THE EFFECT OF ACETYLSALICYLIC ACID ON ANGIOGENESIS *IN VITRO*

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Abstract

Angiogenesis, the formation of new blood vessels, is an essential aspect of, among others, embryonic development, wound healing and the female reproductive cycle. It is also necessary for the expansion of tumour masses beyond a minute volume. Acetylsalicylic acid (ASA) is a non-steroidal anti-inflammatory drug with additional antitumour activity. We tested ASA for its ability to inhibit angiogenesis in a simplified angiogenesis model, hASC+HUVEC co cultured in vitro, using immunocytochemical staining with fluorescence-marked antibodies and observation of tubule-like structures and their branching under a fluorescence microscope. We confirmed that ASA is an efficient and useful angiogenesis inhibitor and deserves further attention. We intend using the designed angiogenesis model and the methods described for observing changes in angiogenesis after anti tumour photodynamic therapy (PDT), and also for enhancing PDT efficiency by addition of angiogenesis inhibitors.

Keywords

angiogenesis, acetylsalicylic acid, hASC+HUVEC co-culture

Introduction

Angiogenesis is the formation of new blood vessels. It is a multistep process, regulated by an interplay of pro- and anti-angiogenic factors involving endothelial cell proliferation, migration, differentiation, and tubule formation, as well as stabilization of newly-formed blood vessels. Angiogenesis is a critical aspect of essential physiological processes such as in embryonic development, wound healing, and the female reproductive cycle, as well as in pathological processes such as in tumor development, macular degeneration, rheumatoid arthritis, ischemic diseases, endometriosis and psoriasis [1-4].

Investigation of angiogenic mechanisms require assays that simulate the key steps in angiogenesis and provide tools for assessing the efficacy of therapeutic agents that either upregulate or down-regulate specific angiogenic mechanisms. The evaluation of factors that affect angiogenesis would optimally be studied *in vivo* due to complex interactions during angiogenesis. Despite the advantage of providing more information on complex cellular and molecular interactions compared to *in vitro* models, animal models have

several disadvantages (such as variability, animal-specificity, and ethics). For this reason, human cell based assays *in vitro* would have more validity and extrapolation to humans. Although tubule formation *in vitro* does not cover the whole angiogenesis process, it effectively imitates the key steps (migration and differentiation of endothelial cells).Moreover, *in vitro* angiogenesis assays provide an opportunity to investigate angiogenic mechanisms and assess the efficacy of therapeutic agents with speed and simplicity that cannot be achieved using *in vivo* assays. The search for anti-angiogenic agents (mainly for the treatment of cancer) is particularly important at the current time. [1,3,4,5]

Acetylsalicylic acid (ASA) is one of the non-steroidal anti-inflammatory drugs (NSAIDs), widely used for the treatment of acute and chronic pain and inflammation. Studies [6-14] have shown that NSAIDs can reduce the risk of many types of cancer suggesting that these drugs may possess tumour-suppressive activity. Recent advances made in a number of laboratories have provided strong evidence that the anti-tumour activity of NSAIDs is associated with suppression of tumour angiogenesis. The formation of a vascular network in the tumour stroma (tumour angiogenesis) plays a critical role in tumour progression and metastasis formation. The absence or destruction of tumour-associated vasculature leads to tumour death via anoxia and lack of nutrients. Hence, inhibition of angiogenesis by NSAIDs may be an important way of combating cancer. [3,15,16]

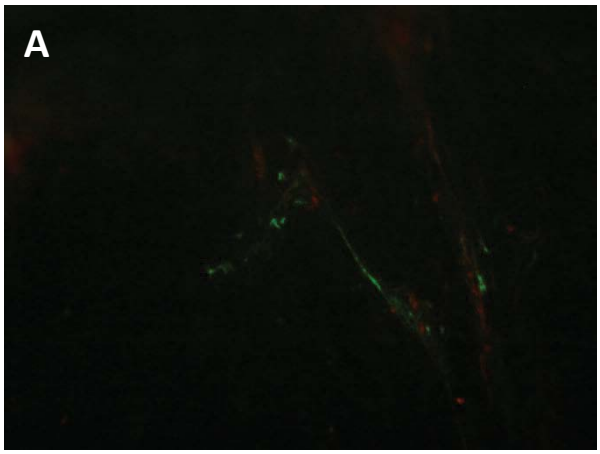
Experiments

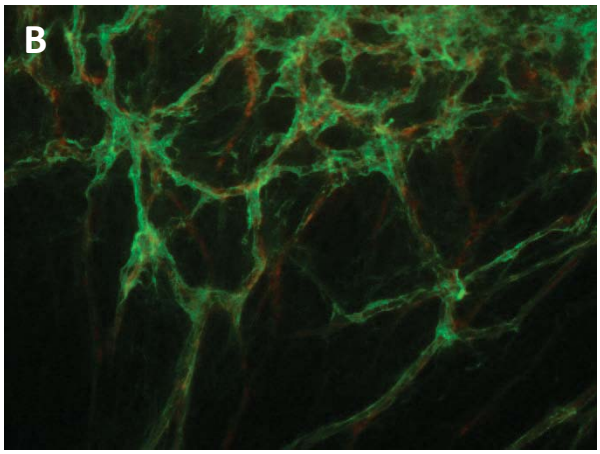
Our aim was to test ASA for its ability to inhibit angiogenesis on a simplified and improved angiogenesis model *in vitro* (designed by the scientific group from FICAM).

For our experiments, we used cell lines hASC (human adipose stem cells, 22 000 cells/well) and HUVEC (human umbilical vein endothelial cells, 4 000 cells/well) and treated hASC+HUVEC co-culture by acetylsalicylic acid (ASA) in a concentration 2, 1, 0.5, 0.25 and 0.125 mmol/l. Further, we stained samples with a staining cocktail of 2 primary antibodies (anti-von Willebrand factor, anti-Collagen) and subsequently by a staining cocktail of 2secondary antibody (anti-rabbit IgG TRITC, anti-mouse IgG FITC). We then observed tubule-like structures and their branching under a fluorescence microscope Nikon Eclipse Ti and software NIS-Elements F 3.0.

Fig 1B shows many tubules in the positive control, grown in the presence of growth factors and no tubules in the negative control grown in simple media without growth factors (Fig 1A).In the sample treated with the highest used ASA concentration, 2mM, fewer vessels can be seen(Fig 1F) whereas the sample with the lowest used ASA concentration, 0.125mM, (Fig 1C) looks similar to the positive control. Thus, 2 mM ASA appears to be effective but 0.125mM was not sufficient to inhibiti angiogenesis.

We confirmed our prediction that ASA (mainly in a concentration of 2 mmol/l) is an efficient angiogenesis inhibitor and deserves our further attention.





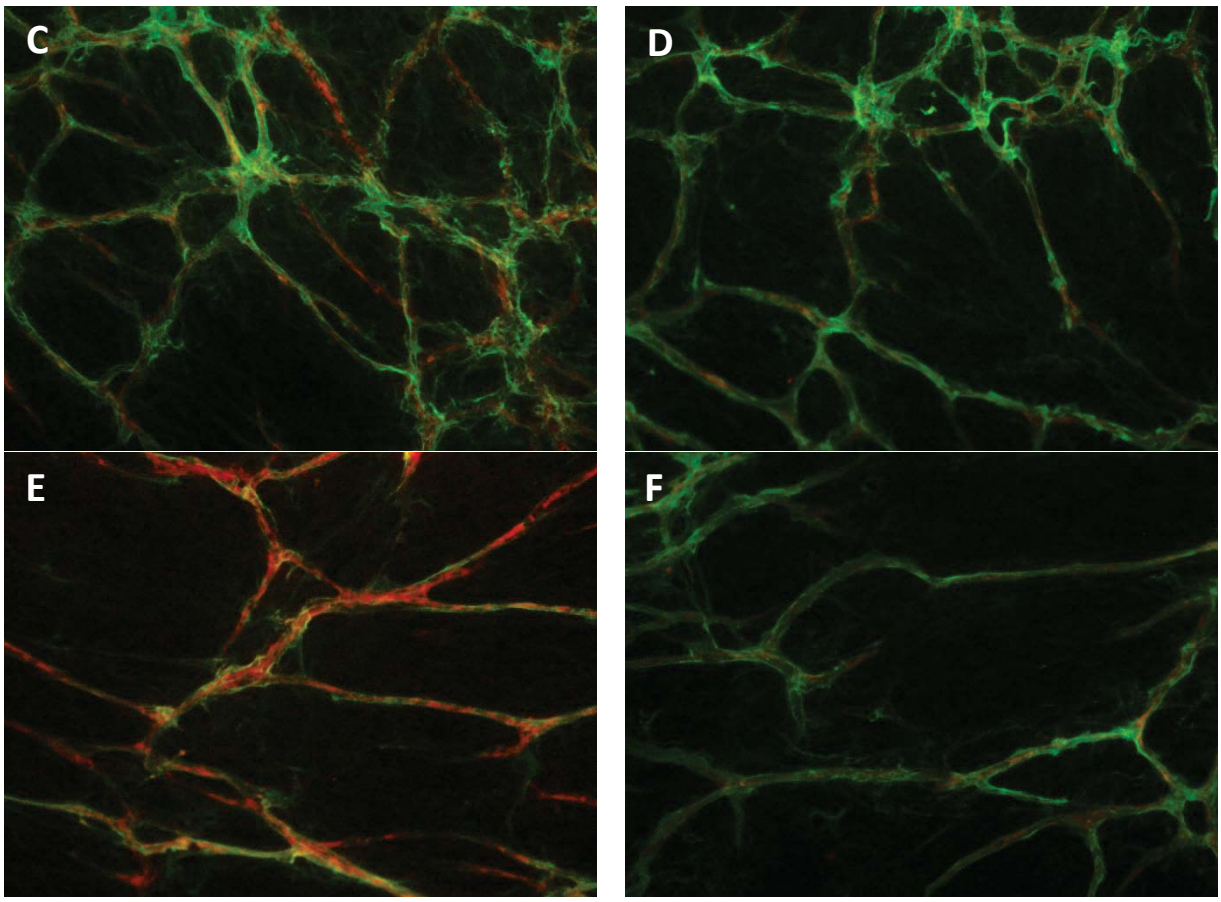


Fig. 1: Formation of tubule-like structures by hASC+HUVEC co-culture incubated in (A) simple medium without growth factors (B) stimulation medium with growth factors VEGF and FGFβ (C) stimulation medium with growth factors and 0.125mM ASA (D) stimulation medium with growth factors and 0.25mM ASA (E) stimulation medium with growth factors and 0.5mM ASA (F) stimulation medium with growth factors and 1mM ASA (G) stimulation medium with growth factors and 2mM ASA. Magnitude 100x.

Conclusion

Our group focus on testing photoactive substances called photosensitizers *in vitro*which in combination with visible light (photodynamic therapy - PDT) destroy cancer cells [17-27]. Search for and developmentof more efficient antitumour therapies is of paramount importance and PDT in particular is attracting much attention owing to its exceptional selectivity and specificity.Angiogenesis plays a critical role in tumour progression. Moreover,it is known that PDT combined with some photosensitiízers (such as Photofrin, Verteporfin, telaporfin, 5-aminolevulinic acid, NPe6, phthalocyanines and others) induces, besides direct cytotoxic effects, destruction of tumour-associated vasculature which can lead to tumour death via lack of oxygen and nutrients [28, 29].

It would be very interesting and useful to combine the experiences of both scientific groups and use the designed angiogenesis model for observing changes in angiogenesis, a major factor in cancer development, after PDT, and for enhancing PDT efficiency by addition of angiogenesis inhibitors.In the future, PDT in combination with antiangiogenic agents such as ASA will offer promising alternatives to currently used treatmentapproaches for malignant tumours, namely, radiotherapy, chemotherapy and surgery.

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