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Role of Inflammation in 20-HETE Regulation of Ischemia-Induced Angiogenesis

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Abstract

Objective: 20-Hydroxyeicosatetraenoic acid (20-HETE), an important bioactive lipid metabolite, has recently been identified to be a novel contributor of angiogenesis secondary to ischemia. Moreover, an inflammatory response is required for the initiation of ischemic angiogenesis, in response to ischemic tissue injury. The goal of this study is to investigate the role of inflammation in 20-HETE regulation of ischemia-induced angiogenesis.

**Methods:** We first established a mouse hind limb ischemia model for immunocompetent Balb/C mice and immunodeficient NOD-SCID mice by femoral artery ligation. Groups of Balb/C and NOD-SCID mice were administered a 20-HETE synthesis inhibitor, DDMS, or saline as a solvent control. Laser Doppler perfusion imaging (LDOI) was used to visualize and quantify blood perfusion on days 0, 1, 3, 7, 14, and 21 post ligation, confirmed by microvessel density analysis. LC/MS/MS analysis was performed on day 3 post ligation on ischemic and non-ischemic controls to measure 20-HETE levels. Additionally, an antibody to lymphocyte antigen 6 complex (Ly6GC) was administered to neutralize the infiltration of neutrophils, macrophages, and monocytes. 20-HETE levels were again measured on day 3 post ligation in these mice.

**Results:** Quantification of the compensatory blood perfusion recovery post ligation by LDOI showed that immunocompetent Balb/C control mice demonstrated a normal course of the compensatory angiogenic response while NOD-SCID immunodeficient mice showed a significantly decreased response. Additionally, DDMS was shown to inhibit the compensatory response in Balb/C mice, while no inhibitory effect was observed in immunodeficient NOD-SCID mice. This observation is confirmed by a marked decrease in microvessel density in SCID mice (1.5±0.2) compared to immunocompetent Balb/C mice (2.5±0.5). As expected, ischemia markedly increased 20-HETE levels in the ischemic gracilis muscle of Balb/C mice by 6-fold (6±2 non-ischemic vs 275±18 pg/mg in ischemic), while levels in NOD-SCID mice showed no change between the ischemic and non-ischemic control. Lastly, Balb/C mice that were treated with Ly6GC neutralizing antibody exhibited significantly decreased 20-HETE levels in their ischemic gracilis muscle compared to the non-ischemic control.

**Conclusions:** Inflammation may be an essential contributor to 20-HETE regulation of ischemia-induced angiogenesis.

Introduction

20-HETE is an arachidonic acid derived eicosanoid, mainly synthesized by the enzyme cytochrome P450 (CYP450) 4A in vivo, based on the following two important findings: 1) Pharmacological 20-HETE interference increases ischemia-induced angiogenesis, 2) Inflammatory cytokines are quickly produced and immune cells are recruited to the site of ischemia when compared to immunodeficient NOD-SCID mice.

Our recent publication (Chen et al, 2016) further demonstrated that 20-HETE is a novel contributor of ischemia-induced angiogenesis in vivo based on the following two important findings: 1) Pharmacological 20-HETE interference significantly inhibited the compensatory angiogenesis secondary to ischemia, and 2) Ischemia markedly stimulated the production of 20-HETE in the hindlimb gracilis muscle where angiogenesis is taken place. The precise cellular origin of the increased 20-HETE and the molecular mechanism underlying 20-HETE regulation in ischemia-induced angiogenesis was unknown.

After ischemic injury, inflammatory cytokines are quickly produced and immune cells are recruited to the site of injury. The first inflammatory cells to arrive at the site of injury are neutrophils, which then recruit macrophages through the release of additional cytokines (Parham, 2014). Thus, inflammatory cells play an important role in mediating ischemia-induced angiogenesis.

In the current study, we aim to determine the role of inflammation in 20-HETE regulation of ischemia-induced angiogenesis. We hypothesized that the inflammatory response may contribute to increased 20-HETE that regulates ischemia-induced angiogenesis in vivo.

Methods

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Results

**Introduction**

20-HETE is an arachidonic acid derived eicosanoid, mainly synthesized by the enzyme cytochrome P450 (CYP450) 4A and 4F. Previous studies by our group (Chen et al., 2014; Guo et al., 2011; Guo et al., 2007) have demonstrated that 20-HETE regulates both endothelial cell (EC) and muscle progenitor cell (MPC) functions that are associated with angiogenesis. Our recent publication (Chen et al., 2016) further demonstrated that 20-HETE is a novel contributor of ischemia-induced angiogenesis in vivo based on the following two important findings: 1) Pharmacological 20-HETE interference significantly inhibited the compensatory angiogenesis secondary to ischemia, and 2) Ischemia markedly stimulated the production of 20-HETE in the hindlimb gracilis muscle where angiogenesis is taken place. The precise cellular origin of the increased 20-HETE and the molecular mechanism underlying 20-HETE regulation in ischemia-induced angiogenesis was unknown.

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Conclusion

Inflammation may be an essential contributor to 20-HETE regulation of ischemia-induced angiogenic response.

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