The Effects of Low pH on Nitric Oxide (NO) Synthesis From Human Monocyte Derived Macrophages*

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✔ The aim of this study was to asses the effects of environmental pH changes to acidic range on nitric oxide (NO) synthesis from macrophages.
Human monocyte derived macrophages were obtained from peripheral blood of healthy human volunteers by using Ficoll-Hypaque density gradient method. Isolated monocytes were seeded into tissue culture flasks and incubated at 37°C in a 5 %CO_2 humidified incubator for 7 days in RPMI (Roswell Park Memorial Institute)-1640 medium with 10% Fetal-calf serum (FCS). Macrophages were then harvested and seeded in 96-well plates at 10^5 cells/well. RPMI 1640 containing 10% FCS at three different pH ranges of 7.4, 7.0 and 6.8 was added to all wells. Samples from each well were taken for determining nitrite concentration at 3, 6, 24 and 48 hours time.
Substitution of the medium of adherent macrophages with media at different pH modified NO production as reflected by changes in nitrite accumulation; as the pH became more acidic, more nitrite was detected in the culture media. The results indicate that the state of pH to which macrophages exposed to causes significant improvement in NO synthesis.
Key words: *Nitric oxide, Macrophage, pH*

✔ **İnsan Monosit-Derive Makrofajların Nitrik Oksit (NO) Sentezine Düşük pH'ın Etkileri**
Çalışmada, asidik karakterde çevresel pH değişikliklerinin makrofajların nitrik oksit (NO) sentezine etkilerinin araştırılması amaçlanmıştır.
İnsan monosit derive makrofajlar sağlıklı gönüllülerin periferal kanlarından Ficoll-Hypaque density graident metod kullanılarak elde edilmiştir. İzole edilen monositler doku kültür flasklarına dağıtıldıktan sonra %10 fetal-calf serumlu (FCS) RPMI (Roswell Park Memorial Institute)-1640 besiyerinde 7 gün 37°C ve %5 CO_2 içeren ortamda inkübe edilmiştir. Oluşan makrofajlar 96 kuyucuklu plaklara 10^5 hücre/kuyucuk olacak şekilde dağıtılmıştır. Tüm kuyucuklara pH 7.4, 7.0 ve 6.8 olan %10 FCS'lu RPMI 1640 ilave edilmiştir. 3, 6, 24 ve 48 saat sonra kuyucuklardan alınan örneklerdeki nitrit konsantrasyonları tespit edilmiştir.
Farklı pH'daki besiyerleri ile adherent makrofajların besiyerleri değiştirildiğinde NO üretiminin bir göstergesi olan nitrit birikiminde değişiklik olmaktadır. pH daha asidik olunca kültür ortamında daha çok nitrit tespit edilmektedir. Sonuçlar göstermektedir ki. makrofajların maruz kaldığı pH'ın durumu NO sentezinde belirgin bir artışa neden olmaktadır.
Anahtar kelimeler: *Nitrik oksit, makrofaj, pH*

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INTRODUCTION

Macrophages are actively phagocytic cells capable of ingesting and digesting exogenous antigens such as whole microorganisms, insoluble particles, injured and dead host cells, cellular debris, and activated clotting factors[1]. Stimulation of macrophages with endotoxin and/or cytokines is responsible for the expression of the inducible isoform of nitric oxide synthase (iNOS). Because macrophages are exposed to low pH within the microenvironment of inflammatory lesions[1,2].

Recent evidence indicates that nitric oxide (NO) may play a part in acute and chronic inflammation. Treatment with inhibitors of NO synthase reduces the degree of inflammation in rats with acute inflammation or adjuvant arthritis. NO is likely to have a multifaceted role in inflammatory reactions, ranging from the enhancement of vasodilatation and the formation of edema[3].

Acidosis is a hallmark of both ischemia and inflammation processes. The decrease of pH in tissue ischemia is secondary to the release of H^+ during ATP hydrolysis and to the accumulation of CO_2. The acidic environment in inflammatory lesions and abscesses is due to increased metabolic acid generation during cell activation. This originates primarily from the hexose monophosphate shunt, by the dissociation of hydrated CO_2[2].

In most cases, acidosis occurs along with NO generation. In ischemia, NO generation is due to in one part to the acidification and reduction of the large pool of nitrite present within tissue[2]. In inflammatory processes, when macrophages are activated with bacterial lipopolysaccharide (LPS), muramyl dipeptide or cytokines such as tumor necrosis factor α (TNFα) and interferon-γ (IFN-γ), they begin to express high levels of NO. Because they causes the expression of the inducible isoform of NO synthase (NOSII or iNOS) that is responsible for high output production of NO[1,2].

The aim of this study was to asses the effects of pH changes to acidic range on NO synthesis from macrophages.

MATERIALS AND METHODS

Human Monocyte-Derived Macrophages

Human monocyte-derived macrophages were obtained by using Ficoll-Hypaque density gradient method[4]. They were obtained from the blood of healthy human volunteers. Isolated monocytes overlaid in tissue culture flasks and RPMI (Roswell Park Memorial Institute)-1640 (Sigma) with 10% Fetal Calf serum (FCS) (Sigma) was added and monolayers were incubated at 37°C, in a 5% CO_2 humidified incubator for 7-12 days. The medium was replaced every 3 days[5-8].

Macrophage monolayers were removed from the surface of culture flasks by treating with ice-cold 0.02% EDTA/PBS for 10 min and by using cell scrapper[4]. Macrophages were seeded in 96-well plates as to be 10^5 cells/ml. The pH of the culture medium adjusted by addition of HCl to pH ranges 7.4, 7.0 and 6.8. The culture supernatants from each well were taken for determining nitrite concentration at 3, 6, 24 and 48 hours time and assayed for nitrite concentration. Each sample points were assessed in five wells.

Measurement of NO production

NO production was assayed indirectly by measuring nitrite production. The amount of NO induced in culture supernatants was quantitated by the Griess reagent method. Briefly, equal volumes of supernatants and the Griess reagent (1% sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride, 2.5% H_3PO_4) (50 µl each) were mixed in

the inducible isoform of iNOS. Because macrophages are exposed to low pH within the microenvironment of inflammatory lesions, the potential role of low pH as an additional regulator of iNOS was investigated[10]. In this study; we found that low pH stimulates NO synthesis by macrophages.

Bellocq et al[2] and Baud et al[10] observed that exposure of macrophages to acidic microenvironment in inflammatory lesions leads to the up-regulation of iNOS activity through the activation of nuclear factor-kappa B (NF-κB). Bellocq et al found that nitrite concentration was high between pH 7.2-6.8 than pH 7.6 and 7.4. Our results were also similar.

Baud et al[10] explain that substitution of the culture medium of rat peritoneal macrophages at pH 7.4 with medium at pH 7.0 up-regulated iNOS activity, as reflected by a 2.5-fold increase in nitrite accumulation. The increase in iNOS activity are associated with a similar increase in iNOS mRNA expression. Low environmental pH-induced iNOS gene expression involved the activation of NF-κB transcription factor since exposure of macrophages to low environmental pH increased NF-κB binding activity in the nucleus, and treatment of macrophages with pyrrolidine dithiocarbamate or n-acetyl-leucinyl-norleucinal, two drugs preventing NF-κB translocation to the nucleus, canceled low pH-induced nitrite accumulation.

Pedoto et al[11] shown that their results have profound implications on the role of acidosis on NO production and lung injury during sepsis.

Hypoxia, LPS, and cytokines, low environmental pH causes amplification of NO synthesis in inflammatory tissues[2]. The present results also indicated that the state of pH to which macrophages, exposed to causes significant improvement in NO synthesis.

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