Effects of Quercetin in transcriptional and post-transcriptional regulation of fetal hemoglobin

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Background

Hemoglobinopathies are a group of inherited blood disorders that primarily affect red blood cells. The most common type is known as sickle cell anemia (SCA). It is characterised by mutations in the HBB gene, which encodes the β-subunit of human haemoglobin, giving rise to hemoglobina S (HbS). When deoxygenated, HbS polymerizes in the red blood cell, giving it a sickle shape and making it rigid and fragile. Fetal hemoglobin (HbF) is the major genetic modulator of the hematologic and clinical features of sickle cell disease, an effect mediated by its exclusion from the sickle hemoglobin polymer. Fetal hemoglobin genes are genetically regulated, and the level of HbF and its distribution among sickle erythrocytes is highly variable. Currently, therapies that induce HbF are promising, such as hydroxyurea (HU). However, due to high costs for underdeveloped countries and to the adverse side effects, it is important to test alternative products, ~~develop new compounds~~, such as Quercetin, a natural flavonoid present in plants that has antioxidant and anti-inflammatory properties.

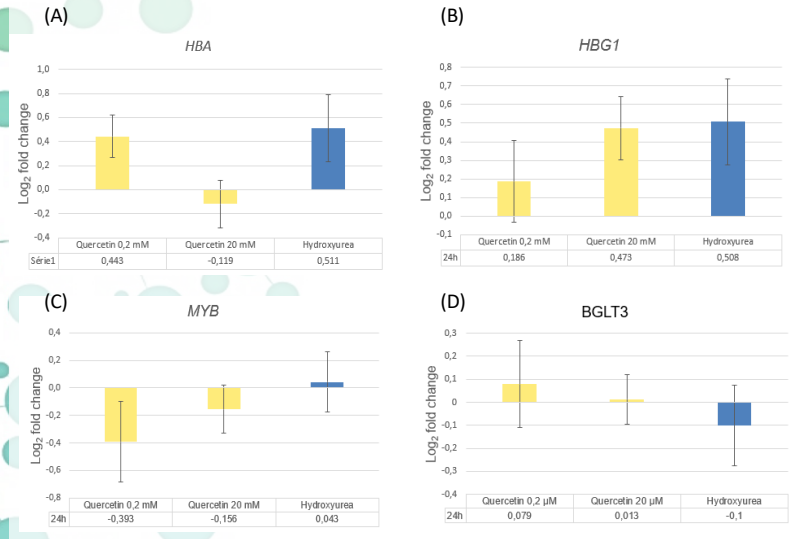
Aim

The main goal of this study is to evaluate the effects of ~~methanolic extracts of Carica Papaya leaves (EMFCP) and~~ Quercetin on transcriptional and post-transcriptional regulation of fetal hemoglobin in the human K562 cell line. These cells will also be exposed to HU, the current therapeutic agent for SCA. In both cases, gene expression of globins, HbF regulators and miRNAs will be assessed by RT-qPCR on cell lysates from exposed cells.

Methods

In this study, K562 cell line was exposed for 24 hours to ~~EMF~~CP, Quercetin and HU. Gene expression was quantified from total RNA by RT-qPCR. The genes subject to analysis of expression levels were globins (*HBA, HBB, HBG1* and *HBG2*); HbF regulatory genes (*MYB, KLF1, BCL11A* and *BGLT3*) as well as the miRNAs involved in the regulation of HbF (*miR-486-3p, miR-24a-5p, miR-210-5p, miR-32-5p* and *miR-96-5p*). The reference gene for coding RNAs was GAPDH and for miRNAs was miR-426-3p.

Results



Quercetin effects on expression levels of HBA (A), HBG1 (B), MYB (C) and BGLT3 (D) genes. RT-qPCR analysis of HBA, HBG1, MYB and BGLT3 expression levels normalised to GAPHD housekeeping gene relative to control cells.

Variations in the expression levels of HBF regulatory genes in K562 cell extracts subject to treatment with Quercetin or HU.

Exposure Time	Treatment	Gene transcription levels
24 hours	Quercetin 0,2 µM	↑HBA,↓HBB,↑HBG,↓MYB,↓KLF1, ↓BCL11A,↑BGLT3
	Quercetin 20 µM	↓HBA,↓HBB,↑HBG,↓MYB,↓KLF1, ↓BCL11A,↑BGLT3
	HU 25 µg/mL	↑HBA,↑HBB,↑HBG,↑MYB,↑KLF1, ↓BCL11A,↓BGLT3

Summary of the effects of Quercetin on the expression of miRNAs and its potential effects on the induction of HbF

miRNA	Target gene(s)	Transcription	Effect on HbF
miR-486-3p	BCL11A	Quercetin increases	Induce
miR-34a-5p		Quercetin at 0,2 µM inhibits	Inhibit
miR-210-5p		Quercetin at 20 µM increases	Induce
miR-32-5p		Quercetin increases	Induce
miR-96-5p	HBG1 and HBG2		Induce

- Data analysis of cell proliferation and viability indicate that, although Quercetin induces a decrease of about 40% in the proliferation rate upon incubation at the 20 µM concentration (when compared to controls), no differences in cell viability were observed for none of the concentrations used in this study. These results indicate that Quercetin has no cytotoxic effects on the K562 cell line.
- Our transcriptional analysis reveals that exposure to Quercetin induces downregulation of BCL11A, MYB, KLF1 and HBB and upregulation of HBG and BGLT3 gene expression levels. In what refers to the miRNAs under study, most of them are induced by Quercetin (miR-486-3p, miR-210-5p, miR-32-5p and miR-96-5p) while miR-34a-5p is inhibited by this natural compound.

Conclusions

- The results obtained indicate that Quercetin appears to induce HbF expression, either by increasing the transcription of γ-globin activating genes (*HBG* and *BGLT3*) or by inhibiting the expression of HbF inhibitors (*MYB* and *BCL11A*) or even by modulating the expression levels of candidate miRNAs.
- Thus, this preliminary study brings a new perspective of natural compounds as potential modulators of γ-globin gene expression. Further studies are needed in order to assess its potential in a future affordable and effective therapy for the treatment of SCD.

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