Epoxide hydrolase inhibition induces an angiogenic response in the chick chorioallantoic membrane

Cristian D. Valenzuela, Alexandra B. Ysasi, Janeil Belle, Robert D. Bennett, Willi Wagner, Kin Sing Lee, Amy Rand, Bodgan Barnych, Maximilian Ackermann, Akira Tsuda, Bruce D. Hammock, and Steven J. Mentzer

Abstract

Omega-6 and omega-3 polyunsaturated fatty acids are precursors to chemical mediators of angiogenesis, important in many human diseases including cancer. Respectively, epoxyeicosatetraenoic acids (EETs) lead to pro-angiogenic effects, whereas epoxydocosapentaenoic acids (EDP) are anti-angiogenic. Epoxide hydrolase catalyzes the breakdown of these endogenously active intermediates, and has been identified as a promising target for angiogenic modulatory therapy. However, in vivo effects of soluble epoxide hydrolase inhibitor (sEHI) remain largely unexplored. The chick chorioallantoic membrane (CAM) is a well-studied and accessible angiogenesis model. Here, we used the CAM at embryonic developmental day 10 to study the morphologic effects of topically applied sEHI and omega-pathway intermediates on blood vessel formation (intussusceptive angiogenesis) and vascular lengthening leading to tortuous vessels (2nd and 3rd order veins and arteries). sEHI treatment for 72 hours significantly increased the density of intussusceptive pillars (1.21 per mm² versus 0.52 per mm² in controls; p<0.0001 two-tailed Student’s t-Test), but did not increase the number, length, or degree of tortuous vessels (p=0.51, p=0.94, and p=0.33, respectively). sEHI applied together with the omega-6 pro-angiogenic intermediate 14,15-EET led to an additional marked increase of pillars (2.86 per um²; p<0.0001), as well as significantly increased tortuous vessels (0.72 per mm² versus 0.40 per mm² with sEHI alone, p<0.001). In contrast, sEHI applied with the omega-3 anti-angiogenic intermediate EDP resulted in the density of tortuous vessels being significantly lower compared to controls (0.182 versus 0.472 tortuous vessels per mm², p<0.01). The pro- and anti-angiogenic morphologic changes in response to omega-6 and omega-3 intermediates with soluble epoxide hydrolase inhibitor suggests this pathway is a potential target for therapeutic intervention in cancer and other diseases.

Support or Funding Information

The work presented here is supported by the National Institutes of Health T32 CA009535-26 and R01 HL94567.
A: Images showing vascular structures under different treatment conditions.

B: Graph showing intussusceptive pillar counts per mm² (± SEM) across different treatment groups: Control, EDP, sEHI, sEHI + EET, sEHI + EDP. Significant differences indicated by asterisks.

C: Graph showing tortuous vessel counts per mm² (± SEM) across different treatment groups: Control, EDP, sEHI, sEHI + EET, sEHI + EDP. Significant differences indicated by asterisks.
Effects of omega-3 and -6 pathway mediators on the chick chorioallantoic membrane. (A) Topical application of soluble epoxide hydrolase inhibitor (sEHI) with exogenous epoxeicosatrienoic acid (EET) of the omega-6 pathway led to pro-angiogenic morphologic changes, and sEHI with epoxydocosapentaenoic acid (EDP) of the omega-3 pathway led to anti-angiogenic changes. Significant changes were found in frequency of (B) intussusceptive pillars and (C) tortuous vessel formation (*p<0.01 or less).