Kafkas Üniv Vet Fak Derg
14 (2): 169-172, 2008
DOI:10.9775/kvfd.2008.35-A

Plasma Nitric Oxide Levels after Injection of Therapeutic Dose of Methylprednisolone Acetate in Rabbits

Metehan UZUN* ✎ Mehmet ÇİTİL** Onur ATAKİŞİ***
Erdoğan UZLU** Kürşad YAPAR**** Hidayet Metin ERDOĞAN**

* Department of Physiology, Faculty of Veterinary Medicine, University of Kafkas, Kars, TÜRKİYE
** Department of Internal Medicine, Faculty of Veterinary Medicine, University of Kafkas, Kars, TÜRKİYE
*** Department of Biochemistry, Faculty of Veterinary Medicine, University of Kafkas, Kars, TÜRKİYE
**** Giresun University, Health Vocational School, Giresun, TÜRKİYE

Yayın Kodu (Article Code): 2008/35-A

Summary

The methylprednisolone asetate (MPA) is an important and long acting member of synthetic glucocorticoids (GC). Although the suppressive effect of GC especially dexamethasone on nitric oxide (NO) synthesis is well known, the effect of MPA on NO production is not fully documented. This study evaluated changes in plasma NO levels before and on day 1, 4 and 7 after injection of two different therapeutic dose of methylprednisolone asetate (MPA; Depo-medrol) in healthy rabbits. The MPA was injected intramuscularly at 2.5 (Group I, n=6) and 5.0 (Group II, n=7) mg/kg doses. Blood samples were collected from auricular vein before and on day 1, 4 and 7 after injections and the NO levels were determined. MPA treatment decreased NO values on day 4 ($P<0.001$) in both groups. In conclusion, the role of therapeutic dose of long acting MPA administration on the decreased plasma NO levels should be considered by clinicians.

Keywords: *Methylprednisolone asetate, Nitric oxide, Rabbit*

Tavşanlarda Tedavi Dozunda Metilprednizolon Asetat Enjeksiyonunu Sonrası Plazma Nitrik Oksit Düzeyleri

Özet

Metil prednizolon asetat (MPA) sentetik glukokortikoitler (GC) grubuna üye önemli ve uzun etkili bir ajandır. Diğer bir sentetik GC olan dekzametazonun nitrik oksit (NO) sentezi üzerine olan baskılayıcı etkisi çok iyi bilinmesine rağmen MPA'nın etkisi tam olarak ortaya konulmamıştır. Bu araştırma ile sağlıklı tavşanlara tedavi dozlarında uygulanan MPA (depo-medrol)'nın enjeksiyon öncesi ve enjeksiyon sonrası 1, 4 ve 7. günlerdeki kan NO düzeylerindeki değişimlerin belirlenmesi amaçlanmıştır. Araştırmada iki farklı deney grubu oluşturulmuş ve 2.5 (Grup I, n=6) ve 5.0 (Grup II, n=7) mg/kg MPA kas içi enjekte edilmiştir. Enjeksiyon öncesi (0. gün) ve enjeksiyon sonrası 1, 4 ve 7. günlerde kulak venasından alınan kan örneklerinde NO düzeyleri belirlenmiştir. MPA uygulaması sonrası her iki grupta da 4. günde NO düzeylerinin daha düşük olduğu belirlenmiştir ($P<0.001$). Sonuç olarak MPA enjeksiyonları sonrası kan NO düzeylerinde bir azalma olabileceğinden, bu durumun klinisyenler tarafından dikkate alınması gerektiği kanaatine varılmıştır.

Anahtar sözcükler: *Metilprednizolon asetat, Nitrik oksit, Tavşan*

✎ İletişim (Correspondence)
☎ + 90 474 242 68 00-1148
✉ metehanuzun@hotmail.com

INTRODUCTION

The long-term administrations of glucocorticoids (GC) are often used in human and veterinary practise and have clear benefits. However, the suppressive effect of GC on nitric oxide (NO) synthesis has been well documented. Nitric oxide is synthesized from L-arginine by a family of isoformic enzymes (eNOS, nNOS and iNOS) known as nitric oxide synthase (NOS). The eNOS is of major importance in the regulation of vascular tone and can be regarded as a vasodilatatory force that helps to maintain equilibrium with vasoconstrictor forces [1]. There was clear evidence for in vivo decrease in blood NO levels after dexamethasone treatment [2-4] and eNOS (endothelial isoform of NO) protein expression and NO levels were significantly reduced by GC in porcine arterial endothelial cell [5]. On the other hand, the decreasing of plasma NO concentration and down-regulation of eNOS have been shown one of the reason in GC induced hypertension [4,6].

The methylprednisolone asetate (MPA) is an important and long acting member of synthetic GC and can be applied as a single intramuscular injection for a week- treatment of some diseases [7]. Although, the suppressive effect of GC, especially dexamethasone on NO synthesis is well known, the effect of MPA on NO production is not fully documented. This study therefore evaluated the effect of MPA on plasma NO levels, a marker of vascular NO production.

MATERIAL and METHODS

Animals

The study involved 13 New Zaeland Rabbits of both sexes, 12-15 months old, weighing between 2-3.1 kg. Rabbits were fed (Special pelleted rabbit diet produced by Bayramoglu Yem AS, Erzurum, Turkey) ad libitum. Animals were kept at room temperature (22-25ºC) with 12:12h light: dark cycle. The Laboratory Animal Care and Use Committe of Veterinary Faculty of Kafkas University approved all experimental protocols.

Study Protocol

Animals were assigned to 2 groups and grouping was based on the dose received. The MPA (Depo-medrol, 40 mg/ml; Pharmacia and Upjohn Company, Kalamazoo, USA; Licenced by Eczacıbaşı®, İstanbul, TÜRKİYE) was injected intramuscularly to animals in Group I (n=6) and Group II (n=7) at dose level of 2.5 and 5.0 mg/kg, respectively. Blood samples were collected from auricular vein before and on day 1, 4 and 7 after injections, namely at the begining, middle and end of the therapeutic use of MPA. The concentration of nitrate and nitrite were determined from deproteinized plasma samples according to the spectrophotometric method of Miranda et al.[8]. The NO values were calculated from the total nitrite and nitrate levels.

Statstical Analysis

The NO values were compared between the groups before injection (baseline) and on day 1, 4, and 7 after injection by repeated measure of ANOVA (Turkey's t-test) using MINITAB statistical package (Version 11.2, 1996). Data are represented as mean±SEM (Standard Error of Mean).

RESULTS

The changes of NO levels were similar in the Group I and II. MPA treatment decreased NO values on day 4 and 7 of injection but only decreases observed on day 4 were statistically significant in group I and II when compared to baseline NO values ($P<0.001$).

In the group, I NO values decreased from 15.4 µmol/L (baseline) to 12.3 and 13.8 µmol/L on the 4^{th} and 7^{th} day of injection, respectively. Similarly, in Group II, a decrease in NO value from 16.4 µmol/L (baseline) to 12.7 and 14.0 µmol/L was observed on the 4^{th} and 7^{th} day after injection, respectively. The changes of NO levels are shown in *Figure 1* and *2* for both groups.

DISCUSSION

The result shown that plasma NO levels decreased on day 4 and 7 in healthy rabbits after injection of two different doses of MPA. These results are the first to demonstrate the effect of MPA, given once at long acting dose, in healthy subjects on blood NO levels.

Plasma concentration of NO values are often used as a marker of vascular NO production. The endothelial isoform of NO (eNOS) is of major importance in the regulation of vascular tone and can be regarded as a vasodilatatory force that

helps to maintain equilibrium with vasoconstrictor forces [1]. Plasma NO levels, a marker of eNOS activities, was therefore determined in the present study. The NO levels determined on day 4, and 7 of the drug administration decreased in both groups. This decrease might be attributed to reducing effect of MPA on eNOS activity in vessel endothelia [5]. Although such effect is expected it has not been disclosed in healthy subjects previously. The suppressive effect of dexamethasone, another member of GC family, on NO synthesis has been well documented. Wallerath et al.[4] have demonstrated that dexamethasone lead to substantial down-regulation of eNOS mRNA and protein and resulted in reduced NO production and in lower serum levels of NO in rats. The reduced transcription of eNOS gene and mRNA destabilization is likely reasons of eNOS down-regulation. Similarly, dexamethasone was shown to decrease eNOS mRNA expression in aortic tissue segments [2] and a dose dependent decrease in blood NO levels was observed in mice after dexamethasone treatment [3]. Similarly, MPA was shown to inhibit eNOS expression in pulmonary artery endothelial cells [5]. The natural or synthetic GC increases blood pressure and NO is an mediator for hypertension produced by GC are also well known [6,9]. Although the present study did not demonstrate the blood pressure upon MPA administration, the possible relation between NO level and blood pressure should also be taken in consideration.

In conclusion, the role of therapeutic dose of long acting MPA administration on the decreased plasma NO levels should be considered by clinicians and further studies are needed to demonstrate the variation in blood pressure during the use of MPA.

REFERENCES

1. **Leclercq B, Jaimes EA, Raij L:** Nitric oxide synthase and hypertension. *Curr Opin Nephrol Hypertens,* 11 (2): 185-189, 2002.
2. **Johns D G, Dorrance AM, Tramontini NL, Webb RC:** Glucocorticoids inhibit tetrahydrobiopterin dependent endothelial function. *Exp Biol Med,* 226 (1): 27-31, 2001.
3. **Schäfer SC, Wallerath T, Closs EI, Schmidt C, Schwarz PM, Förstermann U, Lehr HA:** Dexamethasone suppresses eNOS and CAT-1 and induces oxidative stress in mouse resistance arterioles. *Am J Physiol Heart Circ Physiol,* 288 (1): H436-444, 2005.
4. **Wallerath T, Witte K, Schäfer SC, Schwarz PM, Prellwitz W, Wohlfart P, Kleinert H, Lehr HA, Lemmer B, Förstermann U:** Down-regulation of the expression of endothelial NO synthase is likely to contribute to glucocorticoid-mediated hypertension. *Proc Natl Acad Sci,* 96 (23): 13357-13362, 1999.
5. **Muzaffar S, Shukla N, Srivastava A, Angelini GD, Jeremy JY:** Sildenafil citrate and sildenafil nitrate (NCX 911) are potent inhibitors of superoxide formation and gp91phox expression in porcine pulmonary artery endothelial cells. *Br J Pharmacol,* 146 (1): 109-117, 2005.
6. **Whitworth JA, Schyvens CG, Zhang Y, Andrews MC, Mangos GJ, Kelly JJ:** The nitric oxide system in glucocorticoid-induced hypertension. *J Hypertens,* 20 (6): 1035-1043, 2002.
7. **Ungemach FR:** Pharmaka zur Beeinflussung von Entzündungen. **In,** Löscher W, Ungemach FR, Kroker R (Eds): Grundlagen der Pharmakotherapie bei Haus-und Nutztieren. 325-322, Verlag Paul Parey, Berlin und Hamburg, 1994.
8. **Miranda KM, Espey MG, Wink DA:** A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide,* 5 (1): 62-71, 2001.
9. **Mitchell BM, Webb RC:** Impaired vasodilation and nitric oxide synthase activity in glucocorticoid-induced hypertension. *Biol Res Nurs,* 4 (1): 16-21, 2002.

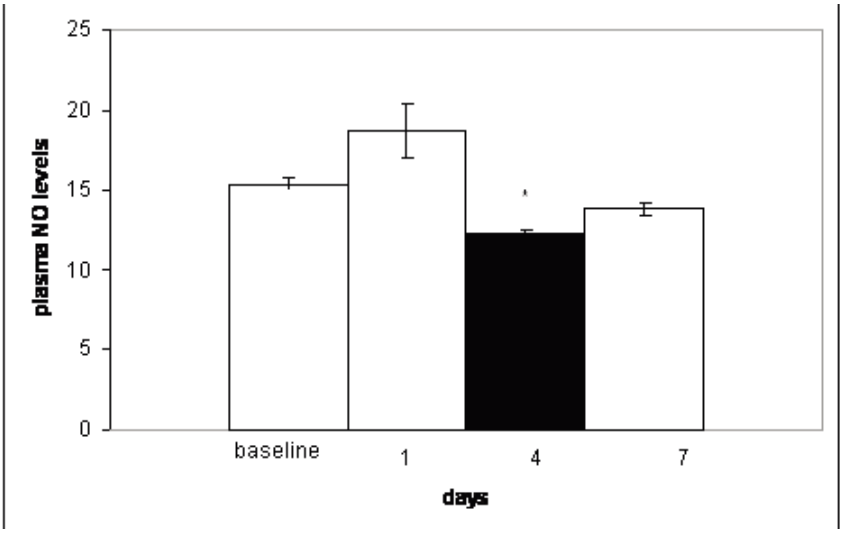

Fig. 1. Effect of the 2.5 mg/kg methylprednisolone acetate (Depo-Medrol) injection on blood nitric oxide (NO) levels in rabbits (n=6). * P<0.001, statistically significant difference compared with baseline values (0. day), all data are means±SEM.

Şekil 1. Tavşanlara (n=6) 2.5 mg/kg dozunda metilprednisolon asetat (Depo-Medrol) uygulamasının kan nitrik oksit (NO) düzeylerine etkisi. *P<0.001, değerler başlangıç değerleriyle (0. gün) karşılaştırıldığında istatistiksel olarak anlamlı düzeyde farklıdır, bütün veriler ortalama±standart hata olarak gösterildi.

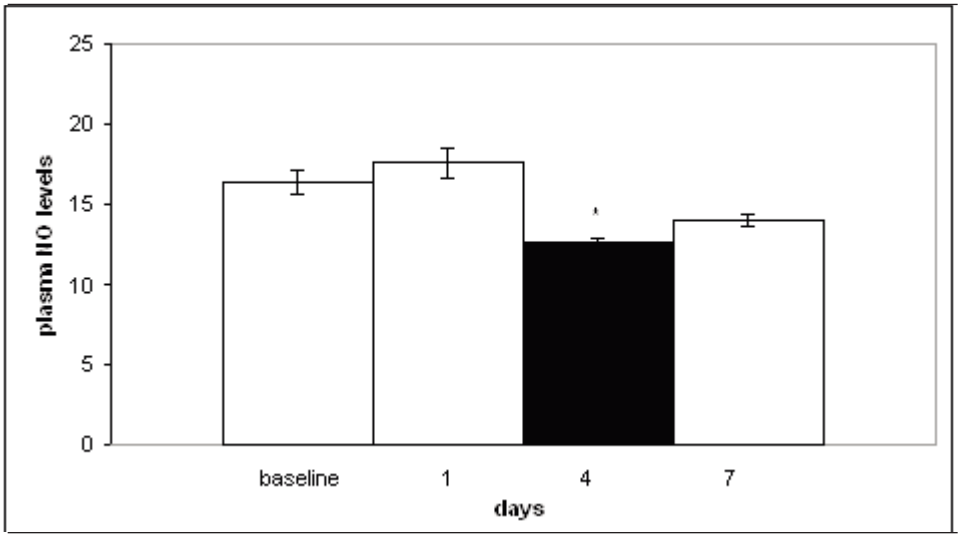

Fig. 2. Effect of the 5 mg/kg methyl-prednisolone asetate (Depo-Medrol) injection on blood nitric oxide (NO) levels in rabbits (n=7). * P<0.001, statistically significant difference compared with baseline values, all data are means±SEM.

Şekil 2. Tavşanlara (n=7) 5 mg/kg dozunda metilprednisolon asetat (Depo-Medrol) uygulamasının kan nitrik oksit (NO) düzeylerine etkisi. * P<0.001, değerler başlangıç değerleriyle (0. gün) karşılaştırıldığında istatistiksel olarak anlamlı düzeyde farklıdır, bütün veriler ortalama±standart hata olarak gösterildi.