Development of an Imaging Toolbox to Study Renal and Cardiac Function, and Cellular Biodistribution for Regenerative Therapies

**Introduction:** Over the last decade there has been an increasing interest in renal regenerative medicine therapies (RMTs). The clinical translation of these therapies requires thorough studies in preclinical species which demonstrate efficacy and safety. Gathering this information is challenging for a number reasons; current biomarkers of kidney injury lack sensitivity and specificity, repeated blood and/or urine sampling in mice is not feasible and the longitudinal assessment of organ function is challenging. Multispectral optoacoustic tomography (MSOT) offers an opportunity to assess the function of the kidney in mice in a single imaging session in a minimally invasive manner longitudinally. This can be combined with ultrasound measurements of cardiac function to assess the progression of kidney and cardiac injury and the efficacy of specific RMTs. These methods can be combined with tracking the regenerative cells using bioluminescence imaging to gather information on their biodistribution. **Methods:** This study utilised an acute model of Doxorubicin-induced multi-organ dysfunction over a period of 4 days with assessment of organ function on days 1 and 4. Mice (male Balb/c, 6-8 weeks) received saline (n=4) or 20 mg/kg Doxorubicin (n=11) on day 0. On day 1 renal function was determined by MSOT (iThera, inVision imaging system) by measuring the clearance of IRDye 800 through the kidney, and cardiac function via ultrasound. Mice which received Doxorubicin then either received saline (n=5) or 107 primary bone marrow derived macrophages (BMDMs) (n=6) via the tail vein on day 1. Renal and cardiac function was again determined on day 4 of the study. In a separate study to examine the biodistribution of the RMT, mice received saline or Doxorubicin as described above. All mice received 107 BMDMs isolated from mice which expressed firefly luciferase. Half (n=7) of the mice were imaged and sacrificed on day 1 using an IVIS imaging system (Perkin Elmer) and the remaining mice (n=7) on day 4. **Results:** Uninjured mice which received saline only, showed no change in renal or cardiac function between days 1 and 4. Mice which received Doxorubicin but no therapy showed significantly impaired renal and cardiac function between days 1 and 4. In mice that received Doxorubicin followed by the RMT, although renal function was reduced between days 1 and 4, this was not significantly different from the uninjured controls. On the other hand, the RMT did not appear to have any beneficial effects on cardiac function over the time course. On day 1 there were no differences in the biodistribution of the RMT in the liver, kidney, heart, spleen or lungs of uninjured mice compared with those that received Doxorubicin. However, on day 4, while uninjured mice showed a similar pattern of RMT biodistribution as on day 1, albeit with a lower signal intensity, while mice that received Doxorubicin showed a significant increase in bioluminescence intensity in the kidneys, but not in the heart. **Discussion:** This study utilised a multimodal imaging strategy to longitudinally assess renal and cardiac function. It was demonstrated that a single high dose of Doxorubicin alters both renal and cardiac function as determined by MSOT imaging. Mice with Doxorubicin-induced kidney and cardiac dysfunction showed increased accumulation of BMDMs in the kidney which was associated with an improvement in function. On the other hand, BMDMs did not accumulate in the damaged heart, nor did they have any beneficial effects on cardiac function. This study demonstrates that by utilising a multimodal imaging approach we can examine the efficacy and biodistribution of a potential RMT longitudinally, and determine the relationship between biodistribution and therapeutic efficacy.