Abacavir stimulates Hsp70 redistribution in antigen presenting cells of patients with hypersensitivity association: with type I alcohol dehydrogenase activity

Annalise Martin, Goyal Anjali Mehta, David Naylor, Paul Cameron, Ian James, A Parash, Tamara McCluskey, Elizabeth Phillips, and Simon Metaal

Centre for Clinical Immunology and Biostatistics (CCIBS), Royal Perth Hospital and Murdoch University, Perth, Western Australia. Department of Clinical Immunology and Biochemical genetics, Centre for Clinical Immunology and Biostatistics, Royal Perth Hospital, University of Melbourne, Victoria, Australia. British Columbia Centre of Excellence in HIV/AIDS, Vancouver, University of British Columbia.

Background: MHC class I molecules, including HLA-B*5701 and beta2-microglobulin (Hsp70) gene, have been shown to be highly predictive of abacavir hypersensitivity (ABC HSR). Here, we dissect the pathways involved in eliciting abacavir-specific immune responses.

Methods: We examined the effect of abacavir on intracellular localization of HLA-B57, Hsp70 in PBMCs, MDDCs by immunofluorescence confocal microscopy from ABC HSR (n=9), ABC tolerant controls (n=9), HLA-B*5701 positive naïve individuals (n=8), and cell line homozygous for HLA-B57 (n=3). Hsp70 redistribution was quantified using ImageJ. The influence of 4-MP mediated inhibition of type I alcohol dehydrogenase (ADH) levels on Hsp70 redistribution and IFN-γ was also studied. RESULTS: Intracellular Hsp70 redistribution after abacavir stimulation in PBMCs of ABC HSR patients (n=8) and ABC tolerant (n=3) compared with controls (n=9), was measured based on average intensity in 0.5 µm x 0.5 µm x 0.5 µm voxels (p<0.02). Intracellular Hsp70 redistribution in ABC naïve individuals (n=8), abacavir specific Hsp70 redistribution decreased from an average of 2.3 arbitrary units (A.U.) (p=0.024). However, ABC exposure did not induce a Hsp70 redistribution in HLA-B*5701 positive naïve individuals (p>0.1). Hsp70 redistribution levels correlated with intracellular IFN-γ (r=0.66, p=0.035). In cell line, the TMF-20A allele was associated with increased Hsp70 redistribution after abacavir exposure. Intracellular Hsp70 redistribution in unexposed ABC naïve individuals depends on ADH metabolism of ABC but is independent of HLA-B*5701. The expression of HLA-B57 was confirmed by flow cytometry analysis using the B17 monoclonal antibody (One Lambda, Inc). A significant increase in fluorescence intensity of HLA-B57 was observed in ABC naïve individuals exposed to 4 µg/ml abacavir for 3hrs compared with unexposed (p=0.001). Abacavir specific Hsp70 redistribution in PBMCs of abacavir hypersensitive patients (n=9), abacavir tolerant patients (n=3) and B cell lines homozygous for MHC haplotypes (n=13).

CONCLUSIONS: These data suggest that Hsp70 redistribution is an early component of the ABC-specific immune response which is sensitive to inhibition of type I ADH, and which influences IFN-γ. The development of hypersensitivity thus represents a multi-step process likely to involve the generation of an abacavir metabolite by ADH followed by intracellular redistribution and finally the development of an HLA-B*5701 restricted immune response mediated by inflammatory cytokines.

REFERENCES