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**Introduction.** Sphingosine-1-phosphate (S1P) is a lysophospholipid mediator that is known to affect diverse vascular functions such as development, remodeling and permeability acting on its G-protein coupled receptors (S1P<sub>1-5</sub>). Its role in the regulation of the vascular tone remains controversial. We aimed to examine the direct effect of S1P and the signaling mechanisms involved on vascular smooth muscle (VSM) contractility under physiological conditions and after increasing the VSM excitability by increasing extracellular K<sup>+</sup> concentration [K<sup>+</sup>]<sub>e</sub>.

**Methods.** Segments of the thoracic aorta were isolated from adult male wild type (WT), as well as S1P<sub>2</sub>, S1P<sub>3</sub> receptor, and Gα<sub>12/13</sub> knockout (KO) mice and their isometric tension was measured in myographs after removal of the endothelium. Vasoactive effect of 10 μM S1P was detected at physiological (4 mM) as well as at elevated (6-8 mM) [K<sup>+</sup>]<sub>e</sub>. Changes of the vascular tension are expressed as mean±SEM percentage of a reference contraction induced by 124 mM K<sup>+</sup>.

**Results.** At physiological [K<sup>+</sup>]<sub>e</sub> S1P slightly increased the vascular tone (21±5%), whereas sphingosine had no measurable effect. Slightly increased [K<sup>+</sup>]<sub>e</sub> (6 mM) failed to influence the vascular tone by itself, but addition of S1P induced marked vasoconstriction (39±7%) that was further increased when [K<sup>+</sup>]<sub>e</sub> was elevated to 8 mM (54±6%).

Further experiments on the signaling of vascular smooth muscle contraction were performed with 8 mM [K<sup>+</sup>]<sub>e</sub>, since it intensified the vasoactive action of S1P without having a significant influence on the resting vascular tone by itself. The vasoconstrictor effect of S1P was diminished in S1P<sub>2</sub>-KO (11±3%) and Gα<sub>12/13</sub>-KO (8±4%) vessels, whereas it remained unchanged in S1P<sub>3</sub>-KO mice (44±7%). S1P-induced vasoconstriction was also strongly decreased by the Rho-kinase inhibitor Y-27632 (10 μM) in WT vessels (17±1%).

**Conclusion.** S1P significantly elevates the VSM tone in isolated mouse aorta under moderately increased K<sup>+</sup>-concentration. This effect is mediated by the S1P<sub>2</sub>-receptor, Gα<sub>12/13</sub> proteins, and the activation of Rho-kinase. This phenomenon may contribute to the pathological increase of the vascular tone under conditions of systemic hyperkalemia or during local elevation of perivascular [K<sup>+</sup>]<sub>e</sub> (eg. due to ischemic tissue infarction).

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**Introduction:** Lipoxygenase activity has been shown to be increased in a number of cancers types and to have an involvement in tumourigenesis and angiogenesis. There is little known on the role of these enzymes in esophageal cancer, a malignancy that is reportedly the 8th most common cause of cancer globally. In this study we aim to investigate the role of 5-lipoxygenase (5LO) and platelet-type 12-Lipoxygenase (12LO) in oesophageal cancer in vitro and in vivo.

**Methods:** Esophageal adenocarcinoma cell line (OE33) were investigated for the expression of 5LO, 5LO associated protein (FLAP) and 12LO and 15-lipoxygenase (15LO), in addition to metabolite receptors BLT1, BLT2 and GPCR31 by qPCR. The effect of novel and commercial 5 and 12LO inhibitors on proliferation of OE33s by BrdU was investigated. The effect of inhibitor treatments to OE33s angiogenic gene profile was conducted using gene arrays. 12LO was over-expressed in OE33s by reverse transfection and proliferative effects were monitored using real-time xCELLigence. Additionally serum levels of LTB4 and 12(S)HETE were measured by ELISA in cancer patients samples (N=68), collected pre-surgery and compared to a small cohort of non-cancer control samples.

**Results:** OE33s were shown to constitutively express high 5LO, 12LO and FLAP but low levels of 15LO. OE33s expressed BLT1 however BLT2 and GPCR31 were not detected. While the FDA approved 5LO inhibitor Zileuton did not affect cell proliferation of OE33s, novel 5LO inhibitor, NIH4, showed a dose response effect (IC<sub>25</sub>= 10μM). A novel 12LO inhibitor NIH13 was shown to have superior anti-proliferative effects compared to commercially available 12LO inhibitor Baicalein, showing an IC<sub>50</sub> of 1μM (p<0.01). This compound was not shown to induce marked apoptosis in OE33s at 5 and 10μM doses at 24-72hr, suggesting another mechanism is responsible for the anti-proliferative effects. Neupilin-1 (NRP1) and VEGFB were found to be significantly down regulated by 5μM NIH13 treatment (P<0.05) while VEGFA was up regulated (P=0.0515). Over expression of 12LO in OE33s resulted in a significant increase in cell proliferation compared to the vector control. Addition of 12(S)HETE displayed mitogenic effects in 12LO over expressing cells and was able to suppress the effect of NIH13 treatment. There was no difference in mean LTB4 levels between the cancer and non-cancer serums. Serum 12(S)HETE was higher in 75% of cancer patients compared to baseline levels of a small non-cancer cohort (p<0.05) and 12(S)HETE levels were associated with recurrence-free survival (p<0.01).

**Conclusion:** The study demonstrates the potential of 12LO activity as biomarker of disease and highlights the potential for a novel class of LO inhibitors as anti-cancer drugs for esophageal cancer. Understanding the molecular mechanisms underlying the anti-tumor effect of these inhibitors may prove useful in the treatment of cancer, either alone or in combination with conventional therapies.

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