A preclinical PK/PD mouse model of LPS induced inflammation permits targeting of inflammatory pathways

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Inflammation models mirror key pathological aspects of human diseases, serving as an integrated platform for preclinical drug advancement. The necessity to enhance therapeutic options and mitigate existing therapy constraints across diverse diseases calls for the innovation of novel anti-inflammatory drugs. Successful drug development hinges on robust preclinical frameworks, making the rodent model of LPS-triggered inflammation a prime choice. In this study, we targeted the TLR4, RIPK1 and NLRP3 pathways using specific antagonists within the well-established LPS-induced mouse model. We then compared their efficacy with that of dexamethasone, a widely utilized anti-inflammatory and immunosuppressive medication.

1 Lipopolysaccharides (LPS) activation of TLR4

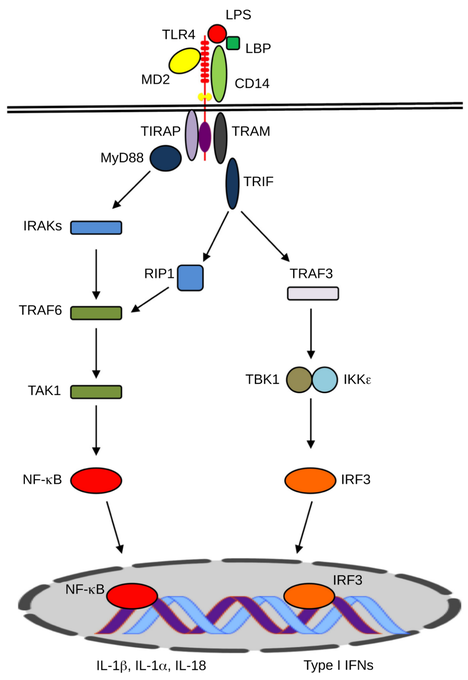


Figure 1: Schematic view of the TLR4 signalling pathway (adopted from ImmunoTargets and Therapy 2015). Lipopolysaccharides (LPS) from Gram-negative bacteria activate the Toll-like receptor 4 (TLR4) pathway in the innate immune response. When LPS binds to TLR4 on immune cells, a conformational change occurs, recruiting proteins like MD-2. This triggers a signalling cascade involving adaptor proteins (MyD88 and TRIF), leading to the activation of transcription factors NF-κB and IRF3. This activation results in the production of pro-inflammatory cytokines and type I interferons, initiating an inflammatory response. This early warning system helps the body combat bacterial infections, but dysregulation can lead to inflammatory disorders.

2 Experimental Design

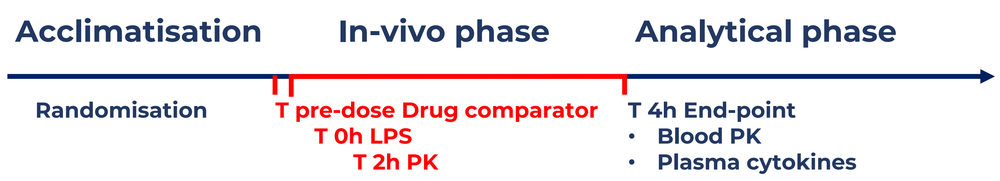
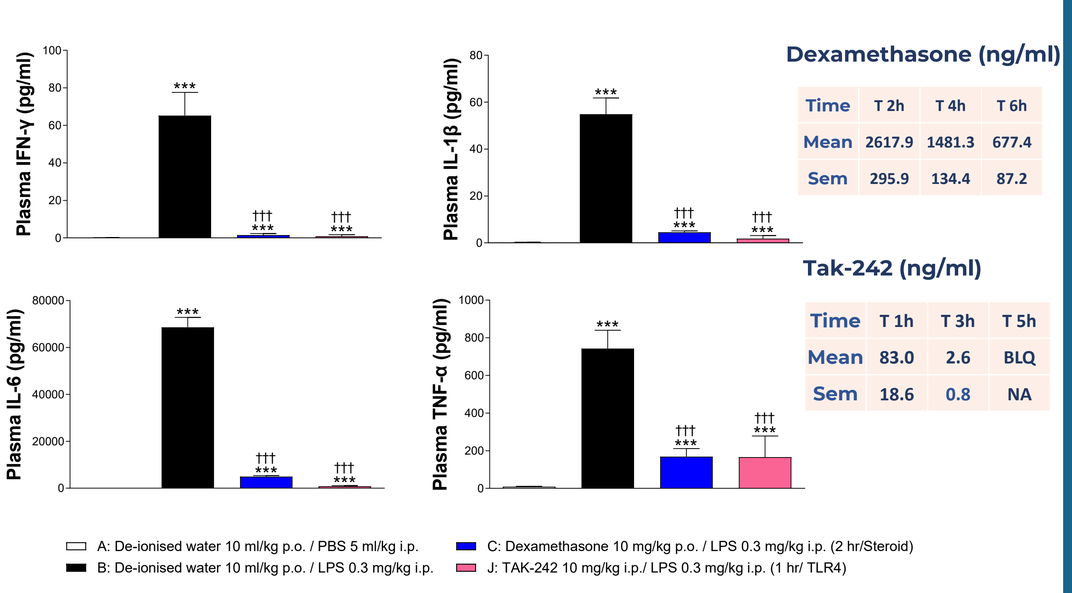


Figure 2: Male C57BL/6J mice, 12-14 weeks of age were dosed with LPS (0.3mg/kg IP). Dexamethasone (10mg/kg, PO), TAK-242, a TLR4 antagonist (10mg/kg IP), GSK2982772, a RIPK1 antagonist (40mg/kg PO) and MCC950, a NLRP3 antagonist (50mg/kg PO) were delivered at predetermined timepoints before LPS injection. Plasma was collected just prior to LPS dose and at 2h and at 4h post-LPS administration for PK analysis (by LC-MS). Plasma cytokines were analysed at the 4h timepoint only (IL-6, TNF-α, IFN-γ and IL-1β by MSD). Brain and kidneys were also collected to measure cytokines and for PK analysis (data not shown).

3 Dexamethasone and TAK-242 (TLR4 inhibitor) reduced LPS induced increase of cytokines in plasma at 4h.



Dexamethasone (ng/ml)

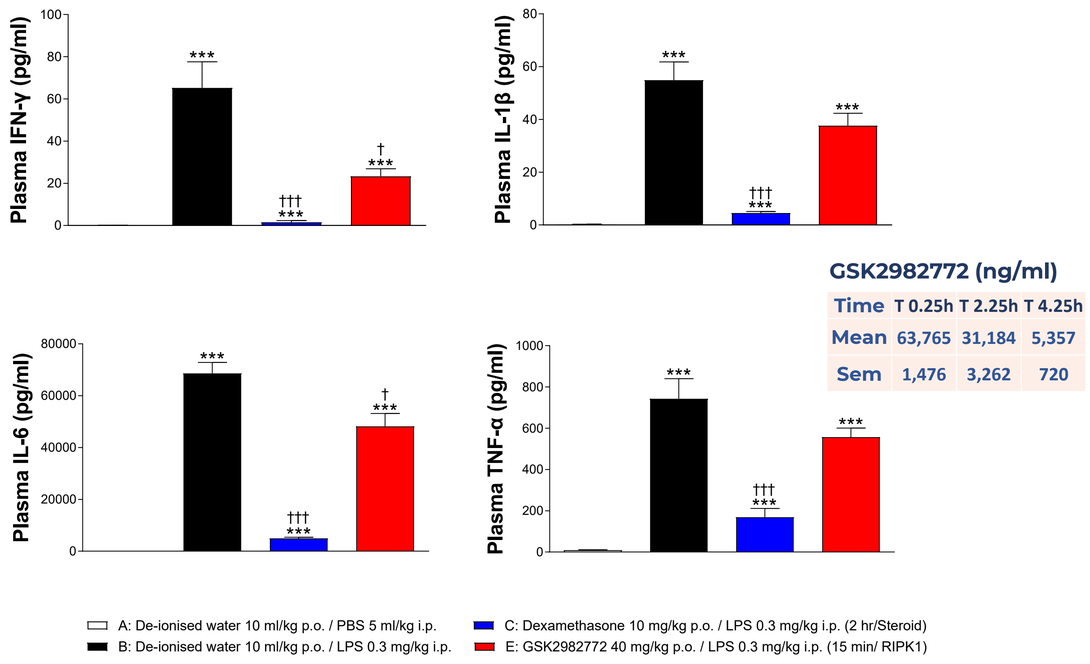
Time	T 2h	T 4h	T 6h
Mean	2617.9	1481.3	677.4
Sem	295.9	134.4	87.2

Tak-242 (ng/ml)

Time	T 1h	T 3h	T 5h
Mean	83.0	2.6	BLQ
Sem	18.6	0.8	NA

Figure 3: Data shown as back-transformed means + SEM (n=4-10). Data was log transformed and analysis was by robust regression with treatment and day as factors. Each treatment was compared to vehicle/PBS and vehicle/LPS by the multiple t test. Significant differences vs. PBS: ***p<0.001. Significant differences vs. LPS: †††p<0.001. Tables show plasma drug levels. Times stated are post drug dose sampling timepoints.

4 GSK2982772 (RIPK1 inhibitor) reduced LPS induced increase of IL-6 and IFN-γ in plasma at 4h.

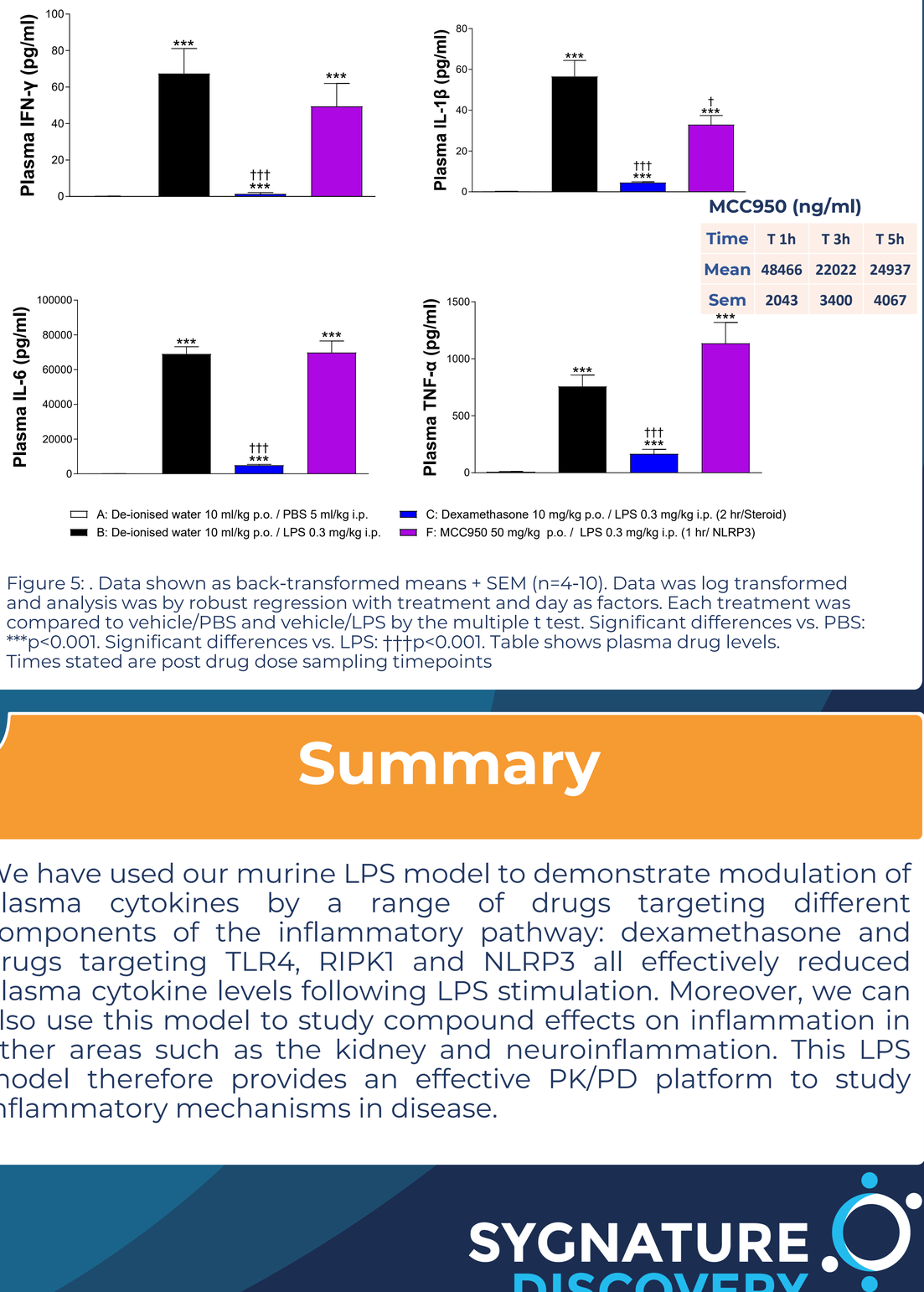


GSK2982772 (ng/ml)

Time	T 0.25h	T 2.25h	T 4.25h
Mean	63,765	31,184	5,357
Sem	1,476	3,262	720

Figure 4: Data shown as back-transformed means + SEM (n=4-10). Data was log transformed and analysis was by robust regression with treatment and day as factors. Each treatment was compared to vehicle/PBS and vehicle/LPS by the multiple t test. Significant differences vs. PBS: ***p<0.001. Significant differences vs. LPS: †p<0.05, †††p<0.001. Table shows plasma drug levels. Times stated are post drug dose sampling timepoints

5 MCC950 (NLRP3 inhibitor) reduced LPS induced increase of IL-1β in plasma at 4h



MCC950 (ng/ml)

Time	T 1h	T 3h	T 5h
Mean	48466	22022	24937
Sem	2043	3400	4067

Figure 5: . Data shown as back-transformed means + SEM (n=4-10). Data was log transformed and analysis was by robust regression with treatment and day as factors. Each treatment was compared to vehicle/PBS and vehicle/LPS by the multiple t test. Significant differences vs. PBS: ***p<0.001. Significant differences vs. LPS: †††p<0.001. Table shows plasma drug levels. Times stated are post drug dose sampling timepoints

6 Summary

We have used our murine LPS model to demonstrate modulation of plasma cytokines by a range of drugs targeting different components of the inflammatory pathway: dexamethasone and drugs targeting TLR4, RIPK1 and NLRP3 all effectively reduced plasma cytokine levels following LPS stimulation. Moreover, we can also use this model to study compound effects on inflammation in other areas such as the kidney and neuroinflammation. This LPS model therefore provides an effective PK/PD platform to study inflammatory mechanisms in disease.

- "Novel perspectives on non-canonical inflammasome activation2 July 2015 ImmunoTargets and Therapy 2015 (Issue 1):131
- Discovery of a first in class receptor interacting protein 1 (RIP1) kinase specific clinical candidate (GSK2982772) for the treatment of inflammatory diseases. J. Med. Chem. 2017 (Issue 60, 1247-1261).
- A small molecule inhibitor of the NLRP3 inflammasome is a potential therapeutic for inflammatory diseases. Nat. Med. 2015 (Issue 21, 248-255)

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