PWE-023

AZATHIOPRINE AND 6-THIOGUANINE BUT NOT 6-MERCAPTOPURINE INHIBIT INTRA-MACROPHAGE REPLICATION OF CROHN'S DISEASE ESCHERICHIA COLI

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Introduction Crohn's disease is associated with increased mucosal colonisation by *E. coli* that have an adherent, invasive phenotype that includes intracellular survival and replication within macrophages. Drugs that prevent this replication might have a therapeutic effect. We previously reported that azathioprine enhances bacterial killing by macrophages[1] and therefore we evaluated the effect of its metabolites, namely 6-mercaptopurine and 6-thioguanine, at clinically relevant concentrations, on replication of *E. coli* within macrophages.

Methods Azathioprine metabolites, 6-mercaptopurine (4.2×10^{-2} to 4.2 μM) and 6-thioguanine (8.2×10^{-5} to 820 fmol/8×10^8 cells) were assessed for their effect on survival and replication of Crohn's *E. coli* isolate HM605 in J774-A1 macrophages in comparison to azathioprine (4.2×10^{-12} to 4.2 μM). Macrophages were pre-treated with drugs for 24 h before bacterial infection. Cells were then infected with HM605 for 2 h to allow for internalisation. Extracellular bacteria were removed and killed with gentamicin (20 μg/mL) for 1 h. Macrophages were then either lysed (at 3 h) or parallel cultures incubated for a further 3 h in the presence of gentamicin (6 h). Internalised bacteria were enumerated by overnight growth on LB agar.

Results As previously seen, Crohn's *E. coli* HM605 significantly replicated within macrophages at 6 h compared to 3 h post-infection levels and azathioprine, at doses of 4.2×10^{-9} to 4.2 μM, resulted in suppression of *E. coli* intramacrophage replication (all $p<0.01$, N=3–8) with a peak effect at 4.2 μM (0.33 fold replication relative to controls; $p<0.001$, N=5 Kruskal Wallis ANOVA). 6-thioguanine also suppressed *E. coli* replication in a dose dependent fashion with peak suppression at 82 fmol/8×10^8 cells (0.47 fold replication relative to control; $p<0.001$, N=4). However, 6-mercaptopurine did not suppress *E. coli* replication at any concentration tested.

Conclusion The enhancement of macrophage killing of intracellular *E. coli* by azathioprine and 6-thioguanine but not 6-mercaptopurine is intriguing. It might reflect the known ability of azathioprine but not 6-mercaptopurine to inhibit inducible nitric oxide synthase[2]. The effect of 6-thioguanine on nitric oxide synthase has yet to be assessed. These effects of azathioprine and 6-thioguanine on bacterial killing by macrophages may be relevant to some of their therapeutic effects, perhaps particularly in fistulating Crohn's disease.

Competing interests P. Knight: None Declared, B. Campbell: None Declared, J. Rhodes Consultant for: Proctor & Gamble and Falk, Speaker bureau with: Abbott, Falk, Ferring, Proctor & Gamble and Schering Plough

Keywords 6-mercaptopurine, azathioprine, Crohn's disease, escherichia coli, macrophages, thioguanine.

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