CHIEF SCIENTIST OFFICE

CODE: TCS 18/15

RESEARCH PROJECT BRIEFING

A new method for pre-clinical drug testing in human kidney tissue

AIMS

There are no drugs to treat acute kidney injury, a common and serious condition. Drugs that work in mice often do not work in humans. We have found a drug that treats acute kidney injury in mice called a RIPK1 inhibitor. Before spending millions of pounds on a drug trial in humans, we wanted to develop ways of testing drugs on human kidney tissue in the lab. This would give us better information about whether the drugs are likely to work if given to people in a trial.

This project aimed to develop an experimental model of acute kidney injury in fresh human kidney tissue and to do preliminary tests of a new drug treatment for acute kidney injury in this model. We also aimed to obtain samples of human kidney tissue from kidney transplant donors to see if the “target” of this drug was present.

KEY FINDINGS

- Precision cut human kidney slices can be obtained and cultured from fresh human kidney that have just been removed
- The kidney tissue in culture contains relevant portions of kidney tubules and intact glomeruli
- The process of obtaining and culturing the tissue slices induces gene changes that are consistent with acute kidney injury
- The slices do not live for long and at 15 hours of culture there is evidence of necrosis in the central portion of the slices
- We were not able to demonstrate clear effects of drug treatment in the kidney slices
- Biopsies obtained from donor kidneys after retrieval demonstrated activation of the pathways that the drugs of interest target

WHAT DID THE STUDY INVOLVE?

- Retrieval of fresh human tissue from nephrectomy specimens followed by the development of a method for “precision cut human kidney slice” culture
- Histological, metabolic and gene expression analysis of cultured human kidney slices
- Drug treatment of cultured human tissue
- Immunohistochemical assessment of donor kidney biopsies

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

1. Precision cut human kidney slides can be obtained and cultured from tumour nephrectomy specimens and are suitable for gene, histological and protein analysis

During the study 6 human kidneys were obtained, cores were taken and sliced for tissue culture. Using a mitochondrial dye, it was demonstrated that the slices had active mitochondria up to 15 hours after retrieval.
After 15 hours of culture high quality, purity and yield RNA, suitable for next generation RNA sequencing was obtained from almost all samples.
ATP levels were measured in the slices demonstrating minimal loss of ATP content in slices at 15 hours compared to retrieval samples.
The expression of an energy-dependent proximal tubular membrane transport protein (OAT-1) was preserved and even increased after 15 hours of culture compared to baseline samples suggesting ongoing tubular viability.

Active mitochondria in precision cut kidney slices

Mitotracker red live tissue staining of precision cut human kidney slices. Mitotracker red stain is only taken up by active mitochondria. Negative control was treated with the mitochondrial posion antimycin A for 2 hours before addition of mitotracker stain.

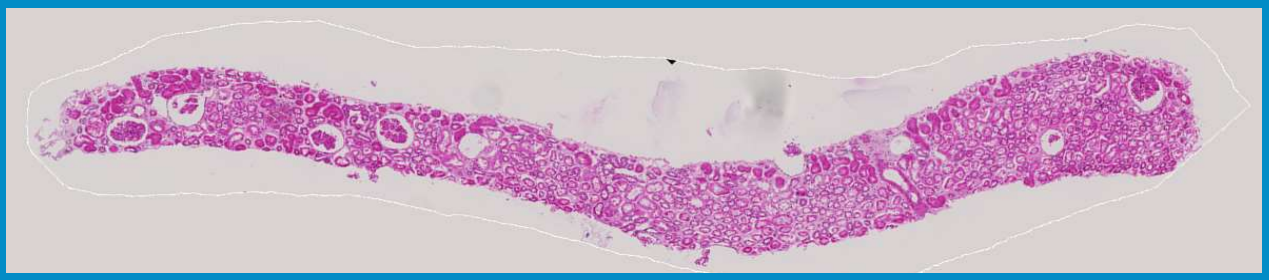
WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

2. Precision cut human kidney slices show evidence of tubular death in the "core" at 15 hours

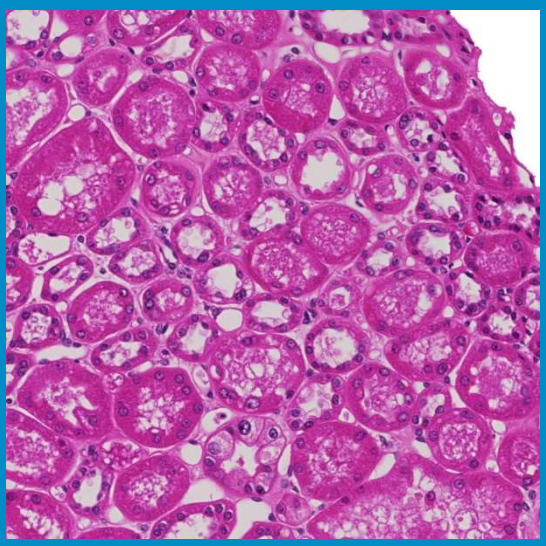
Although there were signs that the tissue was living, histological assessment demonstrated that the tissue slices had evidence of tissue death in the "core" of the slice. Appearances suggested that tubules on the surface were intact, whilst those deeper inside showed signs of death. This may be because oxygen was not able to reach these deeper tubules.

This demonstrates the importance of slicing the kidney as thin as possible. Variation in the thickness of the slices was seen demonstrating the technical difficulties associated with the procedure.

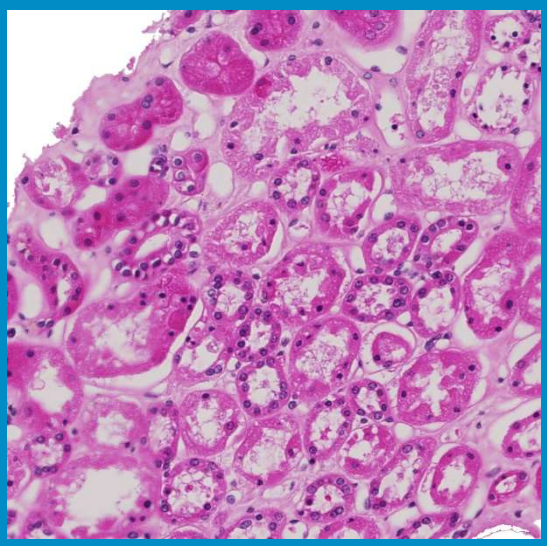
Precision cut human kidney slice in cross section



Baseline sample



Slice after 16H



Histology (H+E) stain of precision cut human kidney slices. The slice at the top is in cross section and is 200µm thick (1/5th of a mm). Slice on bottom right shows evidence of increasing necrosis further deep in the slice.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

3. Precision cut human kidney slices demonstrate features of acute kidney injury

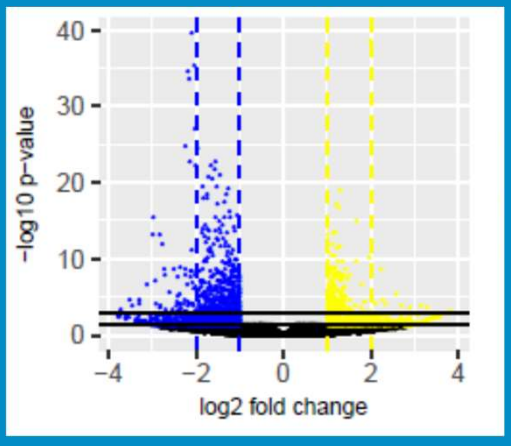
Using targeted quantitative PCR, it was demonstrated that the expression of inflammatory genes associated with acute kidney injury such as monocyte chemoattractant protein and interleukin 1β were markedly increased in samples that had undergone 15 hours of culture compared to baseline samples. TNF-α was also increased although to a lesser extend.

Whole transcriptome sequencing was performed on 3 of the 6 kidneys (3 samples per group per kidney = 9 samples sequenced per group, 36 samples sequenced in total). 1358 genes were significantly regulated after 16 hours of culture compared to baseline controls. Many genes associated with acute kidney injury were significantly regulated. Differences were seen in gene fold-change between drug treated and control samples at 15 hours of culture however no statistically significant differences were found between drug treated and control samples on initial analyses after adjustment for multiple comparisons.

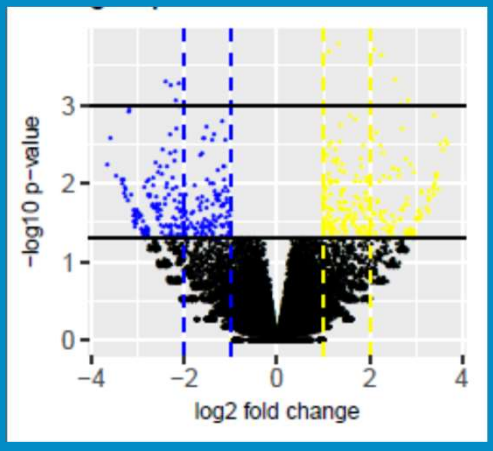
Bioinformatic analysis of sequencing results is ongoing to determine how relevant the transcriptomic signatures found in the precision cut kidney slice model are to human kidney disease.

Next generation RNA sequencing of precision cut human kidney slices: volcano plots

16 hours vs baseline



16 hours vehicle vs 16 hours RIPK1 inhibitor



Each dot on the graphs represents a gene. Blue dots are reduced and yellow dots are increased in expression In the test sample compared to the control. The left gaph shows marked differences in gene expression between The tissue slices at 16 hours of culture vs baseline controls. The right graph show that whilst there were some "fold change" differences in gene expression between samples treated with drugs and those that weren't, we were not able to say for sure if this was due to chance or because of the drug treatment. Further analysis is ongoing to understand these effects.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

4. There is evidence of necroptotic pathway activation in human donor kidney biopsies

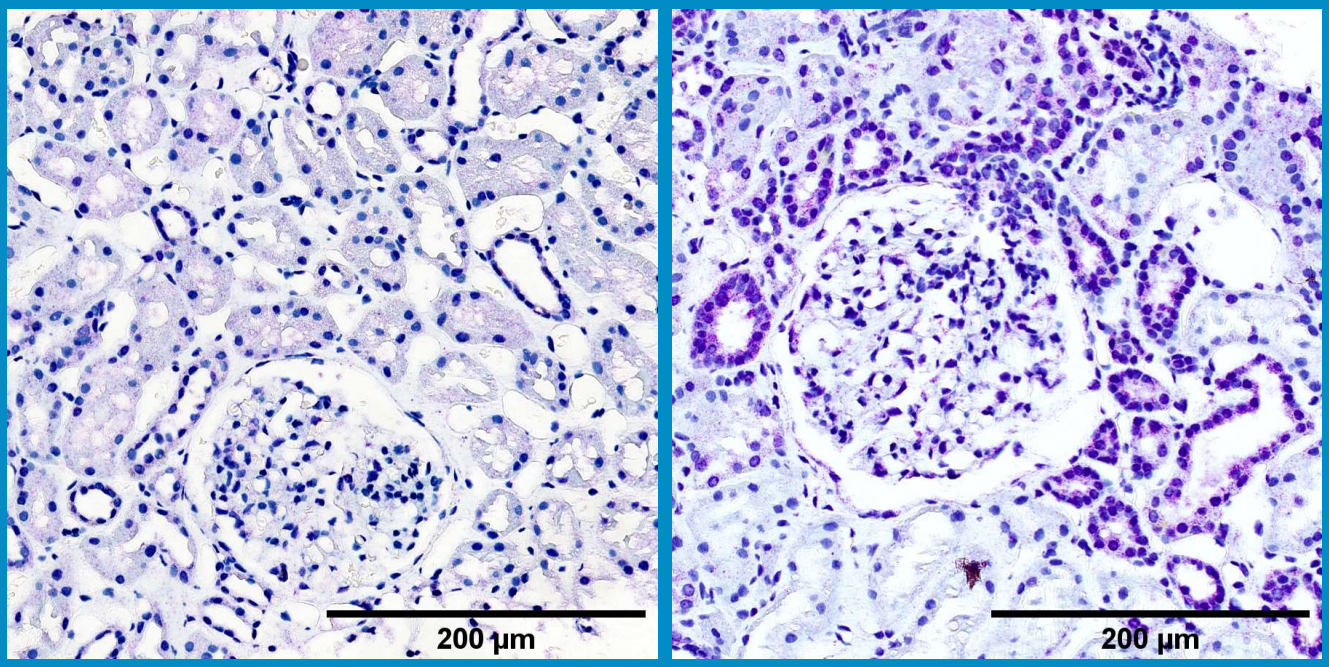
The drug that we are interested in for the treatment of acute kidney injury targets a protein called RIPK1. RIPK1 leads to activation of the necroptotic pathway which results in cell death and inflammation. The protein that executes necroptosis is called MLKL. MLKL activation is seen by its phosphorylation. This has not been identified in acute kidney injury before. Using the quality in organ donation (QUOD) bioresource we obtained 16 biopsies of donor kidneys.

Phosphorylated MLKL was demonstrated in tubules of donor kidneys but was not present in control samples. It tended to be present in tubules that appeared injured. There was no obvious association of MLKL phosphorylation with clinical outcome in these samples however this exploratory data provides the first evidence that the necroptotic pathway may be activated in injured renal tubules in humans.

Phophorylated MLKL (purple)/ necroptotic activation in kidney donor biopsies.

Healthy perfused kidney

Donor kidney biopsy



Healthy perfused kidney biopsy vs donor kidney biopsy after retrieval from donation after cardiac death, phosphorylated MLKL is identified as deep purple, blue dots are cell nuclei.

WHAT IMPACT COULD THE FINDINGS HAVE?

- This project demonstrated that precision cut human kidney slices represent a model of acute kidney injury
- Preliminary data suggest that it can be used to test the impact of drugs on gene and protein expression in primary human kidney tissue
- Testing of drugs on primary human tissue in the lab before clinical trial will improve the success of drugs in clinical trials and increase the speed of development of drugs for kidney failure

HOW WILL THE OUTCOMES BE DISSEMINATED?

- The results will be presented at national and international scientific congresses
- Once analyses are complete, the results will be published in a peer reviewed journal
- Local communication of the precision cut kidney slice model has been undertaken, expertise and learning has been shared with other groups
- The experience and development of human tissue retrieval ethics and procedures have enabled other lines of investigation to be established in kidney disease research at our institution through communication with other scientists at our institution

CONCLUSION

- We have developed a model of acute kidney injury in primary human kidney tissue
- The model developed allows the effect of new drugs for acute kidney injury can be tested in primary human kidney tissue
- The main effects that can be tested are gene and protein expression

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Additional Information