

31P MRS Assessment of Hepatic Mitochondrial Toxicity in Rat

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Introduction: Mitochondria play a complex role in health and disease. The in-vivo assessment of the mitochondrial toxicity will be of a great value for the development of new drugs (1). In this study, we used non-invasive ³¹P MR spectroscopy in rat to assess the high energy metabolite profile after the administration of GSK121A, a known mitochondrial respiratory chain inhibitor. The MRS data was placed into context with in-vitro measurements of isolated mitochondrial function.

Method: All procedures were approved by the Animal Care and Use Committee of GlaxoSmithKline and were specifically designed to minimize animal discomfort. To examine the effect of GSK121A on the liver energetic profile (e.g. ATP, Pi) at 5 hr following a single i.p. dose, Sprague-Dawley rats were treated i.p. with GSK121A (50 mg/kg; n=4 or 25mg/kg; n=9) or vehicle (n=10). Body temperature was measured every 15min for up to 5 hr post treatment. ³¹P-MRS was performed at baseline and 5 hours post-dose using a double tune (¹H, ³¹P) concentric surface coil on a 9.4T/30 cm Bruker system. ³¹P MRS was performed using a 3-D Image Selected *In Vivo* Spectroscopy (ISIS) sequence with outer volume suppression (TR= 0.5s, NS= 2048, SW= 10 kHz, 1 k data). The spectroscopic voxel of interest size was 18×15×18 mm and positioned on the liver on an oblique plane in all three orthogonal directions (Figure). Absolute concentrations of ATP and Pi were extrapolated using an external concentration standard. After the imaging session, terminal blood samples were collected for lactate, blood chemistry and drug concentration measurements. Data are presented as Mean ± SEM.

Results: No mortality was observed in the groups. Body temperature decreased significantly at 5 hours from baseline in the GSK121A treated groups (50 mg/kg, 25mg/kg) compared to the vehicle group from baseline (↓-118% and -84%, p≤0.05). While liver ATP concentrations remained unchanged, Pi and Pi/ATP ratio were increased significantly in the GSK121A treated groups (50 mg/kg, 25mg/kg) compared to the vehicle group (Pi: 8.6±0.3 mM; p≤0.001, 6.8±0.7mM; p≤0.05 vs 5.07±0.5mM, and Pi/ATP: 1.2±0.2, 1.1±0.07 vs 0.8±0.05; p≤0.05, respectively). In addition; the blood lactate level was higher in the GSK121A treated groups (50 mg/kg, 25mg/kg) compared to the vehicle group (29.6±9mg/dl, 17.0±2.9mg/dl vs 12.0±2.2mg/dl, respectively) and correlated with the change in body temperature. These data support ex-vivo results illustrating reduced oxidative capacity in isolated mitochondria treated with GSK121A.

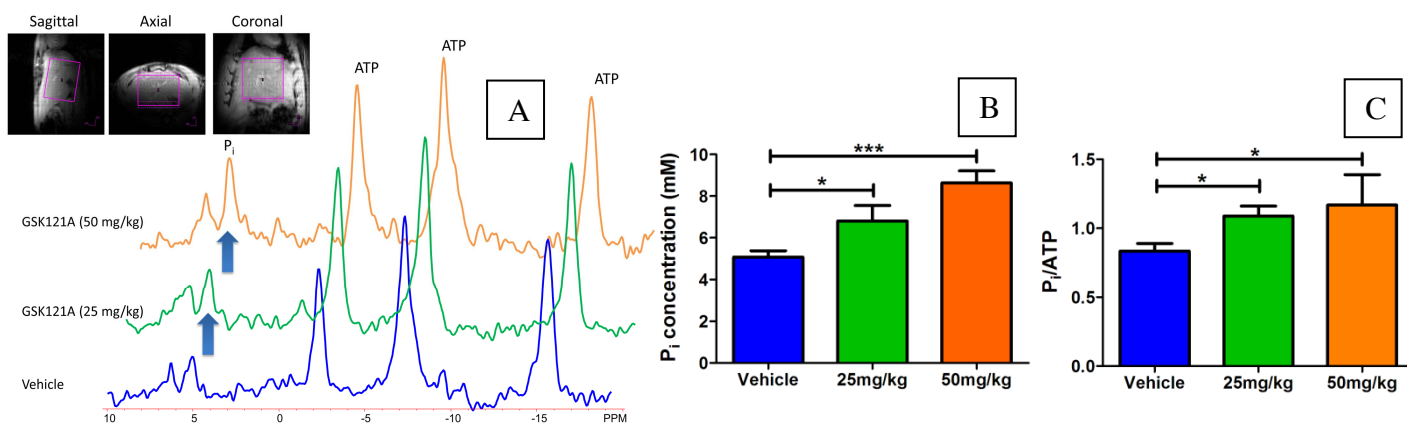


Figure: Representative ³¹P MR spectra (A) acquired 5 hours post treatment of GSK121A (50mg/kg orange, 25mg/kg green) or Vehicle (blue). Pi concentration (B) and Pi/ATP ratio (C) was increased significantly in the GSK121A treated groups compared to Vehicle.

Conclusion: These data suggest that non-invasive ³¹P NMR spectroscopy can be used to assess hepatic mitochondrial dysfunction. While steady state ATP levels appear to be normal, there could potentially be a decrease in ATP synthesis associated with decreased body temperature and increase in Pi. Further studies assessing ATP turnover using saturation transfer techniques would help identify the mechanism for increased Pi/ATP. This clinically translatable MRS method may be used to assess drug effects on hepatic mitochondrial function in patients.

References: 1- Hamilton BF, Stokes AH, Lyon J, Adler RR. In vivo assessment of mitochondrial toxicity. Drug Discov Today. 2008 Sep;13(17-18):785-90.