Department of Surgery
Duke University School of Medicine

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

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

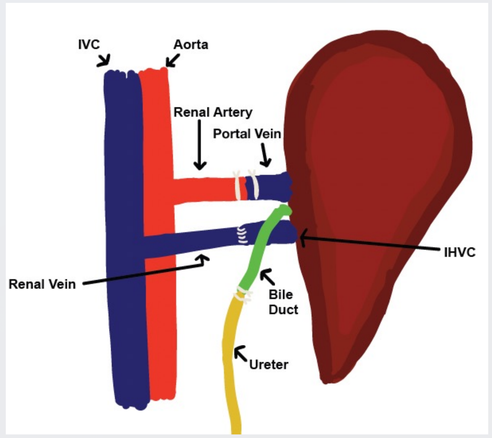
Background

- Laboratory rats are used as the standard animal model for studying liver transplantation (LT) due to:
  1. small size facilitating microsurgery and easy handling
  2. genome is similar to humans
  3. post-LT immune response is similar to that of humans
- Transplants between rat strain mismatches can induce rejection
- Orthotopic model is standard, however our heterotopic model has theoretical advantages:
  - reduce animal suffering – death from liver failure is not the intrinsic endpoint
  - reduce confounders due to biliary dysfunction and anhepatic time
  - facilitate noninvasive monitoring of graft function
- We validate a novel heterotopic LT model proposed by collaborators by U of Wisconsin using different rat strain mismatch

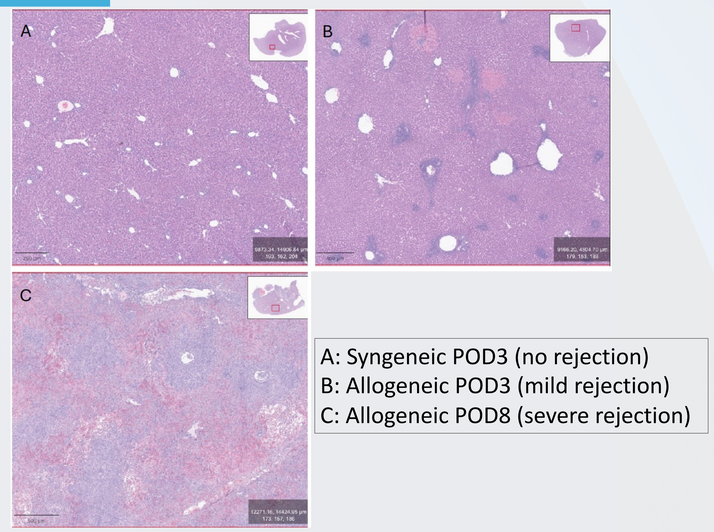
Methods

- Control syngeneic transplants: Lewis donors and Lewis recipients
- Allogeneic transplants: Dark Agouti (DA) donors and Lewis recipients
- Liver donor procurement is performed, then implanted in recipient with following anastomoses:
  1. graft inferior vena cava to recipient left renal vein
  2. graft portal vein to recipient left renal artery
  3. graft bile duct to recipient left ureter
- Rats received subcutaneous heparin and antibiotics in post-op period
- Pre-operative and post-operative AST, ALT, serum T-bili, and urine T-bili were obtained
- Graft histology examined by pathologist using rejection activity index
- Cases:

- 2 syngeneic with endpoint post-op day 8
- 3 allogeneic with endpoint post-op day 3
- 3 syngeneic with endpoint post-op day 8



Results



A: Syngeneic POD3 (no rejection)
B: Allogeneic POD3 (mild rejection)
C: Allogeneic POD8 (severe rejection)

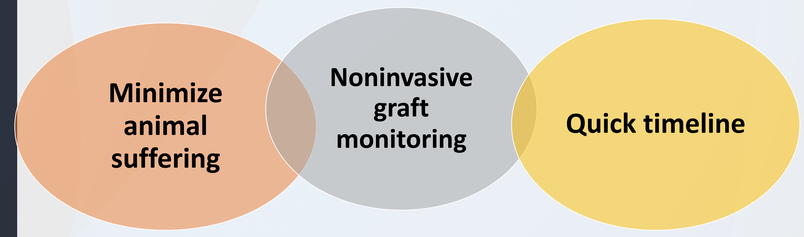
- 2 intraoperative complications -> intraoperative sacrifice
- Remaining 6 rats survived until pre-determined endpoints
- 2 syngeneic transplants were negative for rejection
- 2 POD3 allogeneic grafts had RAI scores of 5 (mild rejection)
- 2 POD8 allogeneic grafts had RAI scores of 9 (severe rejection)
- Rats w/ grafts classified as severe rejection had clear urine at endpoint
- Rats w/ grafts negative for rejection had bile-tinged urine at endpoint
- Mean urine bilirubin was 2.8 with grafts negative for rejection and 0.23 with severe rejection (p = 0.0292)

Sample ID	Portal inflammation	Bile duct inflammation damage	Venous endothelial inflammation	RAI score	Global assessment
Lew-Lew Liver POD8	1	0	0	1	Negative
Lew-Lew Liver POD8	0	0	0	0	Negative
Da-Lew Liver POD3	2	1	2	5	Mild
DA-Lew Liver POD3	2	1	2	5	Mild
DA-Lew Liver POD3	NA	NA	NA	NA	severe
DA-Lew Liver POD8	3	3	3	9	severe
DA-Lew Liver POD8	NA	NA	NA	NA	severe
DA-Lew Liver POD8	3	3	3	9	severe

Sample ID	RAI score	Pre-op ALT	Endpoint ALT	Pre-op AST	Endpoint AST	Pre-op T-bili	Endpoint T-bili
Lew-Lew Liver POD8	1	41	35	72	78	0.3	0.4
Lew-Lew Liver POD8	0	48	45	72	147	0.3	0.3
DA-Lew Liver POD3	5	43	43	64	95	0.3	0.3
DA-Lew Liver POD3	5	40	36	68	96	0.3	0.3
DA-Lew Liver POD3	NA	47	127	76	274	0.3	0.3
DA-Lew Liver POD8	9	42	35	62	131	0.3	0.7
DA-Lew Liver POD8	9	38	74	67	148	0.3	1.2
DA-Lew Liver POD8	NA	43	51	71	131	0.3	1.2

Conclusions

- This study validates the feasibility of a novel heterotopic liver transplant model in rats
- Severe rejection can be achieved within 8 days utilizing DA donors and Lewis recipients, similar to Lewis-to-Brown Norway strain mismatch
- Urine appearance and urine bilirubin content is significantly associated with rejection, providing noninvasive method of monitoring graft survival
- All rats were well-appearing, eating, and had normal activity levels on post-operative day 8, unlike the orthotopic model
- 2 microsurgeons in different labs were able to replicate this procedure, indicating its feasibility
- Short rejection timeline allows for quick iterative experiments



- Future applications are numerous:
  - Studying pathophysiology of acute cellular rejection
  - Testing anti-rejection drugs
  - Experiments with ex vivo liver perfusion
- Future studies should seek to validate and apply this model

Learn more:

Verhoven, B., Zeng, W., Chlebeck, P., et al. Heterotopic Auxiliary Whole Liver Rat Transplant Model Utilizing a Hepaticoureterostomy for Allograft Rejection Studies. JoVE, e66516 (2024).