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Antiangiogenic effects in the modulation of inflammatory pathways. A comprehensive analysis of potential therapeutic substances

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ABSTRACT

Based on current knowledge antiangiogenic compounds are utilized in the treatment of inflammatory-depended diseases such as cancer. It has been shown that these compounds can simultaneously block several signalling pathways regulating angiogenesis. The visualization of fluorescently labelled F-actin and VE-cadherin was allowed to demonstrate that the reorganization of the cell cytoskeleton disrupts cellular processes and consequently inhibits angiogenesis.

Keywords: cell communication, inflammation, antiangiogenesis

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The study aims to assess the effect of compounds that inhibit angiogenesis on the inflammatory response of endothelial cells. The study used EA.hy926 model cells which were treated with tumour necrosis factor-alpha (TNF-α) — a pro-inflammatory cytokine, which is the main modulator of the inflammatory process, and compounds that inhibit angiogenesis [1, 2]. The experiments focused on assessing the effect of Bevacizumab, Pazopanib and KRN-633 on the organization of F-actin and the localization of vascular endothelial cadherin (VE-cadherin) using fluorescence immunostaining and confocal microscopy.

The control cells were distinguished by an organized F-actin network that promoted mutual interactions among adjacent cells and cooperation in mechanical signal transduction. In contrast, VE-cadherin did not show the same continuous membrane expression, although it was strongly expressed at cell-cell interactions and along the F-actin core of filipodia initiating adherence junctions (AJs) (Fig. 1).

After 24 hours of incubation with TNF-α, the organization of F-actin changed. Due to the changed organization of F actin, intercellular spaces were highlighted, which indicates that the studied cells have a limited barrier function. Moreover, the junctional contact between cells assessed using fluorescently labelled VE-cadherin was restricted to point interactions (Fig. 2).

The EA.hy926 cell line incubated with Bevacizumab for 24 hours promoted actin cytoskeleton swelling and complete degradation, which was visible only within vesicular structures on the surface of swollen cells. There were no observed sites of intercellular contact (Fig. 3).

In the case of the combination of TNF-α and Bevacizumab, simultaneous swelling and shrinking cells were observed. However, both of these changes were accompanied by a complete degradation of the actin cytoskeleton and a loss of endothelial layer continuity (Fig. 4).

Treatment of cells with a second antiangiogenic compound - Pazopanib - also revealed swollen cell

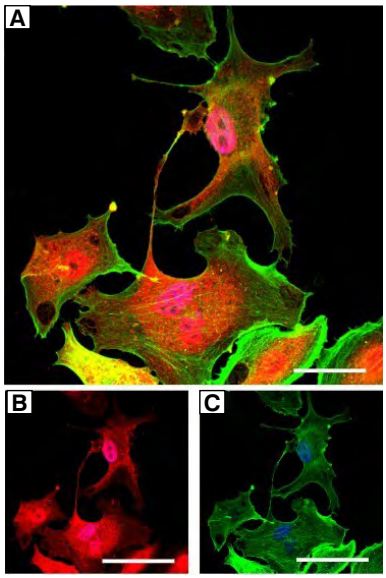

Figure 1. Representative confocal image showing F-actin (green) and VE-cadherin (red) fluorescence in control EA.hy926. Cell nuclei were labelled with DAPI (blue). A. Overlay. B. DAPI + VE-cadherin. C. DAPI + F-actin. (Magnification x100) Bar = 100μm

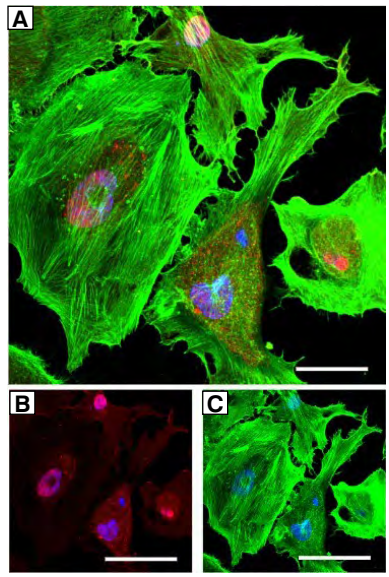

Figure 2. Representative confocal image showing fluorescence of F-actin (green) and VE-cadherin (red) in EA.hy926 treated with 100 ng/ml TNF-α. Cell nuclei were labelled with DAPI (blue). A. Overlay. B. DAPI + VE-cadherin. C. DAPI + F-actin (Magnification x 100) Bar = 100 μm

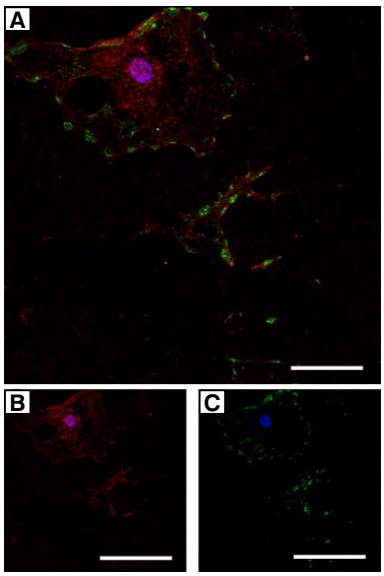

Figure 3. Representative confocal image showing fluorescence of F-actin (green) and VE-cadherin (red) in EA.hy926 treated with Bevacizumab 6.7*10-6 nmol. Cell nuclei were labelled with DAPI (blue). A. Overlay. B. DAPI + VE-cadherin. C. DAPI + F-actin. (Magnification x 100) Bar = 100 μm

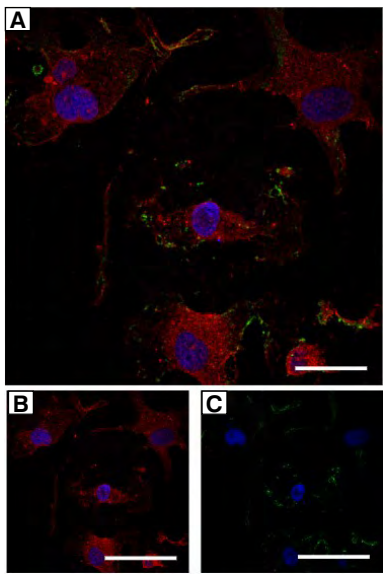

Figure 4. Representative confocal image showing fluorescence of F-actin (green) and VE-cadherin (red) in EA.hy926 treated with 100 ng/ml TNF-α and Bevacizumab 6.7*10-6 nmol. Cell nuclei were labelled with DAPI (blue). A. Overlay. B. DAPI + VE-cadherin. C. DAPI + F-actin. (Magnification x 100) Bar = 100 μm

structures, which were accompanied by significant degradation of the actin cytoskeleton and lack of intercellular interactions (Fig. 5).

The same effect was observed after further incubation of cells with the pro-inflammatory cytokine TNF-α. Nonetheless, the inflammation activity coupled with

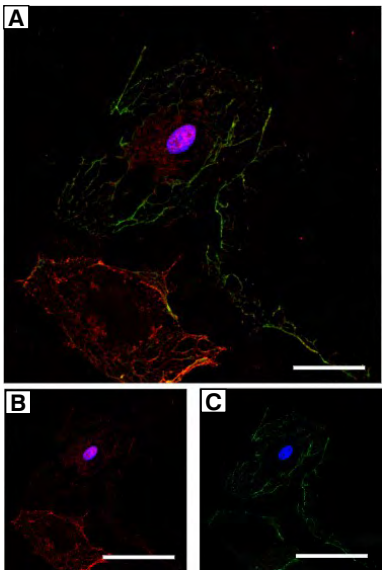

Figure 5. Representative confocal image showing fluorescence of F-actin (green) and VE-cadherin (red) in EA.hy926 treated with Pazopanib 1*10-5 nmol. Cell nuclei were labelled with DAPI (blue). **A.** Overlay. **B.** DAPI + VE-cadherin. C. DAPI + F-actin. (Magnification x 100) Bar = 100 μm

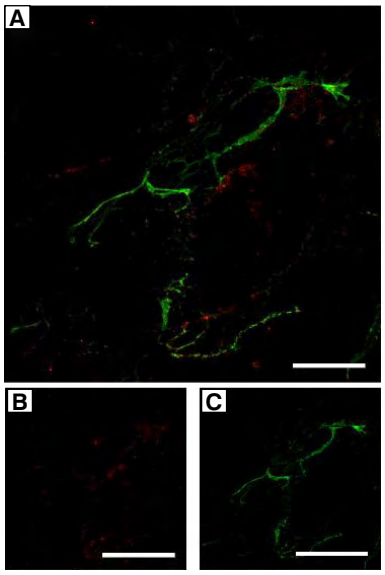

Figure 6. Representative confocal image showing fluorescence of F-actin (green) and VE-cadherin (red) in EA.hy926 treated with 100 ng/ml TNF-α and Pazopanib 1*10-5 nmol. Cell nuclei were labelled with DAPI (blue). **A.** Overlay. **B.** DAPI + VE-cadherin. **C.** DAPI + F-actin. (Magnification x 100) Bar = 100 μm

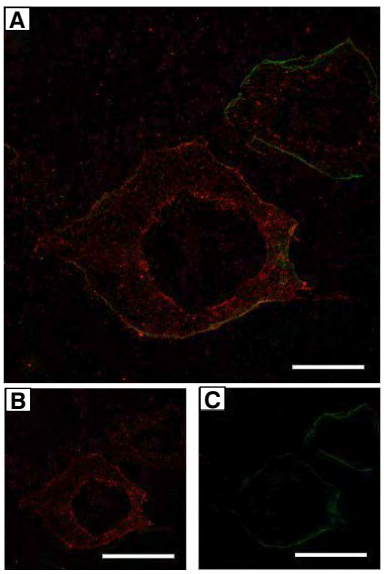

Figure 7. Representative confocal image showing fluorescence of F-actin (green) and VE-cadherin (red) in EA.hy926 treated with KRN-633 1.7*10-4 nmol. Cell nuclei were labelled with DAPI (blue). **A.** Overlay. **B.** DAPI + VE-cadherin. **C.** DAPI + F-actin. (Magnification x 100) Bar = 100 μm

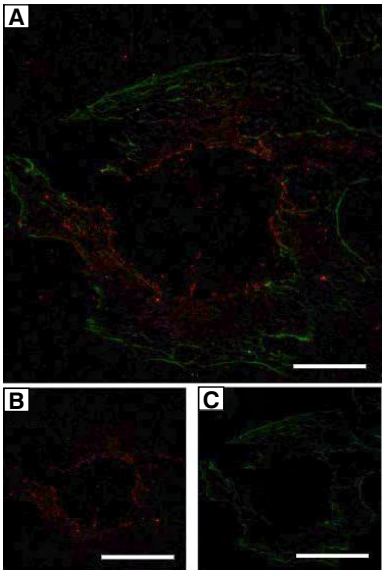

Figure 8. Representative confocal image showing the fluorescence of F-actin (green) and VE-cadherin (red) in EA.hy926 treated with 100 ng/ml TNF-α and KRN-633 1.7*10-4 nmol. Cell nuclei were labelled with DAPI (blue). **A.** Overlay. **B.** DAPI + VE-cadherin. C. DAPI + F-actin. (Magnification x 100) Bar = 100 μm

the antiangiogenic properties of Pazopanib resulted in a complete reduction in the fluorescence intensity of VE-cadherin (Fig. 6).

Finally, the complete disintegration of the cytoskeleton was caused by the application of KRN-633 cells. Incubation for 24 hours both in only

compound presence and in combination with TNF-α showed a visible decrease in the level of fluorescence of actin filaments and intercellular junctions (Fig.7, Fig. 8).

The findings presented in this paper indicate that the drugs employed in the study to inhibit angiogenesis predominantly exert antiproliferative effects, triggering cell survival processes, and also exerting a lethal effect on EA.hy926 cells.

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