must also be assessed in other studies.

Conclusion

Our results have shown that anti-TNF-α treatment with ENC for 12 weeks significantly improves endothelial function in patients with active RA compared to those under conventional therapy. Findings of this preliminary study support the hypothesis that the use of TNF-α blockers in patients with active RA may reduce the high incidence of high cardiovascular complications.

Conflict of interest: None declared

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