22nd June, 2016

2015-2016 Kenneth Warren Fellowship Progress Report

Dear IHPBA Research Committee,

I would like to express my gratitude to the IHPBA Research Committee for awarding me the 2015-2016 Kenneth Warren Fellowship in support of my research on pre-transplant graft therapy by gene silencing during machine perfusion in liver transplantation under the mentorship of Professor Robert Porte at the University Medical Center Groningen (UMCG).

Organ shortage has been one of the most crucial problems in liver transplantation. To expand the donor pool, donation after circulatory death (DCD) donors are considered as promising potential donor sources (1). However, DCD grafts are associated with a high incidence of post-operative graft failure and non-anastomotic biliary stricture (NAS) because of their vulnerability to ischemia reperfusion injury and a particularly prolonged warm ischemic time (2,3). To improve these problems, we have conducted research focusing on pre-transplant graft resuscitation.
Among the therapeutic methods for pre-transplant graft recovery, machine perfusion is one of the most promising technology. It prevents preservation injuries by washing out toxic metabolites and delivering oxygen and metabolic substrates (4). Furthermore, continuous delivery of oxygen and metabolic substances can permit non-hypothermic graft preservation during which a specific biological activity level is maintained in grafts (5). Therefore, machine perfusion is also expected to function as an extracorporeal drug delivery system which can maintain the drug uptake ability of the graft. Thus machine perfusion is a key technology for pre-transplant graft therapy.

The purpose of this study is to develop a pre-transplant graft therapy using the gene silencing effect of siRNA. In this method, siRNA would be transfected into the graft cells via the perfusion fluid during extracorporeal machine perfusion.
In our preliminary study, we found that the efficacy of siRNA transfection is particularly low during machine perfusion and optimization of the perfusion parameters (e.g. temperature, perfusion fluid, oxygenation, perfusion route, perfusion rate) and transfection parameters (e.g. transfection method, molecular size of siRNA) is necessary for the extracorporeal siRNA transfection via machine perfusion.
Therefore, We decided to conduct this study in a stepwise manner. We started this study using metformin which is not only an anti-diabetic drug but also a promising drug for pre-transplant graft therapy because the molecular size of metformin is much smaller than siRNA.
Our mouse model of orthotopic liver transplantation following machine perfusion is an ideal experimental model especially in genetic approaches to machine perfusion research. On the other hand, we have developed a perfusion machine for rat livers in which we can control the perfusion parameters more precisely and obtain more information than that for mouse livers. Therefore, we also developed a rat orthotopic liver transplantation model including machine perfusion procedures for more advanced optimization of perfusion parameters. In this rat model, operative procedures were designed to be suitable for dual machine perfusion and as similar to the human operations as possible (e.g. in situ cold perfusion from the aorta, arterial reconstruction between hepatic arteries, Figure).
The target genes of this treatment would be selected based on our previous studies on pathogenic mechanisms of NAS after DCD liver transplantation (6). We will evaluate the therapeutic significances of the candidate genes using knockout or knockdown mice.
After these fundamental investigations, we will develop the pre-transplant graft therapy by extracorporeal transfection of siRNA.

The UMCG is one of the world leading institutes where machine perfusion research has reached the stage of clinical application after a long research tradition. I am glad to be able to study under this ideal circumstance and hope that this research would contribute to the expansion of the donor pool. I look forward to presenting the completed study at the next IHPBA World Congress.

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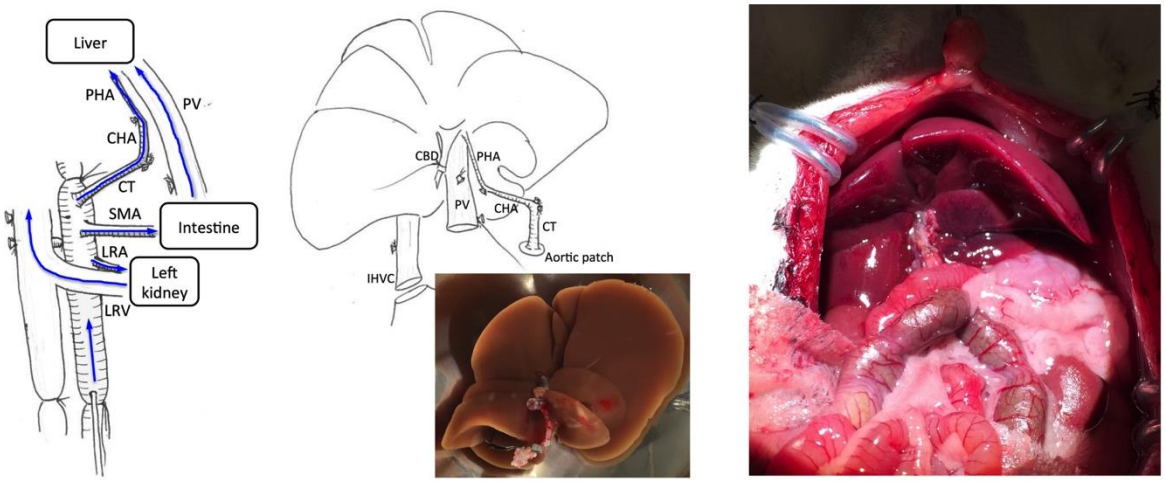
Figure



Reference

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