Testing Rat Cells as a Drug Toxicity Model

Evaluating Rat Primary Proximal Tubule Cells as an *In vitro* Model of Nephrotoxicity Tests

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Introduction

- Nephrotoxicity, which is toxicity of the kidney, is a common adverse reaction to drugs. It needs to be detected early on in pre-clinical screenings before drugs can be used in humans. Nephrotoxicity may occur when toxic compounds from broken-down drugs accumulate in kidney cells.
- The body's response to this damage is to elevate levels of repair molecules, which can sometimes be used as biomarkers of nephrotoxicity, and can be quantified.
- In this project, the utility of rat proximal tubule cells (PTCs) as a model of nephrotoxicity is investigated, by assessing their production of one of the main biomarkers kidney injury molecule-1 (KIM-1), in the presence of well characterised nephrotoxins (cisplatin and polymyxin B).
- Changes in megalin and cubilin levels, both cell receptor molecules thought to play a role in the accumulation of toxins in kidney cells, are considered in this *in vitro* model. The cholesterol-lowering drug rosuvastatin will also be used, since it affects the accumulation of toxins in the kidney.
- This is of importance as the rat PTCs model will allow drug screening studies to be conducted at a reasonable cost and much earlier in the process.

Aims

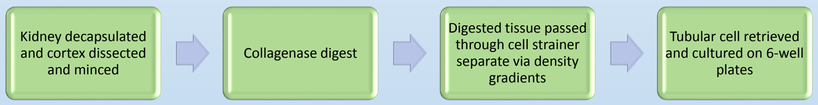
Determine the utility of a nephrotoxicity testing platform by:

- Treating cells with range of concentrations of cisplatin (chemotherapeutic drug) and polymyxin B (antibiotic), in the presence and absence of rosuvastatin (known to have protective effects)
- Measuring the amount of KIM-1, megalin, and cubilin produced at the mRNA level

Methods

- Isolation of rat proximal tubule cells (PTCs) as previously described[1] (figure 1)
- Confluent cells were then treated with cisplatin (5, 10 µM) or polymyxin B (100, 150 µg/ml), in the presence or absence of 50 µM rosuvastatin for 24 hours
- Cell total RNA was isolated and quantified before reverse transcription to cDNA
- qPCR was performed to quantify the relative amount of KIM-1, megalin, and cubilin in the cells

Figure 1



Results

Figure 2: Amount of KIM-1 (% of control)

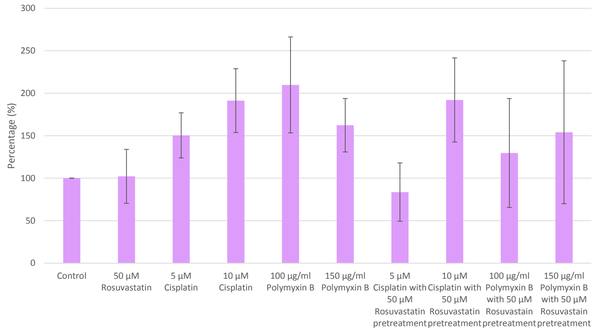


Figure 3: Amount of Megalin (% of control)

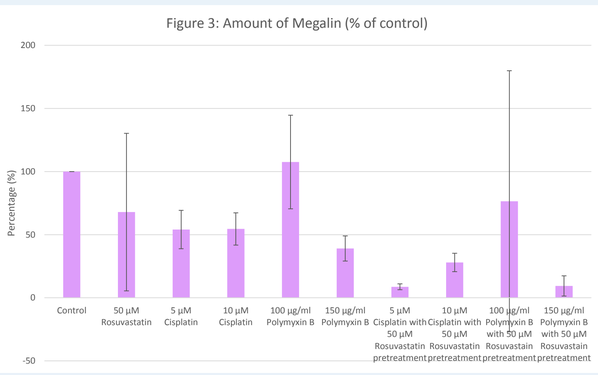
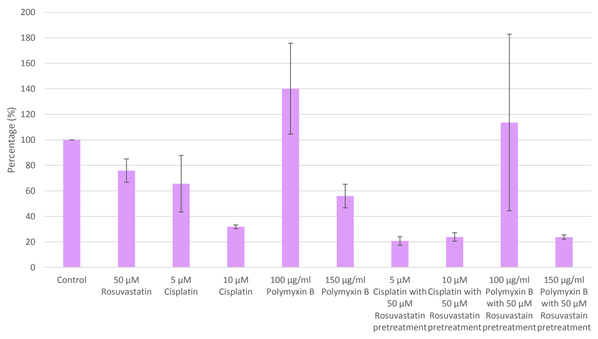


Figure 4: Amount of Cubilin (% of control)



Discussion

- *In vivo* experiments from literature explain that KIM-1 mRNA levels should rise in response to kidney toxin exposure[2,3]. Since the results showed a general rise of KIM-1 mRNA levels between the control and toxin-exposed cells (Figure 2), the PTC model follows the expected trend and thus reacts similarly to *in vivo* models.
- Additionally, the presence of rosuvastatin decreased KIM-1 mRNA levels when cells were treated with both nephrotoxins. This was also expected as rosuvastatin has been shown to reduce the amount of toxin uptake by kidney cells[2].
- Megalin and cubilin levels were lowered (Figure 3 and 4) as a result of rosuvastatin exposure, which may be due to their implications in receptor-mediated endocytosis of toxic drugs[4].
- Since rosuvastatin is already known to reduce cell uptake, it would make sense that megalin and cubilin levels are decreased to allow less receptor-mediated endocytosis. Their levels follow the same trend in response to different treatments, which is also expected as they work together.

Conclusion/Further Study

- It can be concluded that the *in vitro* model tested is a viable testing platform for drug development, because it is reflective of *in vivo* models.
- To gain better understanding of the viability of the rat *in vitro* model, data can be generated from human PTC models and compared to rat data.
- Quantifying other biomarkers may also be considered.

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References

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