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The Mode of Action of Dimethyl Fumarate: Protein Succination and Anti-Pyroptotic Effects.

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Drug products containing dimethyl fumarate (DMF) are currently approved for the oral treatment of *psoriasis vulgaris* and relapsing-remitting multiple sclerosis. However, the mode of action of DMF remains an open question. Cell exposure to DMF is known to increase the level of succinated proteins (1). Protein succination results from the reaction between fumarate and cysteine (Cys) thiol group in proteins *via* a Michael-type addition. Michael adducts formation between electrophilic small molecules and protein-based Cys thiol groups has been postulated to underlie the anti-pyroptotic effects of structurally unrelated compounds including parthenolide (2). According to the assumption of a general reactivity-activity relationship, these findings indicate not only that DMF might exert anti-pyroptotic effects, but also that protein targets of DMF might be involved in the pyroptotic cascade.

To better understand the mode of action of DMF, its effects on pyroptosis in human macrophages were investigated. Furthermore, the reactivity toward succination of Cys residues in proteins involved in the pyroptotic cascade was explored, in order to identify candidate targets of DMF.

The anti-pyroptotic effects of DMF were studied in an *in vitro* model of pyroptosis (3). In particular, phorbol myristate acetate (50 nM; 24 h)-differentiated and lipopolysaccharide (5 μg • ml^{-1}; 4 h)-primed THP-1 cells were pretreated (60-15 min) with DMF (1 nM-10 μM), then exposed to ATP (5 mM; 1 h). Cell death was evaluated by measuring lactate dehydrogenase activity in the collected supernatants. Reactivity of Cys residues in proteins involved in the pyroptotic cascade was predicted by an *ad hoc* sequence-based bioinformatics analysis. Data are given as mean±SEM, and statistical analysis was performed using unpaired Student's *t*-test, as applicable.

The ATP-triggered cell death of THP-1 cells (60.4±4.0% *vs* vehicle alone) was significantly ($p<0.01$) prevented by DMF, in a time- and concentration-dependent manner; when 1 h-pretreatment was adopted, pIC_{50} and maximal effect were 6.5±0.1 and 56.3±4.6%, respectively. An uncommon enrichment of reactive Cys residues was predicted by our bioinformatic analysis of proteins known as components of the

pyroptotic cascade. These sites were distributed on many proteins, thus suggesting a multi-target action of DMF.

In conclusion, our results indicate that DMF is endowed with anti-pyroptotic activity, which is likely related to its action on multiple protein targets. As pyroptosis is a pro-inflammatory form of cell death, possibly implicated in the pathogenesis of diseases characterized by cell loss and chronic inflammation, our findings might contribute to a better understanding of the therapeutic effects of DMF.

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