Poster Session 3 – Drug Metabolism

178
Patient, operative and processing variables: their effect on the quality of isolated human hepatocytes and subsequent function

T. Lloyd, R. Patel*, G. Crees*, S. Chavda*, S. Orr*, D. Berry and A. Dennison

Department of Surgery, Leicester General Hospital, Leicester, LE5 4PW and *UK Human Tissue Bank, Innovation Centre, De Montfort University, Leicester, LE1 5XY, UK

The UK Human Tissue Bank receives donations of human liver from surgical resections and non transplantable livers from cadaveric multi organ donors (MOD) with the approval of NHS Multi Centre and Local Research Ethics Committees. These tissues can be used for the isolation of the functional cells (hepatocytes), which are subsequently used for drug metabolism, toxicity studies, bioartificial liver and cell transplantation research. A multi-variant analysis of the influence of patient and operative factors, nature of tissue and the method of tissue processing is ongoing. The effect of these factors on the quality of human hepatocytes is being evaluated. The results to date reveal optimal donors and conditions for hepatocyte isolation, which will guide the usage of each donation.

Information being recorded to facilitate this ongoing analysis includes patient age, sex, BMI, blood group, smoking and alcohol history, routine pre-operative bloods and pathology. Operative factors of interest include nature of resection, Pringle time and cold ischaemic time. Isolation factors being recorded include size of specimen, time for overall isolation, collagenase digestion time, method utilised including nature of buffers and cannulation type. Results recorded include cell yield, viability (assessed by Trypan blue exclusion) and functional activity. Differences in the nature of the donation in terms of source of tissue; surgical or whole organ, and the distance from theatre to processing laboratory.

From October 2001 to date, data has been collected from donations received from 63 patients undergoing liver resection and 5 whole livers. Mean viability ($\pm$ s.d.) of 83 $\pm$ 7% has been obtained from these specimens with a mean yield of 5.4 million hepatocytes/g of tissue. Multivariant analysis revealed that, hepatocyte yield (cells/g of tissue) was reduced by decreasing body mass index ($P = 0.025$), and visual assessment of the success of tissue digestion (good (1.9 $\pm$ 0.8 cells/g) or moderate (1.22 $\pm$ 0.8 cells/g) gave a good indicator of increased yield ($P = 0.004$). Hepatocyte viability was also improved with decreasing patient age ($P = 0.034$), increased liver steatosis (88.0 $\pm$ 6.5% vs 81.91 $\pm$ 8.7) ($P = 0.035$) and the nature of the cannulation method employed where a venflon (86.88 $\pm$ 7.6%) secured by suturing was superior to an animal catheter (79.89 $\pm$ 8.63%) secured with medical adhesive ($P = 0.002$). Viability from surgically resected liver is approaching significance in superiority over hepatocytes isolated from MOD $P = 0.09$. No significant patient or isolation factors predicted cellular function on culturing.

Our work to date reveals, given the precious nature of human tissue, that all donations should be considered for the isolation of human hepatocytes for research. Human liver donations can also be used for the extraction of subcellular fractions, preparation of precision cut organ slices which can be utilised in pharmacotoxicological research.

179
Human hepatocyte provision for the pharmaceutical industry — consumer views and future challenges

T. Lloyd, S. Orr*, J. Trafford* and A. Dennison

Department of Surgery, Leicester General Hospital, Leicester, LE5 4PW and *UK Human Tissue Bank, Innovation Centre, De Montfort University, Leicester, LE1 5XY, UK

The UK Human Tissue Bank has been established for four years under the approval of NHS multi centre and local research ethics committees. One of its specialist roles is the acquisition of surgically resected and non-transplantable livers from cadaveric organ donors, for hepatocyte extraction. This is now established as a routine technique. A recent 15 month internal review highlighted a yield of 3.1×10^{10} hepatocytes from 7.7 kg of tissue, with acceptable and consistent cell viability. Despite a published demand for hepatocytes for drug development, and an expanding customer base, only 42% of these cells on initial isolation had been utilised. A telephone survey was therefore conducted to explore this paradox.

TL, who has had no previous contact with the end users of the researchers, conducted the telephone survey. The survey asked questions concerning how the researchers used the cells, viability of the cells on receipt (measured using trypan blue exclusion) by the researcher, logistics and problems encountered using human tissue and plans the researchers may have for future use. Researchers were also asked for their opinion on receiving cryopreserved cells and cells plated and transported during working hours. Two groups were identified: group 1 were regular users, including users accepting cells at unsocial hours; group 2 were infrequent users. Five researchers were contacted in group1, and seven in group 2. The results showed that all researchers stated that hepatocytes becoming available at unsocial hours created problems due to personnel and health and safety issues. Last minute complications, such as operation cancellations or patients being inoperable, caused increased frustration for group 2.

There was overall satisfaction from both groups with quality of hepatocytes (cell viability (trypan blue exclusion) and function (measured according to end users internal standard operating procedures). All researchers were satisfied with the notification process of cell availability and transport of cells to their destination. Seven researchers expressed interest in pre-plated cells and three in receiving cryopreserved cells.

Easily accessible good quality human hepatocytes are now routinely available to researchers in the UK. To maximise the use of hepatocytes the problem of availability at unsocial hours after 1700 h and at weekends needs to be resolved. The tissue bank is seeking improvement to this situation with active research into cryopreservation, and the transport of plated hepatocytes to researchers. Cold storage of liver tissue overnight before isolating cells is also being explored. Other future plans will include increasing the number of donations and processing centres to maximise the use of this precious resource.

180
Studies into the cryopreservation of human and hepatocytes

T. Lloyd, J. Wilkinson, S. Orr*, D. Berry and A. Dennison

Department of Surgery, Leicester General Hospital, Leicester, LE5 4PW and *UK Human Tissue Bank, Innovation Centre, De Montfort University, Leicester, LE1 5XY, UK

Cultured human hepatocytes are widely considered the model of choice for pharmacological investigation. Industrial scale hepatocyte isolations have been undertaken at the UK Human Tissue Bank, from discarded surgical liver tissue and multi organ donors, for the use in pharmaceutical and toxicology studies. Many hepatocytes are, however, wasted if an isolation has a particularly high yield or there is low demand for cells. A protocol for the cryopreservation and storage of these excess cells, with preserved functional ability, is therefore desirable. The study was approved by multicentre and local research ethics committee, and all patients were approached 24 h before surgery and informed consent obtained.

Hepatocyte isolations were undertaken using a modified two step collagenase protocol from discarded surgically resected liver tissue and multi organ donors. Hepatocytes were immediately cultured on 12 well plates to act as controls. Two experiments were undertaken, the effect of cellular concentration (2.5×10^6 cells/mL, 5×10^6 cells/mL, 1×10^7 cells/mL and 2×10^7 cells/mL) within the freezing solution and the effect of pre-incubation (for 1 h and 16 h), following the isolation and prior to freezing, on subsequent cell function. Parameters measured in culture were LDH leakage, viability measured by Trypan Blue exclusion, bilirubin conjugation, lignocaine metabolism (CYP 3A4), and attachment by total protein present (BIORAD assay). All cryopreservation was undertaken in 1.8 mL cryovials in a cryopreservation solution containing 10% DMSO and 20% foetal calf serum in

an isopopranol controlled rate device. A total of 16 individual liver specimens were studied for the concentration experiment and 8 liver specimens for the pre-incubation. The result from each liver was expressed as a mean of 6 repeats. Statistical analysis was by General linear model.

A reduction in the viable cells to 61 ± 6% of those pre-incubated for 1 h and 50 ± 18% after 16 h, occurred. Following cryopreservation, the mean return of hepatocytes not pre-incubated was lower than pre-incubation for 1 h (45.6 ± 17.4% vs 48.1 ± 26.0%) but this was not statistically significant ($P = 0.98$). Incubation made no significant impact on attachment, LDH leakage, bilirubin conjugation or lignocaine metabolism. Cell return from the 5×10^6 (35.2 ± 14.8%) was significantly higher than that from 2×10^7 concentration (15.5 ± 11.1%) ($P = 0.01$). In the functional parameters and attachment the wide inter-individual variation revealed no statistical advantage in any of the concentrations.

Pre-incubation showed potential advantages but did not reach statistical significance. The optimal concentration of cryopreservation also needs further investigation.

There was no statistical difference between the functions of the fresh and cryopreserved hepatocytes despite the intervention. Although optimisation of a cryopreservation protocol will continue, efforts should be directed to increase the cell return, as function appears adequate.