Heterotopic Liver Transplant: a Novel Rat Model for Acute Cellular Rejection

Camryn Thompson BS, Min Zhang MD, Isaac Alderete BS, Bret Verhoven BS, David Al-Adra MD, Andrew Barbas MD

Background: Laboratory rats are used as the standard animal model for studying liver transplantation (LT) due to their size facilitating microsurgery and easy handling, genome similarity to humans, and post-LT immune responses being similar to that of humans. Although the orthotopic LT is standard, a heterotopic LT model has several theoretical advantages over the orthotopic model for studying acute cellular rejection. By leaving the native liver in situ to support the LT recipient, researchers can study immune responses to strain mismatch transplants without confounders related to biliary dysfunction and anhepatic time. A heterotopic model could also reduce animal suffering by not relying on severe rejection or death as the experimental endpoint, as is common with the orthotopic rejection model. Herein, we validate a novel heterotopic LT model proposed by collaborators at the University of Wisonsin while also evaluating the kinetics of ACR utilizing a different rat strain mismatch.

Methods: Control syngeneic transplants utilized Lewis donors and Lewis recipients. Allogeneic transplants utilized Dark Agouti donors and Lewis recipients. Auxiliary liver donor procurements were performed, then the recipient procedure began with a left nephrectomy. The following anastomoses were then sequentially performed: graft inferior vena cava to left renal vein, left renal artery to graft portal vein, graft bile duct to left ureter. 2 syngeneic and 6 allogeneic transplants were performed. The 2 syngeneic and 3 allogeneic rats had an endpoint of post-operative day (POD) 8, and the other 3 allogeneic transplants had an endpoint of POD 3. Pre-operative and post-operative AST, ALT, serum T-bili, and urine T-bili were obtained. Graft histology was examined by a pathologist and rated using the rejection activity index (RAI).

Results: 2 intraoperative complications occurred leading to intraoperative sacrifice, and the other 6 rats survived until their endpoints. The 2 syngeneic transplants were negative for rejection. The 2 POD3 allogeneic grafts had RAI scores of 5 which correlated with mild rejection. The 2 POD8 allogeneic grafts had RAI scores of 9, correlating with severe rejection. The rats with grafts classified as severe rejection had clear urine at the endpoint and urine was yellow in rats with grafts negative for rejection. This finding correlated with a mean urine total bilirubin of 2.8 with grafts negative for rejection and 0.23 with severe rejection ($p = 0.0292$). Rats negative for rejection had an average AST increase of 40.5 at the endpoint, and an average ALT decrease of 4.5. Rats with mild rejection had an average AST increase of 29.5 and an average ALT decrease of 2. Grafts with severe rejection had an average AST increase of 75 and an average ALT decrease of 21.5. Total bilirubin increased an average of 0.05 for rats negative for rejection, 0 for mild rejection, and 0.65 for severe rejection.

Conclusions: This study validates the feasibility of a novel heterotopic liver transplant model in rats, which provides a valuable platform for studying acute cellular rejection. Urine appearance and bilirubin content was also significantly different between rats with no rejection and severe rejection, providing a noninvasive method of monitoring graft survival.