

THE WINSTON CHURCHILL MEMORIAL TRUST OF AUSTRALIA

The establishment of Australia's first liver cell bank.

A Bob and June Prickett Churchill Fellowship

A report by

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1. Summary and Acknowledgments

This is the report for the 2002 Bob and June Prickett Churchill Fellowship which encompassed visits to internationally recognised hospitals and laboratories in the United States of America and England which have, or are preparing to, establish facilities for human liver cell banking and transplantation. The specific areas of interest were as follows:

- Methods for organ procurement
- Viewing procedures for liver cell isolation
- Discussion on the practical aspects of the procedures
- Viewing laboratory setups
- Best methods for thawing frozen cells prior to transplantation
- Particular liver cell fractions most suitable for transplantation
- Discussion on details and protocols for treatment of a patient

In addition to the fact-finding goals of the Churchill fellowship tour, was the great benefit of gaining actual hands-on experience with the liver cell isolation and freezing methods of particular laboratories.

It is with heartfelt gratitude that I would like to acknowledge the following people and groups:

- Winston Churchill Memorial Trust for considering this project worthy of financial support.
- Bob and June Prickett for their foresight in supporting projects that relate to community health and for which this particular Fellowship is named.
- The medical scientists, doctors and their research staff at each of the facilities I visited, for sharing their vast experience and their advice with me.
- To everyone who took the time to discuss various aspects of our combined goal of cell transplantation.
- Dr Katrina Allen, Rev. Dr Michael Kelly, Dr Paul Wright and Professor Bob Williamson who have provided support and encouragement for this Churchill Fellowship and the project.
- Emeritus Professor Neil Kirkman and Margaret Kirkman for their generous hospitality in Chapel Hill.
- My entire family, especially my parents and mostly Paul, for their unstinting support throughout the entire Fellowship experience.

2. Executive Summary

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Project Description

To observe the protocols/procedures used to isolate and store human liver cells for the purpose of transplantation.

Highlights of Places and People

Chicago Memorial Institute for Education and Research (research arm of Chicago Children's Memorial Hospital) – Humberto Soriano MD, (Head of the Liver Cell Transplantation program) and Dr Ling-Jia Wang, (Technical Supervisor) and their co-researchers. Participated in a liver cell isolation, freezing and subsequent verification thawing and preparatory discussions for an upcoming liver cell transplantation; observed methods for detection of transplanted liver cells.

Pathology Department, University of Pittsburgh – Associate Professor Stephen Strom and his team, who have isolated liver cells from over 900 livers for both research and clinical applications.

University of North Carolina School of Medicine – Professor Lola Reid and her team who are investigating the source of liver-derived stem cells and their potential for future therapeutic uses. Also included proposed uses of liver-derived stem cells for clinical application and how to isolate them.

Incara Cell Technology, Research Triangle Park, North Carolina – Viewing of their large-scale facility used to isolate liver cells with Dr John Ludlow; discussions of sterility considerations for GMP facility with Dr Bushra Agha; and discussions of expanding donor organ sources with Mr Thomas Asfeldt.

Biological and Biomedical Engineering Department, North Carolina State University – Demonstration of the Mechanical Dispersion Device (liver chopper), designed by students for the purpose of dispersion of a liver with minimal (or no) handling.

King's College, London – Dr Anil Dhawan and his team who kindly showed me their GMP facility for liver cell isolation and storage.

Lessons learnt

Australia's first Liver Cell Bank (sponsored by Rotary) would greatly benefit from the following recommendations:

- Provision of a Good Manufacturing Practice (GMP) facility in which the work is conducted;
- Implementation of sterility tests throughout the cell isolation procedure;
- Informing donor co-ordinators nationally that transplant grade/quality organs used for child transplantation or rejected for transplantation could be utilised for the purpose of cell transplantation, thereby increasing the source of material for the cell bank.

Dissemination of information

It is anticipated that the information gained will be disseminated through the Rotary Clubs in Victoria and more importantly through the Red Cross donor co-ordinators. An article in organ donation newsletter 'Life Gift' highlighting the work of the cell bank has already been published and a follow up article is anticipated. Also this work will be presented at relevant professional society conferences and published in relevant scientific journals.

Implementation

Many of the practical aspects of cell isolation can immediately be implemented. These include basic steps, such as microbiological tests, testing for cell viability of cultured cells after isolation and the methods of maintaining the procedure at 4°C. Construction works are in progress at the Royal Children's Hospital to build a GMP facility that will be operational by mid-2003. A presentation at the organ donation conference in June 2002 has disseminated information nationally and assisted us in approaching all Australian organ donor co-ordinators with the aim of expanding our liver organ sources.

3. Program

United States of America

Chicago, Illinois 3 April – 30 April 2002

Children's Memorial Hospital
Children's Memorial Institute for Education and Research
Dr Humberto Soriano, Dr Ling-Jia Wang, Dr Françoise Smet, Ms Yong Meng Chen

Pittsburgh, Pennsylvania 1 May – 4 May 2002

University of Pittsburgh
Associate Professor Stephen Strom

North Carolina 5 May – 16 May 2002

Chapel Hill

University of North Carolina
School of Medicine
Professor Lola Reid
School of Pharmacy
Dr Ed LeClyse

Research Triangle Park

Incara Cell Technology

Raleigh

North Carolina State University
School of Biological and Biomedical Engineering

United Kingdom

London, England 27 May 2002

King's College, Denmark Hill
Institute of Liver Studies
Dr Anil Dhawan, Dr Robin Hughes, Dr Ragai Mitry

4. Human Liver Cell Banking

4.1 Introduction

Liver disease and liver failure are major causes of death in the Australian community. In many instances the only treatment available for these people is whole liver transplantation. This involves major abdominal surgery, extensive hospitalisation and is an expensive procedure. These difficulties are in addition to the extensive waiting list for an organ, where the wait can be quite considerable because there are simply not enough donor organs for the increasing numbers of ill people.

Liver cell transplantation offers an alternative to whole liver transplantation for some people. This proposed method of treatment involves isolating cells from donated livers which may have been rejected for whole organ transplantation, or for which only half of the organ was used for paediatric transplantation. The cells are then purified and frozen to be thawed at a later stage for infusion into a patient or alternatively, freshly isolated cells may be used. There are multiple advantages/benefits, including:

- (i) cells isolated from one organ may be used to treat many patients, thereby making more efficient use of the donated organ;
- (ii) cells are transfused by a catheter that is inserted radiologically into the portal vein, obviating the need for major abdominal surgery and the subsequent hospital stay.

Liver cell transplantation has been shown to be a safe clinical procedure and effective for people with metabolic liver diseases. It can also serve as a bridging therapy to whole organ transplantation, that is, by extending the life of patients on organ waiting lists until they are able to receive a whole liver transplant (Strom *et al.*, 1997; Fox *et al.*, 1998; Strom *et al.*, 1999). Many successful animal studies have supported the importance of this new clinical procedure (David *et al.*, 2000; Wang, *et al.*, 2001).

A liver cell bank in Australia is urgently needed and would clearly benefit our society. Consequently such a facility is currently being established at the Murdoch Children's Research Institute, in the Royal Children's Hospital, Melbourne, Australia.

4.2 Liver procurement

In the USA, obtaining a liver for liver cell isolation with the end-point of transplantation can be a daunting affair. The majority of liver cell groups use government-recognised Organ Procurement Organisations (OPO) to obtain the tissue. Numerous OPOs exist throughout the USA and the more astute groups have contacted many of the OPOs to inform them of their need for liver tissue. OPOs operate all over the USA and are able to freight tissue across the country.

Research groups also compete for liver tissue (for their own areas of interest) with that obtain livers to derive liver cells that can be sold as cell cultures for different metabolic/toxicological/pharmacological studies. Thus the group best able to reimburse the OPO for their efforts is most likely to obtain the tissue. In many instances, such tissue has already been rejected for whole organ transplantations for a variety of reasons, such as "fatty liver" (a condition termed 'steatosis').

However, not all liver cell groups are in this situation as some are located within a hospital with an active liver transplant unit. The tissue obtained through the hospital is transplant-grade tissue, and is most likely a cutdown liver section where the other half has been transplanted into a child.

The group in North Carolina has proposed the use of cadaveric liver tissue as a source of organs both from adult and neonatal sources. The research group, led by Prof. Reid, also isolate liver stem cells which are reported to be more robust than large normal mature healthy liver cells, have a better survival rate after freezing and are possibly better at regenerating the liver than mature liver cells. The stem cells are purported to tolerate a longer period without oxygen at warm temperature, which may occur while obtaining the liver but is detrimental to mature liver cells.

Recommendation(s)

Raise awareness of the liver cell bank program in other Australian states through their donor coordinators especially those in Qld and NSW, which have the highest rate of donated livers in Australia. Once the program is established, investigate the use of cadaveric liver as a source of liver cells.

4.3 Liver cell isolation procedure

The method for isolating functioning liver cells dates from the 1960's, when it was first performed in animals. Since that time, various modifications have been incorporated to improve the technique and essentially all researchers using isolated liver cells for their studies and for clinical applications use their own adapted version of the original technique. It involves the stripping of calcium from the connective tissue between the liver cells to begin loosening the cells, followed by biological shears ('collagenase enzyme') to destroy collagen fibres connecting liver cells. This process separates the liver into individual cells that are carefully washed before they are frozen in a rate-controlled freezer and stored in liquid nitrogen.

There is much conjecture as to the best method to employ for this work. Everyone is in agreement that calcium must be removed first by using a buffered salt solution lacking calcium (with or without a calcium-chelating agent). All research groups also agree that collagenase is required, however, they differ on the quantity required and the duration that the liver is digested. This may subsequently affect the ability of the liver cells to attach to cell culture plates (known as "plating efficiency", PE) which is one parameter used to assess cell viability, as cells must be functional to actively synthesise proteins. Plating efficiency is a key parameter for many researchers assessing the quality of cells collected. Two clear patterns have emerged from the laboratories visited. Firstly, a shorter collagenase exposure time routinely yields a high number of liver cells with excellent viability however the PE is relatively low (approximately 20%). On the other hand, when the liver is exposed to collagenase for a longer time, slightly lower cell viability is obtained, but the PE is routinely around 60-70%.

The media used for freezing cells also varied in the different laboratories. Cryopreservation is a specialised field and it is an area which only a few liver cell groups have investigated. Most of the groups visited are currently using published methods. Incara Cell Technologies are trialing their own freezing media and also freezing cells within a biological matrix. The latter gives the cell membranes stability but it is presently difficult to retrieve the cells after they are thawed.

Recommendation(s)

No fixed type or brand of collagenase is used by all the groups. The only criterion thus far is that the enzyme is free of endotoxin (bacterial cell wall fragments) to reduce the risk of endotoxic shock in the recipient patient. The type of solution and media utilised for cell isolation appears not to be

as critical. The time of exposure of the liver to collagenase is more of an issue and it is recommended that a longer enzyme digestion time is preferable to a faster digestion time. The freezing media presently used by the groups is performing satisfactorily.

4.4 Dispersion of digested liver

It is desirable that minimal handling of the liver cells is required. Once the collagenase has completed its task, the liver becomes a bag containing floating liver cells and the cells need to be dispersed. Cell dispersion is recognised as one of the most time-consuming aspects of the work. The simplest and least complicated method is to firmly hold the liver and shred it with a scalpel. The second method is to transfer it into a vessel and cut the liver open with a pair of scissors. This method eliminates the need to handle the tissue after the initial procedure. The biomedical engineering students at North Carolina State University have designed the most promising mechanism for dispersing liver cells. It is a self-contained box in which the liver is flushed of organ preservation fluid and liver cell digestion occurs. Once the tubing for that part of the method is removed, the lid with suspended blades is used to chop the organ so that the cells are released. It is similar to hand operated vegetable choppers in domestic kitchens. The cell suspension then drains into a series of sieves with decreasing pore sizes and is then bagged and washed prior to freezing. This design allows the procedure to be recognised as a 'closed' system that is highly desirable because once the liver is placed in the box and the digestion completed, no further handling is done. The cutting motion of the blades work so efficiently that very little intact tissue remains as the entire organ is accessed unlike using scalpels or scissors. The chopping reduces the time of this aspect of work by half.

Recommendation(s)

Reducing the amount of time spent between receiving the organ and the final freezing of isolated cells, is one of the key factors for increasing the viability of the cells. The dispersion of the liver to obtain a cell suspension is recognised as one of the most time consuming parts of the procedure. Prof. Reid has offered that we trial the liver dispersion device in conjunction with Incara Cell Technology and to offer feedback and comments on the system. This offer will be pursued as it would increase the efficiency and efficacy of the overall procedure.

4.5 Liver Cell Bank Database

The database provides information that is of immediate importance for comparisons between banked liver cells and consists of the information relating directly to the donor, the liver after harvesting, the isolation procedure and the yield of cells obtained and banked. All other information linked to the isolation procedure (e.g. chemical and batch numbers) are recorded on proformas and systematically filed.

4.6 Other variables in the technique

There were also small hints and advice offered by different groups which in themselves do not warrant a separate section but as a whole significantly contribute to the liver cell banking procedure. These include:

- Suturing techniques to maintain the position of tubing during liver cell isolation;
- Maintaining the cells at cold temperatures, after the cell isolation procedure, with either trays of ice or a reticulated cooling system;

- The use of three-way taps and syringes to ascertain the correct volume of cells to be frozen and allow the transfer of cells into special cryostorage bags in a sterile manner;
- The use of specific media for the maintenance of cells *in vitro* to check for viability and plating efficiency; and
- The duration which the cells are maintained in tissue culture to check for viability.

5. Conclusions and recommendations

In March 2000, the Rotary Liver Cell Bank was initiated at the Murdoch Children's Research Institute at the Royal Children's Hospital in Melbourne. Over the past two years, we have been able to inform liver transplant teams and co-ordinators of our program, as well as the wider community groups. This education is continued through the media, our own institute newsletters and annual report, and at scientific conferences and Rotary Club meetings.

In the future, we need to:

- implement the best practices observed in other countries, that would also comply with Australia's own Therapeutic Goods and Administration guidelines and the internationally-recognised Good Manufacturing Practises guidelines.
- continue the new collaborations with our overseas colleagues as it forms an immediate line of communication with international leaders in this field.

By progressing under the guidance of our international and local collaborators, it should be possible to establish the Rotary Liver Cell Bank as one of the best programs for human liver cell banking and transplantation in the world. This should ensure that Australians would benefit from this recently validated cell therapy. In time, the program could be expanded nationally and perhaps cater to parts of South East Asia as well.

This report provides an overview of the liver cell bank programs overseas and in Australia. Details of the actual protocol for liver cell isolation procedures may be obtained directly from the author. The Churchill Memorial Trust, through the sponsorship of Bob and June Prickett, has substantially contributed to the advancement of the liver cell transplantation program in Australia.

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