Rosiglitazone inhibits MMP-9 protein expression through NF-κB and AP-1 dependent mechanisms in human THP-1 cells

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Altered expression of matrix metalloproteinases is associated with the development of atherosclerosis. Macrophages, a key component of the atherosclerotic plaque not only secrete MMP-9 but also express high levels of the peroxisome proliferators-activated receptor-γ (PPARγ) nuclear receptor. Interestingly, glitazones (PPARγ agonists) inhibit MMP-9 expression in macrophages and vascular smooth muscle cells. However, the underlying mechanism is less clear, with NFκB, AP-1 and Stat pathways being implicated using luciferase reporter assay (Ricote *et al.* 1998). The aims of the present study were to investigate the direct effects of rosiglitazone (RGZ) and troglitazone (TGZ) on NFκB, AP-1 and GSK-3B signalling with regard to the inhibitory effect of the glitazones on PMA-induced MMP-9 expression in THP-1 macrophages.

THP-1 cells were cultured in RPMI 1640 medium with 10% FBS and antibiotics. Cells were incubated in serum-free medium supplemented with medium (untreated control), or phorbol 12-myrisate 13-acetate (PMA; 50ng/ml) for 48 hrs. RGZ or troglitazone (TGZ) were added to the medium prior to stimulation with PMA; cells were pre-exposed to GW9662 (PPARγ antagonist) for 1hr where appropriate. MMP-9 expression was determined by ELISA and real time PCR. AP-1 transcription factors were analysed ELISA; NFκB, IKBα and GSK-3B were analysed by Western blot. Data were analysis by oneway-ANOVA with post hoc analysis (Bonferroni) and expressed as mean ± SE. A value of $P<0.05$ was taken to indicate statistical significance.

TGZ and RGZ inhibit PMA-induced MMP-9 mRNA and protein expression by 50 and 60% respectively. Furthermore, GW9662 reversed the inhibitory effect of RGZ (1 μM) on MMP-9 protein expression (0.37 ± 0.02 *vs* 0.58 ± 0.036), but not mRNA expression; no effect was observed at higher concentrations of RGZ (10 μM) or with TGZ. RGZ inhibited ($P<0.05$) PMA-induced NFκB phosphorylation (2.0 ± 0.15 *vs* 1.4 ± 0.09), but did not affect IκBα degradation after 48 hr exposure; no effect were observed at earlier time points. PMA alone or in combination with RGZ did not alter GSK-3B phosphorylation. RGZ inhibited PMA-induced expression of c-Jun, JunD, cFos, Fra1 and Fra2 members of the AP-1 transcripition family by between 40-75%.

In conclusion, we demonstrate that RGZ and TGZ regulate MMP-9 protein and mRNA expression in THP-1 cells. In the case of RGZ this effect could be partially reversed by inhibition of PPARγ. Furthermore, RGZ mediated inhibition of MMP-9 involved downregulation of NFκB and multiple members of the AP-1 transcription family.

Ricote, M. *et al* (1998) Natr. **391**: 79-82.