

Influence of anthocyanins on the adipogenic and chondrogenic differentiation of human adipose mesenchymal stem cells

Līga SAULITE^{1*}, Kaspars JEKABSONS¹, Ineta POPENA¹, Ruta MUCENIECE¹, Maris KLAIVINS², Una RIEKSTINA¹

Faculty of Medicine, University of Latvia, Raiņa blvd. 19, Riga, Latvia LV-1568

Faculty of Geography and Earth Sciences, University of Latvia, Raiņa blvd. 19, Riga, Latvia LV-1568

Corresponding author: Līga SAULITE*, Faculty of Medicine, University of Latvia, Raiņa blvd. 19, LV-1568, Riga, Latvia. E-mail: liga.saulite@lu.lv

Introduction

Mesenchymal stem cells (MSCs) are adult stem cells which can be extracted from different tissue types such as bone marrow, dental pulp, skin and adipose tissue. MSCs can be differentiated in various cell types such as glial cells, neurons, adipocytes, osteocytes and chondrocytes [Saulite et al. 2017, Mol. Neurobiol., Saulite et al. 2017, Beilstein J. Nanotechnol.]. Due to their multipotency, MSCs can be used as a suitable model system for biological active compound screening for pharmaceutical and pharmacological purposes.

Anthocyanins are flavonoids responsible for the pigmentation in plants [Konczak et al. 2004, J Biomed. Biotechnol.]. Anthocyanins are known for their anti-oxidative, anti-inflammatory and anti-tumor properties [Suzuki et al. 2011, Nutrition&Metabolism]. The influence of anthocyanins on the reduction of obesity and diabetes has been a subject of discussion in recent years. It has been shown that a consumption of anthocyanins lower the risk of obesity and type 2 diabetes [Guo et al. 2015, Rev. Endocrin. Metab. Disord.]. However, the effects of anthocyanins on the chondrogenic differentiation has not yet been studied in detail.

Materials & Methods

Adipose MSCs (ATCC) were used in this study. Malvidin, Cyanidin and Delphinidin were used to evaluate the impact of anthocyanins (Sigma-Aldrich). Cytotoxicity of anthocyanins was evaluated by cck8 kit. Adipogenic and chondrogenic differentiation of MSCs was done for 21 and 14 days respectively, using Gibco StemPro differentiation kits. Medium was changed every 4 or 3 days respectively. Anthocyanins were added to the differentiation medium at 25 μ M concentration each. Cytochemical staining was done by Oil Red and Alzian Blue for adipogenesis and chondrogenesis respectively.

The expression of adipogenesis genes *Adiponectin*, *FABP4*, *LPL* and chondrogenesis genes *Sox9*, *Col2a1*, *Aggrecan* and *TGF- β 1* were analysed by qPCR using SyberGreen (Solis BioDYNE).

Results

Cytotoxicity of anthocyanins

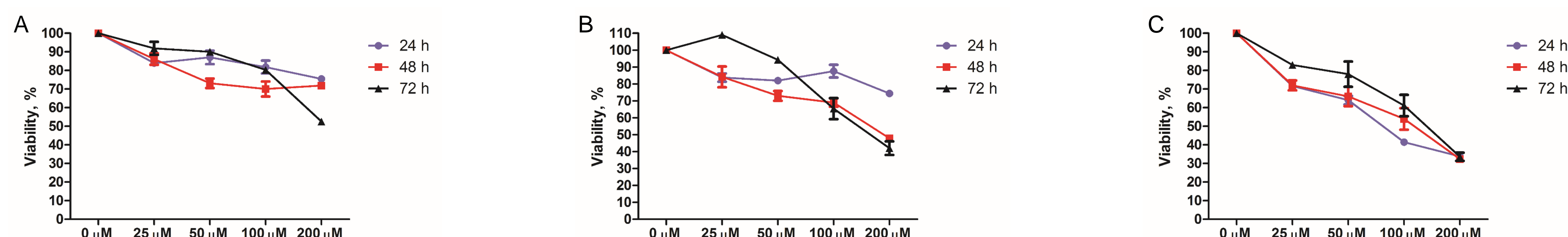


Fig. 1. Cytotoxicity of anthocyanins on MSCs. A) Malvidin at concentrations higher than 25 μ M exerted 20 – 30 % cytotoxicity with a more prone decrease in cell viability after 72 h. B) Cyanidin induced a slight cytotoxicity (20 %) already at 25 μ M concentration. Cytotoxicity was concentration and time dependant reaching IC50 at around 200 μ M after 48 and 72 h. C) Delphinidin demonstrated concentration dependant cytotoxicity in all tested time points, reaching IC50 at approximately 100 μ M.

Influence of anthocyanins on the adipogenic differentiation of MSCs

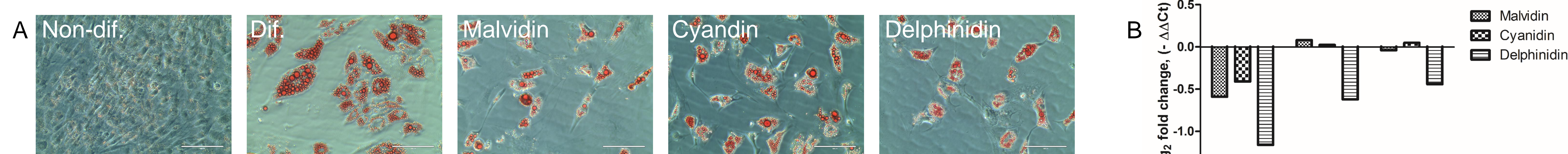


Fig 2. Influence of anthocyanins on the adipogenic differentiation of MSCs. A) After the differentiation cells were stained for lipid inclusions. B) Gene expression analysis revealed that Delphinidin was the only anthocyanin able to reduce the expression of all tested adipogenesis markers, however Malvidin and Cyanidin reduced only the expression of *Adiponectin*. Oil Red Staining. Scale bar – 100 μ m.

Influence of anthocyanins on the chondrogenic differentiation of MSCs

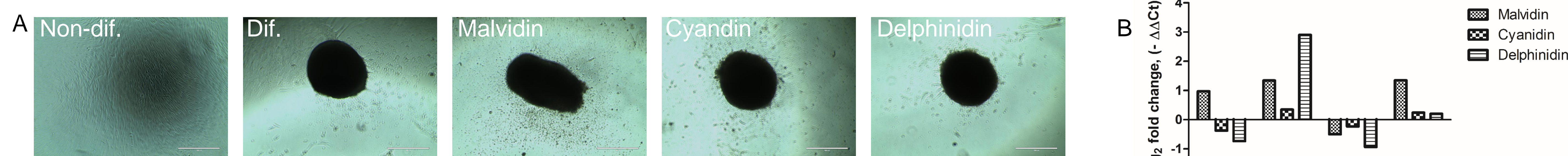


Fig 3. Influence of anthocyanins on the chondrogenic differentiation of MSCs. A) After the differentiation cells were stained for glycosaminoglycans. B) Gene expression analysis revealed that Malvidin was the only anthocyanin able to increase the expression *Sox9*, *Col2a1* and *TGF- β 1*. Delphinidin highly increased *Col2a1* expression, but had no effect on *Sox9* and *TGF- β 1*. Cyanidin did not impact the expression of non of the tested genes. Alzian Blue staining. Scale bar – 400 μ m.

Conclusions

- Anthocyanins at 25 μ M exerted almost no cytotoxicity and therefore this concentration could be declared as optimal for the current experimental setting.
- Malvidin, Cyanidin and Delphinidin decreased the expression of *Adiponectin*, however only Delphinidin additionally decreased the expression of *FABP4* and *LPL*, indicating a promising adipogenesis preventive effect.
- Malvidin decreased the expression of chondrogenesis markers *Sox9*, *Col2a1* and *TGF- β 1* indicating a promising chondrogenesis promoting effect.

Acknowledgements: This work has been supported by the European Regional Development Fund within the project No. 1.1.1.1/16/A/047 "Genus *Vaccinium* berry processing using "green" technologies and innovative, pharmacologically characterized biopharmaceutical products".