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

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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 gial cells, neurones, adipocytes, osteocytes and chondrocytes [Saullite et al. 2017, Mol. Neurobiol., Saullite 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 cell 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 µM concentration each. Cytochemical staining was done by Oil Red and Alzian Blue for adipogenesis and chondrogenesis respectively. The expression of adipogenesis genes Adiponecin, FABP4, LPL and chondrogenesis genes Sox9, Col2α1, Aggreccan and TGF-β1 were analysed by qPCR using SyberGreen (Solis Biodyne).

Results

Cytotoxicity of anthocyanins

Fig 1. Cytotoxicity of anthocyanins on MSCs. A) Malvidin at concentrations higher that 25 µM 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 µM concentration. Cytotoxicity was concentration and time dependant reaching IC50 at around 200 µM after 48 and 72 h. C) Delphinidin demonstrated concentration dependant cytotoxicity in all tested time points, reaching IC50 at approximately 100 µM.

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 Adiponecin. Oil Red Staining. Scale bar – 100 µm.

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, Col2α1 and TGF-β1. Delphinidin highly increased Col2α1 expression, but had no effect on Sox9 and TGF-β1. Cyanidin did not impact the expression of non of the tested genes. Alzian Blue staining. Scale bar – 400 µm.

Conclusions

- Anthocyanins at 25 µM 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 Adiponecin, 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, Col2α1 and TGF-β1 indicating a promising chondrogenesis promoting effect.

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