A41 TRANSIENT EXPOSURE OF MACROPHAGES TO P38 MAP KINASE INHIBITION CONDITIONS CELL RESPONSES THROUGH MAPK ACTIVATED PROTEIN KINASE-2 REGULATION

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Background and objectives Previous work by the authors suggests that selective p38 mitogen-activated protein kinase (MAPK) inhibitors, SB203580 and ML3403, have inhibitory effects on adherent accessory cells such as macrophages that last up to 120 h after the drugs are washed off, whereas no such effect is seen in lymphocytes.[1] This raises the question as to whether p38 MAPK signalling remains inhibited after washout or whether there is a long term priming effect on the macrophages. To answer this, intracellular p38 MAPK activity of human macrophages transiently exposed to the p38 MAPK inhibitor, SB203580, was determined with a substrate phosphorylation assay for different time-points after washout. Tumour necrosis factor α (TNFα) synthesis was also assayed.

Methods U937 cells, a human monocytic cell line, were differentiated to the macrophage phenotype with PMA (80 nM) for 48 h (PMA-U937 cells). The cells were trypsinised and plated in 12 well plates at 5×10^5/well. After overnight serum starvation, the cells were incubated with SB203580 for 2 h before being washed four times and rested for various time-points. Cells were then stimulated with 100 ng/ml LPS for 30 min and supernatants were collected for TNFα measurement by ELISA and whole cell lysates were prepared for western analysis of downstream targets of p38, transcription factors ATF-2 and MK-2.

Results PMA-induced differentiation of U937 cells to macrophages was confirmed by extracellular staining with macrophage markers (CD16 and CD51/61) and flow cytometry. LPS stimulated TNFα production by PMA-U937 cells, which was completely inhibited by SB203580. LPS stimulated a concentration-dependent increase in the phosphorylation of ATF-2 and MK-2. However, only phosphorylation of MK-2 was inhibited by SB203580. Washing the cells after drug incubation restored the ability to stimulate phospho-MK-2 in the macrophages shortly after washout (1 min and 2 h), however the inhibition of MK-2 phosphorylation by p38 MAPK was reasserted 4 h after drug withdrawal. Phospho-ATF-2 remained unaffected throughout, indicating messenger selectivity.

Conclusions This data confirms that p38 inhibition has a selective effect on accessory cells that persists after washout, and that this could be regulated through MAPK activated protein kinase-2. In addition, SB203580 preconditioning appears to have a biphasic effect on macrophages, with inhibition of phospho-MK-2 when the drug is present, no inhibition shortly after washout as would be expected, but with an unexpected secondary inhibition of P38 MAPK phosphorylation of MK-2 at longer time-points. Since preconditioning is not witnessed in T lymphocytes, the inhibition of p38 MAPK may have different anti-inflammatory actions depending on the time elapsed after drug administration.

REFERENCE

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