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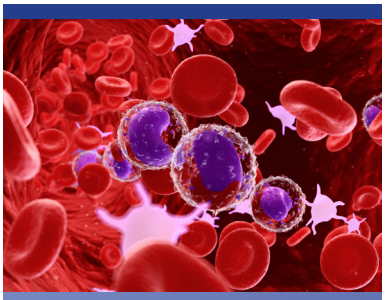
In vitro **Toxicology**

PBMC Cytotoxicity

Background Information



'*In vitro* high throughput assays utilising primary human immune cells will significantly enhance our capabilities to predict candidate drugs with potentials to cause rare but occasionally fatal hypersensitivity reactions during early stages of preclinical drug development.'

- Drugs designed for their therapeutic benefits can unfortunately also be associated with unwanted hypersensitivity reactions, which can be fatal in a small proportion of susceptible individuals[1].
- Drug hypersensitivity is an unintended adverse drug reaction with an immunological aetiology to an otherwise safe and effective therapeutic agent.
- In addition to inter-individual differences in detoxification pathways, the immunoregulatory system that preserves tolerance to neoantigens varies between individuals and can be influenced by genetic and environmental risk factors as well as disease.
- Drug-specific T-cells are implicated in drug-induced end organ damage and have been isolated from cutaneous blister fluid and liver biopsies[2, 3].
- Human peripheral blood mononuclear cells (PBMC) primarily consist of lymphocytes (B-cells, T-cells and NK cells). Due to the overwhelming abundance of T-cells within the PBMC population (70-85%), these cells provide a useful and appropriate model with which to study the immunological mechanisms associated with drug-mediated hypersensitivity *in vitro*.
- The PBMC cytotoxicity assay, which utilises cells isolated from multiple individuals, provides a high throughput assessment of the cytotoxicity of candidate compounds *in vitro*. It can also provide an initial insight into how immune cells from different donors respond to candidate drugs in development.

Protocol

Cell Type
Human peripheral blood mononuclear cells (PBMC).

Donors
>6 donors available for multi-donor studies.

Analysis Platform
Cellular ATP – Cytation 3 Cell Imaging Multi-Mode reader.
LDH release – SpectraMax ABS absorbance microplate reader.

Test Article Concentrations
8 point dose response curve with top concentration based on 100x C_{max} or solubility limit.
3 replicates per concentration*.

Test Article Requirements
Maximum (dependent upon number of repeat doses) 150 µL of a DMSO* solution to achieve 200x top concentration maintained at 0.5% DMSO or equivalent amount in solid compound.

Time Points
24-72 hour pre-incubation*.

Quality Control
Negative control: 0.5% DMSO (vehicle)*.
Positive controls: 2 appropriate compounds.

Data Delivery
Minimum effective concentration (MEC) and AC_{50} value for cellular ATP content and LDH release.

*Other options available on request.

The PBMC cytotoxicity assay provides a high throughput assessment of the cytotoxicity of candidate compounds *in vitro* and measures drug-induced cellular ATP depletion and LDH release.

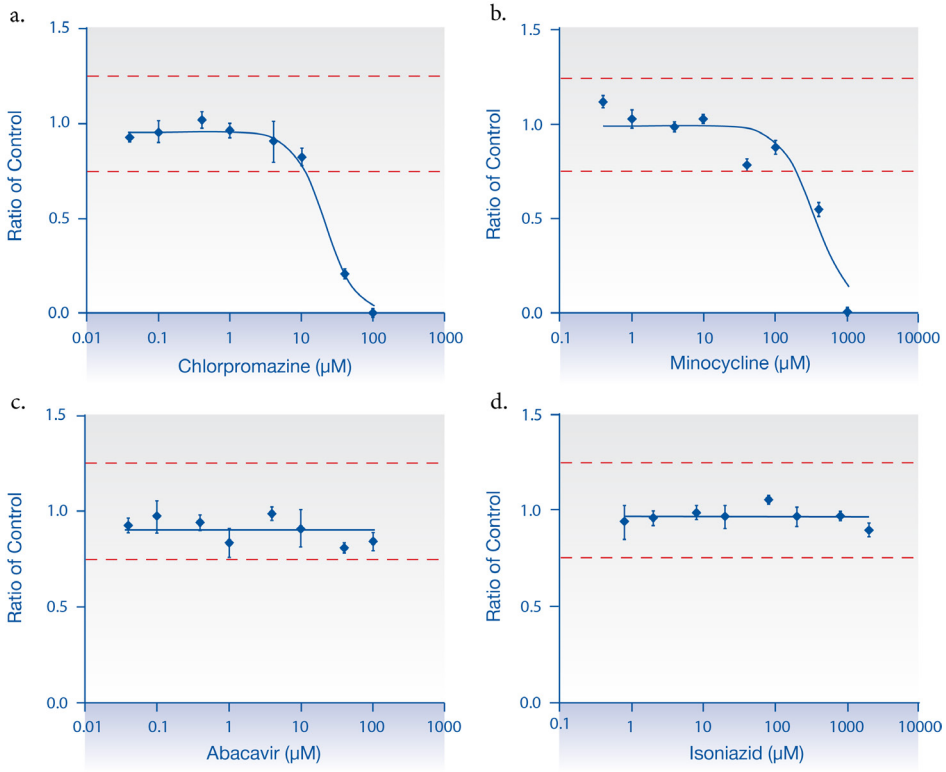


Figure 1

Representative cellular ATP dose response graphs for (a) chlorpromazine, (b) minocycline, (c) abacavir and (d) isoniazid.

Treatment of PBMC with chlorpromazine (AC_{50} =17.1 µM; MEC = 8.6 µM) and minocycline (AC_{50} = 336 µM; MEC = 196 µM) for 24 hours resulted in a decrease in cellular ATP.

Abacavir and isoniazid showed no cytotoxic effect at 100 µM and 2000 µM top concentrations respectively.

Error bars represent ± SD of the measurement while red dotted lines represent the range of control values..

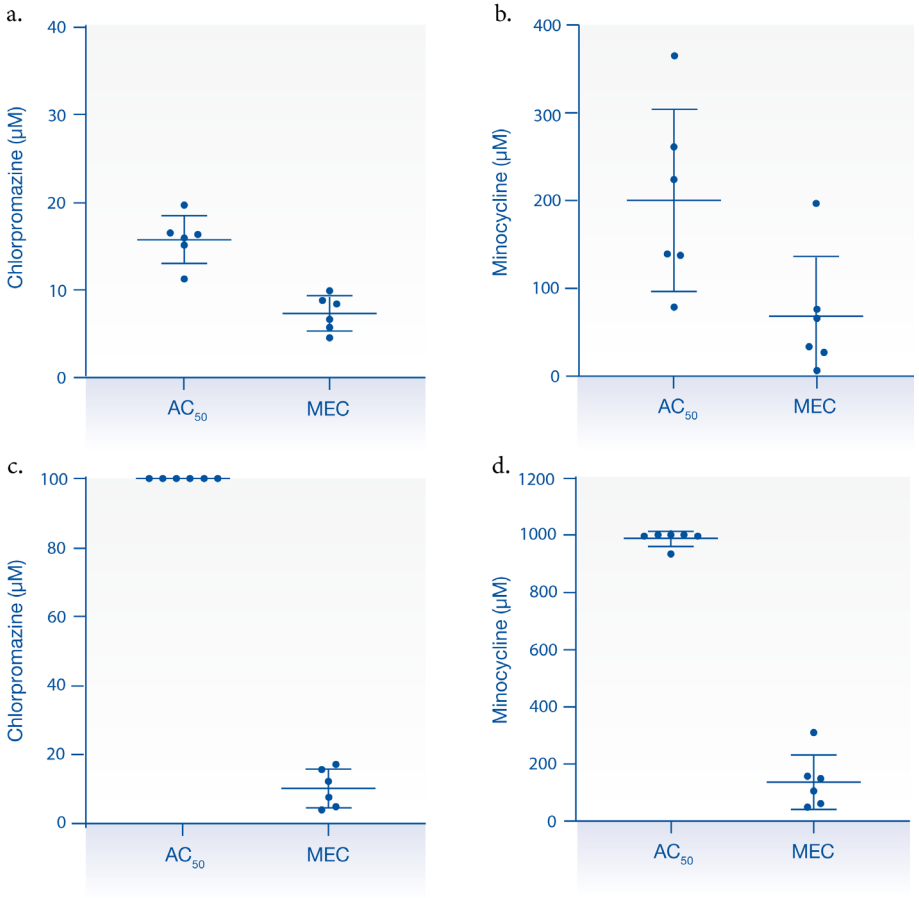


Figure 2

Dot plots demonstrating the AC_{50} and MEC values collected for chlorpromazine and minocycline across six individual PBMC donors.

Figures A & B and C & D show data obtained using cellular ATP and LDH assay respectively.

Solid black lines represent the average of the measurement while error bars represent ± SD..

References

[1] Naisbitt DJ *et al.*, (2020). Immune dysregulation increases the incidence of delayed-type drug hypersensitivity reactions. Allergy **75(4)**; 781-797

[2] Sullivan A *et al.*, (2018). β-Lactam hypersensitivity involves expansion of circulating and skin-resident TH22 cells. *J Allergy Clin Immunol* **141(1)**; 235-249

[3] Mennicke M *et al.*, (2009). Fulminant liver failure after vancomycin in a sulfasalazine-induced DRESS syndrome: fatal recurrence after liver transplantation. *Am J Transplant* **9(9)**; 2197-2202

[4] Guo GW & Liang YX (2001) Aluminium-induced apoptosis in cultured astrocytes and its effect on calcium homeostasis. *Brain Res* **888;** 221-226

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