Orijinal Araştırma / Original Investigation
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The Effect of Iloprost on Nitric Oxide, Asymmetric Dimethyl Arginine and Serotonin in the Treatment of Peripheral Vascular Disease

Periferik Vasküler Hastalığın Tedavisinde İloprostun Nitrik Oksit, Asimetrik Dimetilarjinin ve Serotonine Etkisi

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ÖZET

AMAÇ: Bu çalışmada periferik arter hastalarında iloprost kullanımının dolaşımdaki asetil dimetil arjinin (ADMA), Serotonin ve Nitrik Oksit (NO) gibi endotelyal fonksiyonlarda görev alan parametreler üzerindeki etkinliğinin araştırılması hedeflenmiştir.

GEREÇ VE YÖNTEM: Çalışmaya Fontaine III-IV hastalığı tanısıyla takip edilip medikal tedavi kararı verilen 30 olgu (19'u erkek, 11'i kadın, yaş aralığı ise 60.7 ± 13.7) alındı. Hastalara iloprost infüzyonu ön kol venlerinden 0.5-1.5 ng/kg/dk dozunda 16 saatlik intravenöz infüzyon şeklinde başlandı ve 7 gün verildi. Tedavi öncesi ve sonrası (8. günde) ADMA, Serotonin ve NO sonuçları için kan alındı. Total nitrit (nitrit + nitrat) konsantrasyonu modifiye kadmiyum redüksiyon metodu ile, ADMA ve serotonin düzeyleri High Performance Liquid Chromotography (HPLC) yöntemiyle ölçüldü.

BULGULAR: İloprost tedavisi öncesi ve sonrası ADMA değeri tedavi sonrası düşmüş olup bu istatistiksel olarak anlamlı iken (p=0.001), Serotonin (p=0.82) ve NO (P= 0.16) değerlerindeki değişiklikler istatistiksel olarak anlamsız bulunmuştur.

SONUÇ: Periferal arteriyel hastalıklarda iloprost tedavisi sonrası endotelyal disfonksiyon göstergesi olarak kabul edilen ADMA'nın azaldığını ve periferik arteriyel hastalarında kullanılan tedavinin değerlendirilmesinde dikkate alınabilecek bir parametre olabilir.

Anahtar Kelimeler: iloprost, nitrik oksit, ADMA, serotonin

ABSTRACT

OBJECTIVE: In this study, it is aimed to investigate the efficacy of iloprost use in peripheral artery disease patients on the parameters involved in endothelial functions such as circulating acetyl dimethyl arginine (ADMA), serotonin and nitric oxide (NO).

MATERIALS AND METHODS: 30 patients (19 male, 11 female, age interval 60.7 ± 13.7) who were followed-up with the diagnosis of Fontaine III-IV disease and decided to receive a medical treatment were included in the study. Iloprost infusion was initiated as a 16-hour intravenous infusion at the dose of 0.5-1.5 ng / kg / min from the forearm veins and was given for 7 days. Blood was taken for ADMA, Serotonin and NO results before and after the treatment (8th day). Total nitrite (nitrite + nitrate) concentration was measured by the modified cadmium reduction method, ADMA and serotonin levels were measured by High Performance Liquid Chromatography (HPLC) method.

RESULTS: ADMA value before and after the iloprost treatment was decreased after the treatment and while this was statistically significant (p=0.001), the changes in serotonin (p=0.82) and NO (P= 0.16) values were found statistically insignificant..

CONCLUSION: Peripheral arterial disease may be a parameter that can be taken into account in the evaluation of treatment for peripheral arterial disease and ADMA, which is considered to be an endothelial dysfunction indicator after iloprost treatment.

Keywords: iloprost, nitric oxide, adma, serotonin

INTRODUCTION

Peripheral artery disease (PAH) is a disease characterized by narrowing or obstruction of the arteries distal to the abdominal aorta and the aortic bifurcation level as a consequence of progressive atherosclerosis (1). Impaired endothelial structure and inflammation play an important role in the initiation and progression of the atherosclerotic event. A complex inflammatory and fibro-proliferative response occurs against the accumulation of atherogenic lipoproteins caused by the plasma in the arterial intima (1-2).

Iloprost is the synthetic analogue of prostacyclin and has effects such as arterial vasodilatation, anti-inflammatory, ischemic reperfusion injury inhibition, fibrinolytic effect, inhibition of platelet activation (3-5). Nitric oxide (NO) is a material that is produced from L-arginine, which is an amino acid, via endothelial nitric oxide synthase (NOS) and is playing an important role in maintaining vascular

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homeostasis by regulating vascular tone (6). It has an indirect effect on NO production rate by affecting the cell entrance pathway with arginine and ADMA (7). ADMA is an endogenous competitive inhibitor of endothelial NOS (nitric oxide synthase) and it reduces the production and bioavailability of endothelial NOS (8). In vascular diseases, ADMA levels increase without any clinical symptoms (9,10). Endothelial dysfunction is correlated with ADMA levels (9), indicating that is a better marker of endothelial damage than cholesterol (11).

In this study; ADMA, serotonin and NO rates before and after the treatment are compared in peripheral arterial disease patients receiving iloprost treatment.

MATERIAL & METHODS

Thirty patients with Fontaine III-IV peripheral artery disease were included in the study. (19 male, 11 female, age interval 60.7 ± 13.7). Iloprost infusion (Ilomedin®, Bayer Schering Pharma AG, Berlin, Germany) was initiated as a 16-hour intravenous infusion of 0.5 ng / kg / min from the forearm vessels (12). During the first day of treatment, the dose was increased to 1.5 ng / kg / min by increasing 0.5 ng / kg / min every 30 minutes. The side effects that may arise during the treatment were closely monitored. The treatment dose was then continued for 7 days at a dose of 1.5 ng / kg / min according to tolerability of the dose. Blood was taken on the 8th day after the treatment. At least one of invasive and non-invasive methods such as arterial ultrasonography (USG), digital subtraction angiography (DSA), magnetic resonance angiography (MRA) and computerized tomographic angiography (BTA) was used for diagnosis for the patients. Physical examination of all the patients was performed and demographic features, cardiovascular risk factors, and medications were recorded. Patients with a fasting blood glucose level of 126 mg / dl or greater or those taking oral antidiabetic drugs and those using insulin; those having a systolic blood pressure of 140 mmHg or greater, having diastolic blood pressure of 90 mmHg or greater, or those taking antihypertensive drugs were accepted as hypertensive. Blood samples were collected from the patients for routine hematologic and biochemical tests following a 12-hour fasting. Patients were included in this clinical trial that had been diagnosed with a peripheral disease, who could go to a major amputation, and who did not have any vascular reconstruction or lumen-opening intervention (angioplasty) or fibrinolytic therapy, and who quit smoking for at least 2 weeks before. Patients who had a history of myocardial infarction during the previous month, patients having an acute coronary syndrome, renal and hepatic dysfunction or any known systemic disease, and those who did not tolerate the iloprost dose were excluded from the study. Patients were informed about the study, their consent was obtained and the study protocol was approved by the university ethics committee.

ADMA, Serotonin, and NO measurement: Blood samples taken from the patients via antecubital vein were placed in the tubes containing pre-cooled ethylenediaminetetraacetic acid (EDTA) and then placed in ice. The samples were centrifuged at 3000 G for 10 minutes. Serum and plasma were then removed from these tubes and stored at -80 ° C until the day of study, and serum and plasma were immediately thawed on the day of study. Nitrite and nitrate amounts from the serum samples was assigned by the Griess reaction after deproteinization and total nitrite (nitrite + nitrate) concentration was assigned by the modified cadmium reduction method. Among the thawed plasma samples, ADMA and serotonin levels were studied in High Performance Liquid Chromatography (HPLC) (Shimadzu 10 AVP, Japan) (13) according to the kit procedure with suitable commercial kits (20).

The study was approved by Local Ethical Committee of Medical School of Fırat University (Approval No. 2010-23). All steps of study were designed according to Helsinki Declaration and in adherence to local guidelines for good clinical practice.

Statistical Assessment:

SPSS (Statistical Packages for the Social Sciences) 12.0 software was used for the statistical analyses. The data obtained was taken as mean ± standard deviation. T-test was used to compare the data obtained before and after the treatment. Significance level was accepted as $p<0,05$.

RESULTS

Thirty patients (19 male, 11 female, age interval: 60.7 ± 13.7), who had no chance for surgical or endovascular intervention as determined as a result of the doppler and computerized tomographic (CT) angiography as a result of ischemia, were included. Age interval of the cases was 38-90 and mean age was 60.7 ± 13.7. 6 of the cases (20%) were diabetic. As an additional disease, hypertension (HT) was present in 5 cases (16.6%) (HT) and hyperlipidemia (HL) was

present in 11 cases (36.6%). Other demographic characteristics of the patients were given in Table 1.

After the treatment, all of the 30 patients, who were enrolled in the study, completed their treatment at the indicated dose and time. In 11 (36.6%) cases, side effects were observed during the treatment. The side effects observed are given in Table 2.

Two patients (6.6%), who were included in the study and had tissue necrosis, were amputated after the iloprost treatment. ADMA value before and after the iloprost treatment was decreased after the treatment and while this was statistically significant (p=0.001), the serotonin (p=0.82) and NO (P= 0.16) values were found statistically insignificant. Blood levels before and after iloprost are given in Table 3.

Table 1. *Demographic characteristics of the patients*

Demographic characteristics	number	%
Age 38-74 mean 58.73 ± 15.75		
< 50 years old	8	26.6
> 50 years old	22	73.3
Gender		
Male	19	63.3
Female	11	36.6
Smoking		
Active smoker	12	40
Quitted	4	13.3
Non-smoker	14	46.6
Additional diseases		
Diabetes Mellitus	6	20
Hypertension	5	16.6
Hyperlipidemia	11	36.6
Clinical grade		
Grade III	22	73.3
Grade IV	8	26.6

Table 2: *Side effects observed in the cases after iloprost treatment*

Side effects	number	%
Headache	5	16.6
Phlebitis	1	3.3
Nausea	3	9.9
Flushing	1	3.3
Rash	1	3.3

Table 3. *ADMA, Serotonin, NO levels of the cases (µmol/L)*

	Before iloprost	After iloprost	P value
ADMA	2.78 ± 1.01	1.3 ± 0.41	0,001
Serotonin	81.15 ± 54.40	80.16 ± 45.58	0.82
NO	62.46 ± 21.4	57.8 ± 16.7	0.16

ADMA: asetil dimetil arjinin NO: nitric oxide

DISCUSSION

Peripheral artery disease is a disease with high incidence in the population with a high prevalence and morbidity (14,15). When there is no appropriate care, the patients with PAD have ischemic conditions that lead to amputation and that increase morbidity and mortality (16). More than 30 million people worldwide are estimated to be affected from PAH, and the prevalence of peripheral arterial disease is reported to be 17% in women and 20% in men over 65 years old (15).

In the literature, there are limited number of studies evaluating ADMA, serotonin and NO after the ilomedin treatment. In a study, ADMA values were evaluated after the iloprost treatment in obstructed peripheral artery patients and plasma ADMA and serotonin levels were found to be low after the iloprost treatment (17). In this study, plasma ADMA values after iloprost treatment were decreased as in this study.

The most important pathway in the continuation of endothelial function is the presence of Nitric Oxide (NO) produced by NOS. The most effective molecule on this pathway has been accepted as ADMA that is the junction point of the effects of the risk factors (9,18). Serotonin is an indol amin that is synthesized from L-tryptophan, which is an essential amino acid. Serotonin is a strong smooth muscle stimulant and a vasoconstrictor (19). Prostacyclin and prostaglandin reduce the release of E2-like vasodilator agents by the release of local vasoconstrictor substances such as serotonin (5-hydroxytryptamine = 5-HTI) (20). The function of the serotonin carrier in the tissues is considered to be responsible for cardiac and smooth muscle contractility, platelet aggregation, cellular mitogenesis and regulation of neuronal activity (21). Thrombocytes do not synthesize serotonin but only store them and mean serotonin is released in a thrombotic event due to an endothelial damage. It increases the platelet aggregation by affecting the receptors on the platelet in the serotonin (22,23). Through to the 5-HTI-like receptor in the vein endothelium, NO is released by serotonin and thus, vasodilatation occurs. In the studies conducted by using a NO blocker, it is observed that blood flow is accelerated by the vasoconstriction effect of serotonin (23). It is seen that the vasoconstriction effect of serotonin in the vessels with impaired endothelium due to advanced atherosclerosis is not masked (24). In addition, ADMA inhibits NO and

reduces vascular compliance, increase vascular resistance, and limit blood flow (7). It is shown that angiogenic growth factors stimulate NO release (24) and require NO in order to perform angiogenic functions (17,25,26). Jacobi et al. (27) have demonstrated that the inhibition of ADMA has increased angioadaptation against ischemic and inflammatory stimulants in mice and angiogenesis and arteriogenesis has recovered.

In the present study, ADMA value before and after the iloprost treatment in peripheral artery patients is decreased after the treatment and while this was statistically significant (p=0.001), and NO (P= 0.16) values are found statistically insignificant. According to the study of Hara et al., it is emphasized that the level of whole blood plasma serotonin can be a marker for atherosclerosis (19). In the studies conducted, the treatment of cancer in atherosclerotic patients does not reduce atherosclerotic vascular complications, but is effective in reducing mortality (24,28,29). In this study, it is seen that the values before the iloprost treatment (81.15 ± 54.40) and after the iloprost treatment (80.16 ± 45.58) are statistically insignificant (p = 0.82).

This study has investigated the efficacy of parameters related to the endothelial functions such as ADMA, Serotonin and NO in iloprostin circulation in the patients using iloprost due to peripheral artery disease. As a result, it is seen that ADMA that is accepted as a good indicator of endothelial damage in peripheral arterial diseases has decreased after the iloprost treatment. It may also serve as a parameter that can be considered in the assessment of treatments in peripheral arterial disease patients. One of the problems in this study is the lack of numerical insufficiency, and the studies involving more quantities could increase the statistical power.

Limitations of Study:

The main limitation of study is related low sample size. The results of study should be confirmed with larger series. The second limitation is concerning about the elder age of study subjects. The response of iloprost infusion should be clarified in younger ages.

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Etik: Bu çalışmanın etik kurulu alınmıştır.

Ethics committee approval had been taken.

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Yazar katkı durumu; Çalışmanın konsepti; SŞ, dizaynı; SŞ, Literatür taraması; SŞ, verilerin toplanması ve işlenmesi; SŞ, istatistik; SŞ, yazım aşaması; SŞ,

Author contribution status; The concept of the study; SŞ, design; SŞ, literature review; SŞ, collecting and processing data; SŞ, statistics; SŞ, writing phase; SŞ,

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