Systemic VEGF Inhibition Accelerates Experimental Atherosclerosis and Disrupts eNOS-mediated Endothelial Homeostasis

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\textbf{Background:} Pharmacological inhibition of \textit{vascular endothelial growth factor} (VEGF), a major mediator of angiogenesis, has become a widely accepted and effective treatment of \textit{age-related macular degeneration} (AMD). Recent meta-analyses of prospective clinical trials, however, raise concern for systemic vascular off-target effects.

\textbf{Objective:} We investigated the vascular effects and molecular mechanisms of systemic VEGF inhibition in a mouse model of atherosclerosis and a possible role of \textit{endothelial nitric oxide synthase} (eNOS) as downstream target of VEGF.

\textbf{Methods:} 8-week old male apolipoprotein E knockout mice were fed a high cholesterol diet (1.25\% w/w) for 24 weeks and were exposed to a systemic pan-VEGF-receptor inhibitor (PTK787/ZK222584, 50mg/kg/d, n=10) or placebo by gavage for the last 10 weeks. Atherosclerotic plaque formation was quantified \textit{en face} in thoraco-abdominal aortae; cellular plaque composition, proliferation, and eNOS expression were assessed by immunohistochemistry in aortic arches. Further expression analyses, endothelial proliferation, and \textit{nitric oxide} (NO) availability were assessed in cultured human aortic endothelial cells.

\textbf{Results:} \textit{In vivo} systemic VEGF inhibition increased atherosclerotic plaque formation by 33\%. Plaque-resident macrophage content was increased 2.2 fold, whereas the number of proliferating cells was decreased in the intervention group. \textit{In vitro} we observed a dose-dependent decrease in total eNOS, resulting in a reduced NO availability and endothelial proliferation with increasing doses of PTK787.

\textbf{Conclusion:} Our data provide a novel mechanism for cardiovascular off-target effects of systemic VEGF inhibition. Given the inherent increased cardiovascular risk of AMD-patients and their need for chronic therapy, cardiovascular safety should be specifically addressed in prospective clinical trials.