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In vitro study on the effects of inhibitors of angiogenesis in atherosclerosis

Introduction

- Atheromatous plaques can be classified in two main types: stable and unstable.
- Unstable plaques often show plaque angiogenesis and are prone to rupture, promoting thrombus formation which can lead to a myocardial infarction, stroke, and sudden death[1].
- Inhibition of intraplaque angiogenesis is a strategy to reduce atherosclerotic plaque size and stabilize the plaques.

AIM OF THE CURRENT PROJECT

Use anti-angiogenic strategies to prevent intraplaque angiogenesis studying the effects of the **inhibition of bFGF (using K5) and PFKFB3 (using AZ33)** on plaque angiogenesis.

INHIBITION OF bFGF

K5 is a molecule able to bind the angiogenic growth factor bFGF, to block its interaction with the receptors on the surface of endothelial cells and block its biological activity. The anti-angiogenic effects of this compound were measured as the ability to stimulate the proliferation and migration of ECs.

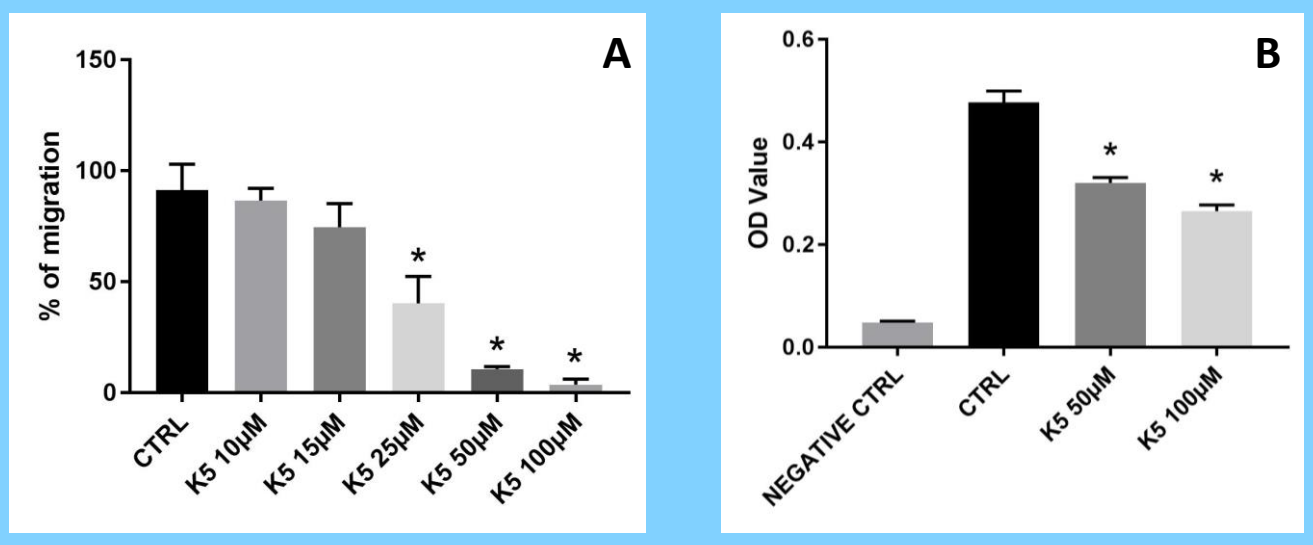
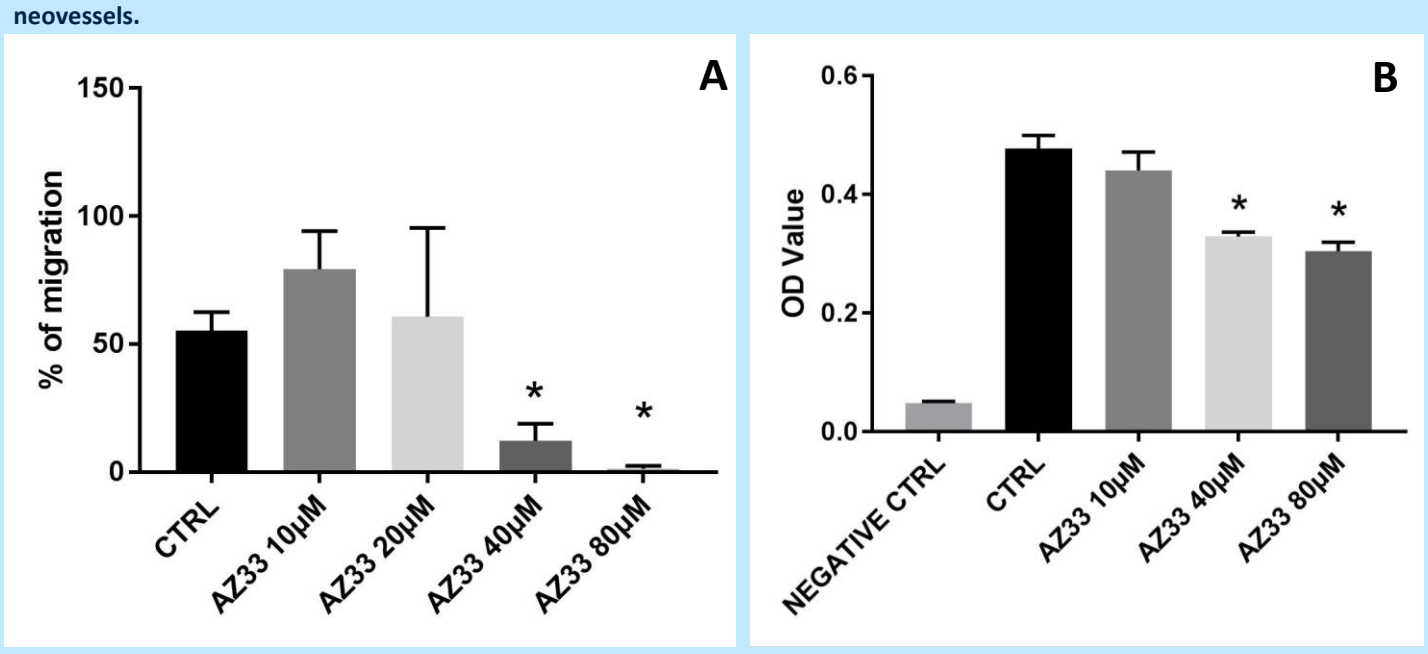


Figure A. **K5** is able to reduce migration of ECs cells in a scratch migration assay and **Figure B.** is also able to reduce proliferation of ECs measured in an MTT assay.

INHIBITION OF GLYCOLYSIS

In angiogenesis endothelial cells rely of glycolysis and PFKFB3 is a key enzyme in this metabolic pathway. AZ33 is a selective inhibitor of the kinase part of the PFKFB3 enzyme, a key regulator of the glycolytic process. The effects on the inhibition of glycolysis in ECs was evaluated by measuring their proliferation and migration capacity, as well as their ability to form neovessels.



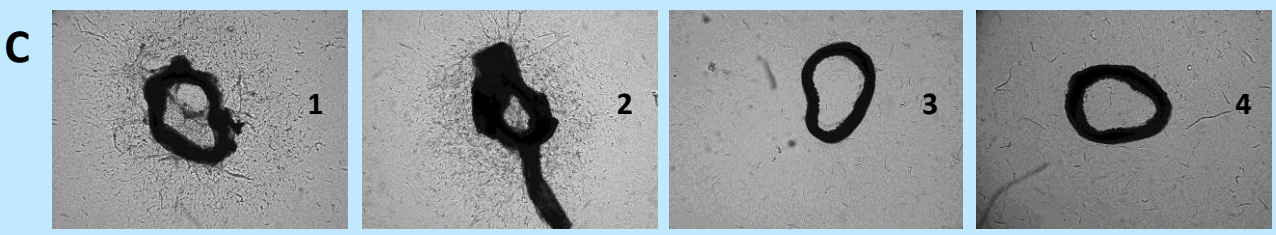
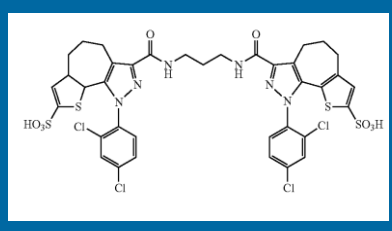


Figure A. In a scratch migration assay using H5V cells, **AZ33** resulted able to inhibit the capacity of ECs to migrate and in **Figure B.** resulted also able to inhibit ECs proliferation evaluated as the OD resultant value of an MTT assay. **Figure C.** In an aortic ring assay AZ33 inhibited the formation of neovessels at concentrations of 40µM (**Figure C 3**) and 80 µM (**Figure C 4**) compared to the control (**Figure C 1**), while lower concentration didn't show any effect (**Figure C 2**).

MATERIAL AND METHODS

- **Synthesis of K5, a bFGF inhibitor:**



- **Scratch migration assay**: to evaluate the migration of ECs
- **MTT assay**: to evaluate the proliferation of ECs
- **Aortic ring assay**: to evaluate ability of ECs to form neovessels

CONCLUSIONS

In vitro inhibition of bFGF and inhibition of glycolysis resulted in a lower migration and proliferation of ECs, and the inhibition of PFKFB3 showed also ability an impaired formation on new vessels.
Inhibition of bFGF and PFKFB3, via targeting molecules, are two promising strategies to prevent intraplaque angiogenesis and increase plaque stability.

References

1. Parma L, Baganha F, Quax PHA, de Vries MR, Plaque angiogenesis and intraplaque hemorrhage in atherosclerosis. Eur J Pharmacol. 2017

The project leading to this application has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 675527