CO-releasing fumarate complexes show superior anti-inflammatory effects compared to dimethyl fumarate treatment

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1 Introduction

Inflammatory autoimmune diseases like psoriasis and multiple sclerosis are characterized by the aberrant induction of interleukin (IL-) 12 and IL-23-producing type I dendritic cells (DC), resulting in pathogenic Th1/Th17 cell responses.

We previously showed that the small molecule dimethyl fumarate (DMF) is able to improve both of these inflammatory diseases by depletion of intracellular glutathione and subsequently the accumulation of reactive oxygen species (ROS), the induction of heme oxygenase-1 (HO-1) and the inhibition of STAT1 phosphorylation. This results in the generation of type II dendritic cells which in turn induce an anti-inflammatory Th2 response [1].

In addition, carbon monoxide (CO) is an emerging therapeutic agent for various conditions characterized by a hyperactivated immune system [2]. We therefore reasoned to chemically combine fumaric acid and CO in a single molecule and synthesized CO-releasing molecules linked to methyl fumarate (FumET-CORMs).

2 FumET-CORMs

Fig. 1: Structure of FumET-CORMs: ANGO146, ANGO371, ANGO351 and ANGO360

To overcome the difficulties associated with oral CO administration by inhalation of the gas, CO-releasing molecules linked to methyl fumarate (FumET-CORMs) were developed. These compounds can either release methyl fumarate and CO directly when they are dissolved in an aqueous solution (A+B) or they have to be transported into the cell first, where they are then cleaved by esterases (C+D). The mechanism of this intracellular and enzyme-triggered release is shown in E.

3 Results

Fig. 2: Cytotoxicity of FumET-CORMs and their effects on the glutathione system.

(A) Cells were isolated from bone marrow of C3H mice and treated with DMSO, 1 µg/ml lipopolysaccharide (LPS) or with the indicated concentrations of DMF or FumET-CORMs + LPS for 24h. Cells were then harvested and counted with a Neubauer chamber. Live/death discrimination of cells was assessed by trypan blue staining. Shown is the percentage of living cells in each group. (B) BMDCs were incubated with DMSO or with the indicated concentrations of DMF or FumET-CORMs for 4h and GSH content was analyzed by a spectrometric assay (mean ± SD). (C) DCs were incubated with DMSO, 70 µM DMF or 25 µM FumET-CORMs for 4h, and intracellular ROS levels were assessed by staining with 2,7-dichlorofluorescein (H₂DCFDA). Intracellular ROS, gray; DMSO-treated controls, open.

Fig. 3: Induction of HO-1 by FumET-CORMs blunts inflammatory cytokine secretion.

For monitoring of protein expression DCs were treated with DMSO, 70 µM DMF or the indicated concentrations of FumET-CORMs and stimulated with 1 µg/ml LPS. Cell lysates were prepared after 2h and analyzed for HO-1 expression by Western Blot (A). Densitometric analysis of Western Blot is shown. (B) BMDCs were treated with DMSO or with the indicated concentration of DMF or FumET-CORMs and then stimulated with 1 µg/ml LPS for 24h. Cell culture supernatants were harvested and IL-23 levels were determined by ELISA (mean ± SD).

Fig. 4: Decrease of intracellular IL-12 levels through inhibition of STAT1 phosphorylation.

Cells were treated as described in Fig. 3. Protein expression of STAT1 and pSTAT1 was monitored by Western Blot analysis (A). Densitometric analysis of Western Blot is shown. For quantification of IL-12 cytokine levels cell culture supernatants were analyzed by ELISA (B).

4 Methods

Bone marrow derived dendritic cells (BMDCs) were isolated as shown in Fig. 5 and purity of DC culture was determined by FACS analysis. For functional analysis of DMF-treated DCs, glutathione levels were determined by a spectrometric assay (Fig. 6). Furthermore, interleukin levels were measured by ELISA and Western Blot analysis was performed to monitor HO-1 and STAT1 expression.

Fig. 5: Isolation of BMDCs.

BMDCs were isolated from femurs of C3H mice and cultured in DMEM media supplemented with 20 ng/ml GM-CSF in uncoated culture dishes. Replenishment with fresh culture medium took place on day 3 and 6. Immature BMDCs were harvested on day 7.

Fig. 6: Enzymatic recycling assay of GSH.

First step is the oxidation of GSH by DTNB [5,5'-dithio-bis (2-nitrobenzoic acid)] to form the derivative TNB (5-thio-2-nitrobenzoic acid) and GS-TNB. GS-TNB is then reduced to GSH by glutathione reductase and NADPH. The TNB formed can be quantified at 412 nm.

5 Conclusion

- ANGO351 and ANGO360 exhibit a substantially improved anti-inflammatory activity as compared to the clinically used drug dimethyl fumarate and were able to induce type II dendritic cells.
- Functionality of FumET-CORMs is dependent on their intracellular enzymatic activation by esterases as the non-activated control compounds ANGO146 and ANGO371 did not interfere with LPS-induced inflammatory cytokine production.

6 References

- [1] Ghoreschi, K. et al. (2011): Fumarates improve psoriasis and multiple sclerosis by inducing type II dendritic cells. *J Exp Med.* 208:2291-2303
- [2] Motterlini, R. et al. (2010): The therapeutic potential of carbon monoxide. *Rev. Drug. Discov.* 9:728-743