Abstract: P1494

Title: THE ROLE OF INDUCIBLE NITRIC OXIDE SYNTHASE IN HYDROXYUREA IMPAIRMENT OF ANGIOGENESIS

Abstract Type: Poster Presentation

Topic: Sickle cell disease

Background:

Hydroxyurea (HU) is a chemotherapeutic acting mainly through the inhibition of ribonucleotide reductase and DNA synthesis, while nitric oxide (NO) inducer/donor properties contribute significantly to its cytostatic properties. Therapeutic effects of HU are accompanied with reduction of angiogenic factors in patients with sickle cell anemia and myeloproliferative neoplasms. We have shown that HU increases inducible nitric oxide synthase (NOS2) levels and enzymatic activity. Besides endothelial nitric oxide synthase (NOS3) with a predominant role in angiogenesis, NOS2 has been also related with proangiogenic properties.

Aims:

The aim of this study was to investigate the mediation of NOS2 in HU inhibition of angiogenesis.

Methods:

To relieve NOS2 implication in HU caused angiogenesis inhibition, we used human microvascular endothelial cells (HMEC-1) treated with HU alone or in combination with NOS inhibitors and performed Matrigel®-based vascularization assay followed by staining with vital fluorescent dye and quantification of angiogenesis by Image J. Effects of NOS2 ablation on HU-induced angiogenesis impairment were assessed by per os HU treatment of WT and Nos2-/- mice for 7 days, followed by subcutaneous injection of VEGF and heparin supplemented Matrigel®. After plugs were extracted from mice, hemoglobin concentration was evaluated by Drabkin's method. Microstructural characteristics of the plugs were further estimated by hematoxylin and eosin staining. Vessel differentiation was appraised after immunofluorescence staining against CD31, whereas novel vessels were stained using anti-CD105 antibody, a marker of actively proliferating endothelial cells.

Results:

HU treatment caused changes in endothelial cell network quantified as 36±0.86% increase in mesh number and consequently 25±0.52% decrease in the mesh size indicating diminished angiogenesis. Co-treatment with pan-selective NOS inhibitor L-NAME, or NOS2-selective inhibitor 1400W, suppressed these effects. Hemoglobin concentration was dramatically reduced after HU treatment of WT mice and Nos2 ablation was not able to rescue it since Nos2 deficiency alone decreased hemoglobin levels. Plugs from WT mice were characterized by complete endothelial ingrowth into the Matrigel® with visible round vessel-like formations that was strongly impaired by HU. Structural characteristics of the vessels were sustained in Nos2-/- mice but decreased CD105 expression was observed. However, area of CD105 fluorescence was ~ 6 times higher in HU-treated Nos2-/- mice compared to HU treated WT mice ($p < 0.01$). Expression of angiogenesis marker CD31 decreased after HU treatment but was preserved in HU-treated Nos2-/- mice.

Summary/Conclusion: HU-induced decrease in angiogenesis upon *in vitro* and *in vivo* treatment can be rescued by NOS inhibition or Nos2 knock-out, indicating the involvement of NOS2 in the molecular mechanism of HU activity.

Keywords: Hydroxyurea, Angiogenesis, Nitric oxide synthase, Nitric oxide