Characterization of inflammation and immune cell modulation induced by low-dose LPS administration to healthy volunteers

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Human endotoxin model – introduction

- A model of systemic inflammation
  - Flu-like symptoms
  - ↑ CRP production
  - ↑ Concentrations pro- and anti-inflammatory cytokines

- Administration of purified LPS (endotoxin) from *E. coli* or other Gram-negative bacteria

- *E. coli*: high reproducibility of effects (*Andreasen et al.*)

- High LPS doses, not preferred
  - Potential effects of immune-modulating interventions might not be observed
  - Not free of risk for the volunteer (a.o. cardiovascular)
  - Other homeostatic mechanisms may be temporarily impaired
LPS hyporesponsiveness

- Follows upon \textit{in vivo} LPS challenge
  - Altered cytokine production
  - ↓ Inflammatory response following LPS rechallenge

- Many negative regulators (e.g. SOCS-1, IRAK-M, and SHIP) of the TLR4 signaling pathway \textit{(Fu et al. 2012, Morris et al. 2011)}
LPS hyporesponsiveness – *Kox et al. 2011*

- **T=0:** *in vivo* challenge
- **T=4hrs and 4 wks:** *ex vivo* challenge

*Ex vivo* LPS hyporesponsiveness
- Resolved 1 week after *in vivo* LPS challenge
- Exact time course unclear
- Possible differences between read-outs
Study objectives

• To assess the relationship between administration of low doses of LPS (0.5, 1 and 2 ng/kg) and the inflammatory response (cytokine levels and CRP) in healthy male volunteers

• To assess the duration of hyporesponsiveness of the immune system after *in vivo* LPS administration, as determined by *ex vivo* LPS challenges
Study outline (1)

- Randomized, blinded, placebo controlled, sequential-group study
- 24 healthy male subjects
  - 3 cohorts (active-pl: 6-2)
- Ascending single iv doses of 0.5-2ng/kg LPS
  - U.S. Reference *E. Coli* endotoxin Lot#3 (O113:H, 10:K negative, ~10IE/ng)
- IV hydration
  - Pre-hydration 2hrs pre-dose: 1500mL glucose/saline
  - Hydration 6hrs post-dose: 150mL/hr
Study outline (2)

- **Inflammatory response, *in vivo***
  - CRP
  - IL-1β, IL-6, IL-8 and TNF-α (human 4-plex, MSD)

- **Inflammatory response, *ex vivo***
  - Whole blood cultures with LPS (*E. Coli*, O111:B4, ~10IE/ng)
    - 24 hrs incubation at 37°C, 5% CO₂
  - -2hrs, 6, 12, 24, 48 and 72hrs
  - IL-1β, IL-6, IL-8 and TNF-α (human 4-plex, MSD)

- **Safety**
  - AEs / vital signs / ECG / routine labs
Study results – Safety

• Well tolerated, no clinically relevant changes or unexpected treatment-related trends in
  - Urinary or blood laboratory parameters
  - ECG recordings
  - Vital signs
  - AEs: mild severity and self-limiting

• Most frequent reported AEs
  - Headache; 66.7% of the LPS-treated subjects, 33.3% of the placebo-treated subjects
  - Feeling cold; 44.4% of the LPS-treated subjects, none of the placebo-treated subjects
Study results – Safety – Temperature and HR

- BP highly variable over time, max. mean decreases in the range of 0 to -13 mmHg
Study results – *In vivo* CRP

- **Statistical analysis**
  - Significant contrasts, dose groups (0.5, 1, 2ng/kg) vs pl: p<0.0001
Study results – Circulating cytokines (TNF-α, IL-6, IL-8)

• **T\_max**: 1.5-3hrs post-dose

• **Statistical analysis**
  - Significant contrasts up to t=6hrs: (0.5, 1, 2ng/kg) vs pl: p<0.0001

• **IL-1β**: ↑ 3-6hrs post-dose (2ng/kg)
Study results – LPS Hyporesponsiveness

- Statistical analysis

|                | Contrast at 6hrs versus pl | Estimated difference (%) | p-value  |
|----------------|----------------------------|--------------------------|----------|
| **IL-1β**      | 1ng/kg                     | -65.8                    | <0.0001  |
|                | 2ng/kg                     | -84.7                    | <0.0001  |
| **TNF-α**      | 1ng/kg                     | -66.4                    | 0.0005   |
|                | 2ng/kg                     | -74.7                    | <0.0001  |
Study results – LPS Hyporesponsiveness

- Statistical analysis

|       | Contrast at t=6hrs vs pl | Estimated difference (%) | p-value |
|-------|--------------------------|---------------------------|---------|
| **IL-6** |                          |                           |         |
| 1ng/kg | -31.3                    |                           | 0.0283  |
| 2ng/kg | -41.3                    |                           | 0.0024  |
| **IL-8** |                          |                           |         |
| 0.5ng/kg | 55.1                    |                           | 0.0879  |
| 1ng/kg | 19.2                     |                           | 0.4961  |
| 2ng/kg | -4.8                     |                           | 0.8475  |

- [IL-8] and [IL-6] response ≠ [TNF-α] and [IL-1β]: indication for priming?
Conclusions

• LPS doses 0.5-2ng/kg: well-tolerated

• PD parameters: cytokine release and safety markers (temperature and heart rate)

• LPS doses ≥ 0.5ng/kg: distinct inflammatory response

• LPS dose-dependent hyporesponsiveness observed for IL-1β, IL-6 and TNF-α after ex vivo LPS stimulation:
  - Max. measured 6hrs post-dose
  - Total duration of ~12hrs

• Clinical pharmacology studies: application of a combination of in vivo LPS administration and repeated ex vivo LPS challenges
unlocking the true potential
• Monocyte count: dose-dependent decrease with a minimum change from baseline at 6hrs post-dose, returning to baseline 12-24hrs post-dose
Study results – Safety – Neutrophils / Leucocytes

- Neutrophil count: peak levels at 4hrs post-dose
- Leucocyte count: peak levels at 4-6hrs post-dose

returning to baseline 12-24hrs post-dose
Study results – Safety – Thrombocytes

- Blood platelet count (thrombocytes): dose-dependent decrease with min. mean levels ~4hrs post-dose, returning to baseline levels around 12 (0.5 and 1ng/kg) and 48hrs post-dose (2ng/kg)
Study results – Safety – BP

![Graph showing systolic and diastolic blood pressure over time for different treatments: Placebo, LPS 0.5 ng, LPS 1.0 ng, and LPS 2.0 ng.](image)

- **Systolic BP (mmHg)**
  - Placebo
  - LPS 0.5 ng
  - LPS 1.0 ng
  - LPS 2.0 ng

- **Diastolic BP (mmHg)**
  - Placebo
  - LPS 0.5 ng
  - LPS 1.0 ng
  - LPS 2.0 ng
LPS signaling pathways
Human endotoxin model – Priming

• Follows upon *in vivo* LPS challenge with very low doses
  - ↑ Inflammatory response following LPS rechallenge
  - May enable the immune system to elicit a strong inflammatory response against potential pathogens (*Fu et al.*)

• Priming in animals described extensively, but underlying mechanisms poorly understood
Power calculation (TNF-α, IL-6 and CRP)

- Parallel study design, LPS dose level: 0.5 ng/kg, sample size of 8 subjects per treatment group
  - 80% power to detect (two-sided significance level of 0.05)
    - 28% inhibition in the LPS-induced TNF-α response;
    - 53% inhibition in the LPS-induced IL-6 response;
    - 49% inhibition in the LPS-induced CRP response.

- Inter subject variability on log scale is well comparable between different LPS doses - this power calculation also applies for LPS doses of 1 and 2 ng/kg