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

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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 control. Laser Doppler perfusion imaging (LDPI) was used to visualize and quantify blood perfusion on days 0, 1, 3, 7, 14, and 21 post-ligation, confirmed by microwave density analysis. LC/MS/MS analysis was performed on day 3 post ligature on ischemic and non-ischemic control gracilis muscles to measure 20-HETE levels. Additionally, an antibody to lymphocyte antigen 6 complex (Ly6C) 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 ischemia by LDPI 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 microwave density in SCID mice (21.0±2.2) post ischemia compared to immunocompetent Balb/C mice (35.0±3.2). As expected, ischemia markedly increased 20-HETE levels in the ischemic gracilis muscle of Balb/C mice by 1.6-fold (237±29 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 Ly6G/C neutralizing antibody exhibited significantly decreased 20-HETE levels in their ischemic gracilis muscle compared to the non-ischemic control.

Conclusion: Inflammation may be an essential contributor in 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 while NOD-SCID mice showed a significantly decreased response. Interestingly, we see no effect of DDMS on blood perfusion post-ischemia in NOD-SCID mice, supporting a decreased angiogenic response in this immunodeficient model. Taken together, these data strongly support a potential role of immunity in 20-HETE regulated ischemic angiogenesis.

Methods

Experiment 1: Measurement of 20-HETE Levels

Experiment 2: Laser Doppler Perfusion Imaging

Results

Figure 1A: Representative Laser Doppler Perfusion Imaging Scans for Balb/C and SCID mice are shown from days 0, 1, 3, and 21 post-ligation. Blood perfusion was used as one of the indexes of compensatory angiogenesis. Results: Ischemic muscles in Balb/C mice have a 6-fold increase in 20-HETE levels (pg/mg protein) compared to non-ischemic muscles. Balb/C mice have a significantly higher MVD than SCID mice, consistent with a decreased angiogenic response in SCID mice.

Figure 1B: Quantification of blood perfusion ratio secondary to ischemia. Blood perfusion for days 0, 3, 7, 14, and 18 were quantified and plotted for Balb/C control, Balb/C with DDMS, Balb/C with DDMS and SCID controls, and SCID DDMS groups. Results: The Balb/C controls underwent normal courses of compensatory angiogenesis as we have previously published, whereas SCID controls showed a significantly decreased compensatory response. As previously demonstrated, DDMS decreased compensatory blood perfusion in Balb/C mice. Interestingly, we see no effect of DDMS on blood perfusion post ischemia in NOD-SCID mice. On the third day after ligation, SCID mice were shown to have an increase in blood flow, but the delta of the SCID curve is skewed by this increase. Without day 3 data, there is no change in blood flow.

Figure 2: Comparison of 20-HETE levels in hind limb gracilis muscles of Balb/C, SCID, and Ly6G/C treated Balb/C mice before and after ischemia. Femoral artery ligation was performed in Balb/C, SCID, and Balb/C mice treated with Ly6G/C antibody (i.p.). Ly6G/C targets and depletes neutrophils, macrophages, and monocytes. On day 3 post-ligation, gracilis muscles were excised from ischemic and non-ischemic hind limbs of Balb/C and SCID mice and were quantified for 20-HETE levels by LC/MS/MS analysis. 20-HETE levels were normalized to total protein. Results: Ischemic muscles in Balb/C mice have a 4-fold increase in 20-HETE compared to contralateral non-ischemic muscles, while SCID mice show no difference in 20-HETE levels between ischemic and non-ischemic muscles. Balb/C mice treated with Ly6G/C antibody showed decreased 20-HETE production post ligation in the ischemic limb. 20-HETE levels in Ly6G/C treated Balb/C mice were closer to basal levels of 20-HETE production.

Figure 3: Proposed model of ischemia induced production of 20-HETE leading to compensatory neovascularization. After injury, 20-HETE levels in ischemic tissues are increased which leads to production of angiogenic factors such as VEGF and progressive compensatory neovascularization. The source of 20-HETE is unknown, but proposed potential sources may be inflammatory cells, EC, or EPC.

Conclusion

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

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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 mobilization of progenitor (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 regulating 20-HETE regulation in ischemia-induced angiogenesis remains 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 (Palhalm, 2014). Thus, inflammatory cells play an important role in mediating ischemic 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.