Activators of the transcription factor Nrf2 as new regulators of hepcidin expression

Hepcidin, an acute phase protein, is regarded as central mediator of the cytokine-induced anemia of chronic inflammation (ACI), one of the most common causes of iron deficiency anemia in patients with inflammatory bowel disease and obesity. Identified as primary trigger is the STAT-3 mediated induction of hepcidin synthesis in the liver and macrophages by cytokines of the interleukin-6 family (IL-6, Oncostatin M [OSM]) (1-6).

1. Background and Aim

Hepcidin cells were transfected with a hepcidin promoter luciferase construct and treated with IL-6 (10µM) or OSM (10ng/ml). After 16h of incubation a luciferase assay was performed. Both cytokines significantly induced hepcidin promoter activation (**p<0.001) (Figure 2).

2. Materials and Methods

- The current study aimed to determine additional signal transduction pathways of cytokine-induced hepcidin synthesis, with particular focus on a possible role of the transcription factor NF-E2-related factor 2 (Nrf2).
- HepG2 cells were cultivated under standard conditions and treated for 6 or 16h with either IL-6 (10 ng/ml) or OSM (10 ng/ml) alone or in combination with STAT-3 inhibitor (50 µM), suramin (SPH) (10 µM), dimethyl fumarate (DMF) (100 µM) or 15-Desoxy-Delta-12,14-prostaglandin J2 (PGJ2).
- For reporter gene assays, the cells were transfected by Lipofectamine with hepcidin promoter plasmid. Luciferase activity was measured luminometrically.
- Quantitative real-time PCR was performed for quantitative determination of mRNA.
- Proteins were detected by Western Blot analysis.

3. Results

The Nrf2 activators suramin (A), a natural Nrf2 activator found in broccoli, dimethyl fumarate (B), used as therapy for psoriasis and multiple sclerosis, and PGJ2 (C), an Nrf2 positive control, were used for coinoculation with the cytokines (Figure 3). All three Nrf2 activators significantly inhibited not only IL-6- and OSM-induced hepcidin promoter activation, but also intracellular hepcidin mRNA levels, observed by qRT-PCR (Figure 4).

4. Conclusions

As shown for DMF and SFN (Figure 5), simultaneous addition of PGJ2 to IL-6 in HepG2 did not inhibit the phosphorylation of STAT3. However PGJ2 did induce Nrf2 in the nuclear protein faction as shown in figure 6, suggesting possible involvement of Nrf2 in the inhibitory effect of hepcidin expression seen following simultaneous addition of Nrf2 activators to interleukins.

5. References and Acknowledgements


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