Novel anti-inflammatory molecule DMFO targets Nrf2 in modulating proinflammatory & antioxidant mediators

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Background

Inflammation is a complex pathology → plethora of signalling pathways & multiple mediators

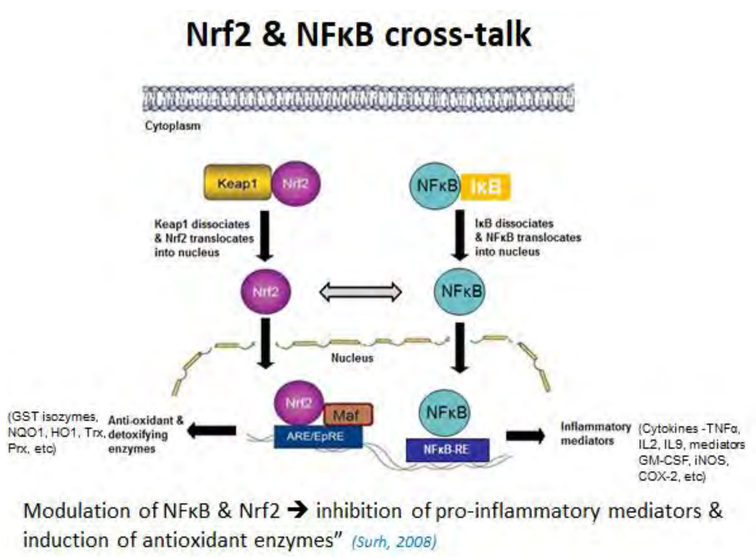
Acute inflammation : short-term response→ healing

Chronic inflammation : prolonged, dysregulated involve tissue destruction & attempts to repair

Inflammation: "disease of modern civilization" underlies atherosclerosis,cancer,arthritis,allergies,metabolic disorders, etc

Currently available anti-inflammatory drugs:

- NSAIDs(Non Steroidal Anti-inflammatory drugs) – GI (gastrointestinal) adverse effects
- Selective COX(cycloxygenase)2 inhibitiors (coxibs) - adverse CV (cardiovascular) effects
- Corticosteroids - long-term adverse effects - osteoporosis,diabetes,bone marrow suppression,etc
- Protein therapies - expensive,not orally bioavailable



Modulation of NFκB & Nrf2 → inhibition of pro-inflammatory mediators & induction of antioxidant enzymes" *(Surh, 2008)*

We serendipitously identified an indanedione derivative DHPO

- powerful antioxidant & dual COX-LOX (lipoxygenase) (IC_{50} (inhibitory concentration) 5LOX:37μM; COX1: 186μM; COX2:78μM)
- AMPK (AMP-activated protein kinase) activator (phosphorylates Thr172)

Our lab synthesized 14 analogs of DHPO (orally active small molecules)

Single-step one-pot synthesis, → stable compounds; meets Lipinski rules

characterised IR, MS, NMR (C13, H1), Purity by HPLC >99%; PK on 2 analogs (HPLC)

Acute toxicity studies : safe up to 2g/kg p.o. (OECD TG420)

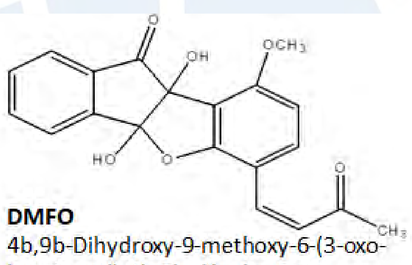
All compounds: *in-vivo* anti-inflammatory, non-ulcerogenic, *in-vitro* antioxidant

Analog-specific activity: *in-vivo* peripheral analgesic, radioprotective, anti-diabetic (AMPK activation), anti-allergic (mast cell stabilizing); *in-vitro* anti-oxidant, T helper cell modulation, LOX/COX inhibition *(Mathew et al 2015)*

Aims

To evaluate the anti-inflammatory activity of one promising analog namely, DMFO in animal models of acute & chronic inflammation

To explore the Nrf2-targeted cellular & molecular mechanism of action of DMFO

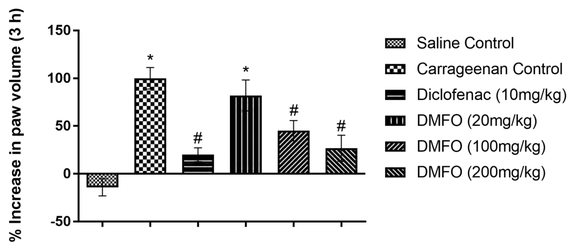


DMFO
4b,9b-Dihydroxy-9-methoxy-6-(3-oxo-but-1-enyl)-4b,9b-dihydro-5-oxa-indeno[2,1-a]inden-10-one

Results & Discussion

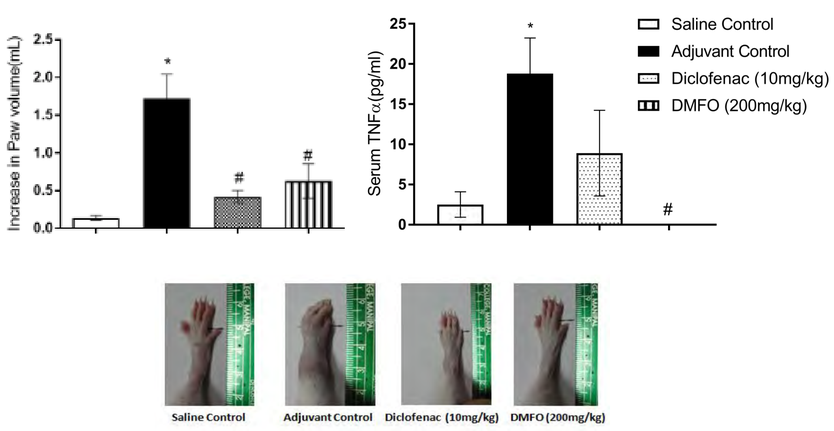
Results are mean±SEM, n:6-8;
*: p < 0.05 Vs saline (negative) control; #: p < 0.05 Vs positive (inflammation) control

DMFO dose-dependently reduced Carrageenan-induced rat paw oedema (model of acute inflammation)

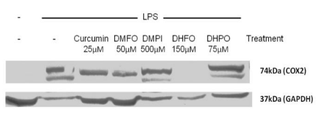


DMFO reduceded ovalbumin (antigen)-induced mast cell degranulation in mice without affecting IgE levels

DMFO reduced Freund's adjuvant-induced arthritis in rats (model of chronic inflammation)



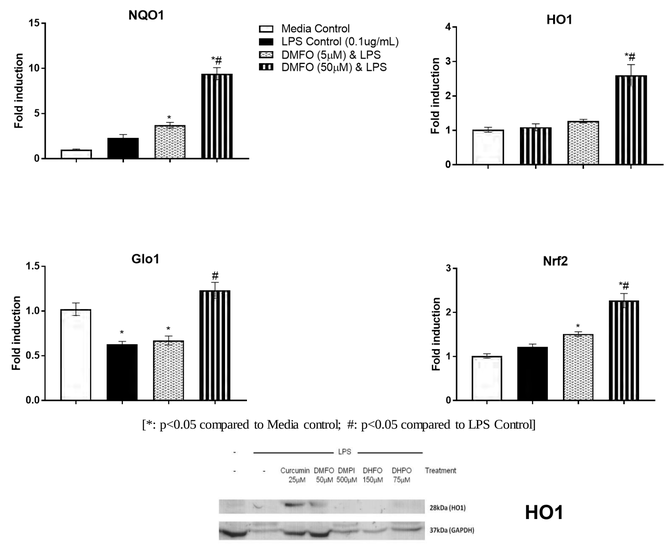
DMFO reduced ROS and inflammatory markers in LPS (lipopolysaccharide)-stimulated primary macrophages



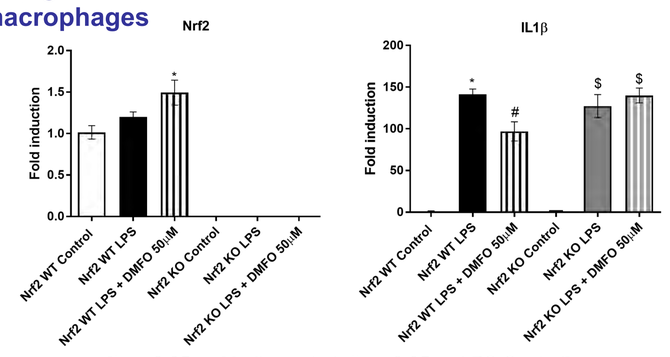
Compound	DCFH-DA assay IC_{50} for ROS (μM)
DHPO	19.05 ± 4.30
DHFO	145.97 ± 2.52
DMFO	71.86 ± 4.02
DMPI	243.64 ± 8.50

DPI (5 μM) : 62.18 ± 1.78 % ROS inhibition

DMFO enhanced anti-oxidant markers in LPS-stimulated primary macrophages

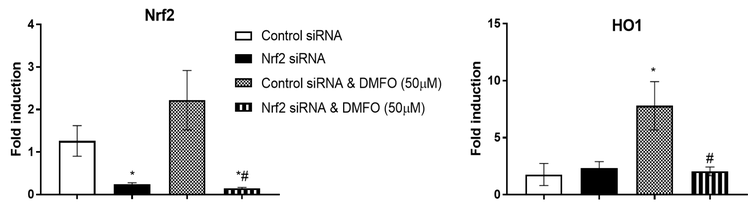


DMFO reduced pro-inflammatory IL1β gene expression in LPS-stimulated primary macrophages without affecting IL1β gene expression in LPS-stimulated Nrf2-KO macrophages



[*: p<0.05 vs Media control; #: p<0.05 vs LPS Control]

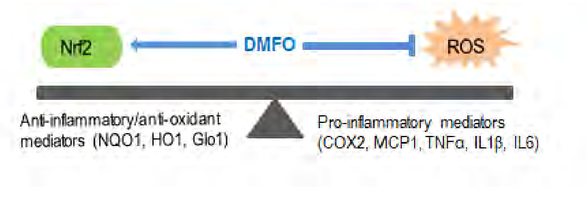
DMFO did not inhibit Nrf2/HO1 gene expression in Nrf2-silenced RAW264.7 macrophages → demonstrates Nrf2-targeted activity



[*: p<0.05 vs Control siRNA; #: p<0.05 vs Control siRNA + DMFO (50 μM)]

Conclusion

- DMFO : promising, novel, safe (up to 2 g/kg), orally active, easily synthesizable, stable, pleiotropic, Nrf2-targeting, non-ulcerogenic, anti-inflammatory lead molecule suitable for long term dosing in chronic conditions *(Mathew et al, 2018)*



References

- Surh YJ. NF-kappa B and Nrf2 as potential chemopreventive targets of some anti-inflammatory and antioxidative phytonutrients with anti-inflammatory and antioxidative activities. Asia Pac J Clin Nutr. 2008;17 Suppl 1:269-72.
- Mathew G, Unnikrishnan MK. Multi-target drugs to address multiple checkpoints in complex inflammatory pathologies: evolutionary cues for novel "first-in-class" anti-inflammatory drug candidates: a reviewer's perspective. Inflamm Res. 2015 Oct;64(10):747-52.
- Mathew G, Sharma A, Pickering RJ, Rosado CJ, Lemarie J, Mudgal J, Thambi M, Sebastian S, Jandeleit-Dahm KA, de Haan JB, Unnikrishnan MK. A novel synthetic small molecule DMFO targets Nrf2 in modulating proinflammatory/antioxidant mediators to ameliorate inflammation. Free Radic Res. 2018 Oct;52(10):1140-1157.

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