Detection of corticosteroid-induced changes in thymus: a comparison between MRI-evaluated volume changes and wet weight.

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Introduction: High-resolution non-invasive imaging monitors changes in organ volume in disease, and therapy. It may also be sensitive to the pharmacology of novel drugs in specific organs. Corticosteroid hormone analogues exert extensive anti-inflammatory and immunosuppressive actions so are widely used clinically to treat inflammatory conditions including cerebral oedema, rheumatoid arthritis, musculoskeletal injury and some forms of asthma. Undesirable, apoptotic side effects of corticosteroids may be profiled by measuing thymus involution. Conventional *post mortem* wet weight measurements may be prone to inter-subject and inter-operator variability, increasing numbers required for statistical confidence. Here we show that 2D multislice MRI is an efficient, effective tool to show mouse thymus volume dose-responses to a model corticosteroid, dexamethasone (Dex).

Methods: Animals were housed and maintained and procedures conducted in accordance with the Home Office Animals (Scientific Procedures) Act 1986, UK. Animals (24 balb/c female mice (16-18g)) were anaesthetised (isoflurane) and the free breathing animals placed in a 7T Bruker Biospec (35mm coil), homeostatically maintained with respiratory monitoring under 2% isoflurane anaesthesia (800ml/min in O_2). 2D sagittal multislice multispin-echo images were acquired (FOV 3 x 3cm, 128 x 128 matrix, slice thickness 0.75 mm, TR 1200 ms,TE 10 ms, SW 50 kHz). 4 Groups were used: 1: Vehicle (saline + cremophor,) 2: 0.1mg/kg Dex. 3: 1mg/kg Dex. and 4: 10 mg/kg Dez. Baseline images were acquired, animals were dosed s.c., imaged 48 h later, euthanased (pentabarbitone overdose) and thymus glands carefully removed and weighed. Cross sectional areas of the thymus were measured manually using the Bruker ParaVision ROI tool, summed, and multiplied by slice thickness to give a measure of thymus volume, the whole analysis taking ca. 5 minutes per thymus. Statistica V.6 was used for statistical analysis.

Results: Figure 1 is a typical image of a control mouse thymus. Figure 2 depicts MRI volume, and wet weight, responses to incresing Dex. doses; bars show 95% confidence intervals. There was a significant decrease ($p < 0.001$) in both volume and wet weight after the higher two Dex. doses. However the MRI volume measurements were more sensitive when detecting smaller differences, between lower doses, *eg.* Between 0.1 & 1mg/kg MRI volume measurements are associated with $p = 0.00061$ against $p = 0.081294$ for wet weight. Confidence intervals for all Dex. doses were consistently tighter for MRI volumes than for wet weights, and statistical power consequently greater. Thus, Figures 3 and 4 plot predicted group sizes against the statistical confidence with which differences of 1/3, 2/3 and 3/3 of maximal response to Dex. should be detectable: For 90% confidence over the 1/3 interval, wet weight analysis would require over 130 animals, against only 30 with the use of MRI volumes. This data suggests that, given equal response variabilities, MRI volume is associated with significantly lower measurement variability (*cf.* standard deviations of 0.006 (MRI volume) and 0.26 (weight)), increasing statistical power with significantly reduced numbers of animals. The greater precision and reproducibility of MRI volume measurements should confer greater confidence to the assessment of selective corticosteroid profiles, for instance by enabling measurements at more doses to characterise subtle differences between compound.

Conclusion: MRI measurements accurately reflect apoptotic changes in the mouse thymus in response to corticosteroids. Compared with conventional wet weight measurements, MRI volumes appear to be less variable, and to confer greater statistical power with fewer animals. These properties may provide new opportunites for more precise differentiation between subtly different compounds in studies which would otherwise be prohibitive due the need for impractically large animals groups.

Figure 1 MRI of mouse thymus.

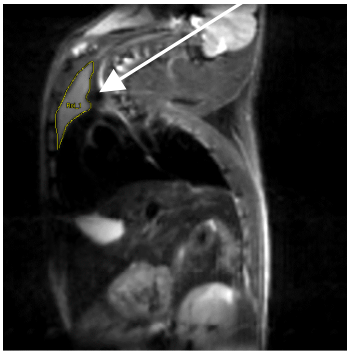

Figure 2 Dose response curves for MRI volumes and wet weights.

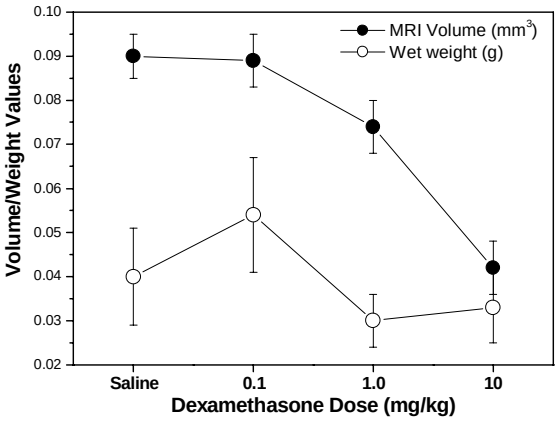

Figure 3 Sample size power calculations using thymus weight.

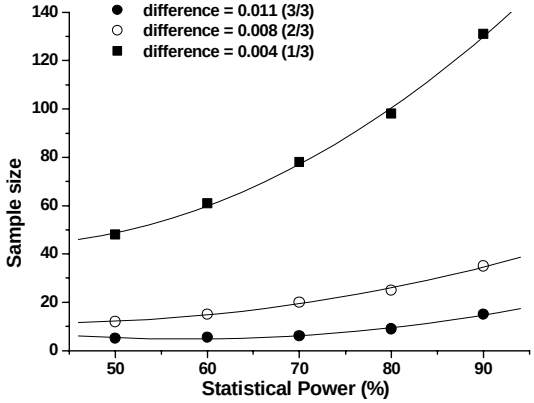

Figure 4 Sample size power calculations using thymus volume.