Interferons

I-01 NOVEL MECHANISM OF ACTION OF ANTI-MALARIAL DRUGS IN THE INHIBITION OF TYPE I INTERFERON PRODUCTION
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Background Anti-malarial drugs (AMD) such as Hydroxychloroquine (HCQ) and Quinacrine (QC) are effective in the treatment of skin rash and arthritis in systemic lupus erythematosus (SLE). However, which mechanism(s) are responsible for their beneficial action is uncertain. Type I interferon, (IFN-I) is strongly implicated in the pathogenesis of SLE and ‘interferonopathies’ such as Aicardi-Goutieres Syndrome (AGS). A new DNA activated IFN-I pathway, cyclic GMP-AMP (cGAMP) synthase (cGAS), was recently discovered and linked to AGS and mouse models of Lupus. Preliminary data indicate that a subset of SLE patients also have elevated cGAS and cGAMP (the cyclic dinucleotide responsible for activation of STING and IFN-I).

Materials and methods In silico structure-based drug screening were provided by the CANDO docking algorithm. Predictions made by CANDO were confirmed by Autodock Vina and analysed via PyMOL. cGAS activity/cGAMP production was analysed by Thin Layer Chromatography (TLC). DNA-binding to cGAS in the presence or absence of AMD was determined by an Electrophoretic Mobility Shift Assay (EMSA). Following DNA cell transfections, cytokines were quantified by qPCR, ELISA or an ISRE-luciferase reporter assay. cGAMP in patient samples was quantified by mass spectrometry.

Results In silico screening of drug libraries identified several antimalarial drugs (AMD) which could potentially inhibit cGAS activity by interacting with cGAS/DNA dimer complex. Electrophoretic Mobility Shift Assay revealed that AMD disrupted the double stranded DNA-cGAS complex in a dose dependent manner. These AMD also inhibited IFN-I expression in THP1 cells transfected with dsDNA and in 293 T cells transfected with cGAS/STING plasmids validating that cGAS is a target of AMD. We synthesised several new AMD. One of these compounds, X6, had excellent water solubility and cell penetration. X6 localised to the cytosol and had a lower toxicity profile compared to QC. Biochemical and cellular assays revealed that X6 was a more potent inhibitor of IFN-I production than HCQ. We also validated mechanism of action and proof of concept in the animal model of AGS.

Conclusions Our studies identify new DNA sensor cGAS as a target of AMD activity, which provide a novel mechanism of action of these AMD. We have synthesised new AMD like drugs that are also able to inhibit cGAS as well as Toll pathways. These drugs could be beneficial for the treatment of AGS and/or Lupus.