LPS-induced cytokine storm produces QTc prolongation which can be prevented by an anti-inflammatory compound.

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Introduction

Increases in the levels of pro-inflammatory cytokines play a role in the morbidity of numerous diseases. Marked elevations in levels of IL-1β, IL-6 and TNF-α, in particular, have been found in diseases as diverse as diabetes, Alzheimer's disease, inflammatory bowel disease and cancer. There is now increasing evidence which suggests over-expression of IL-1β, IL-6, TNF-α, and other cytokines plays a role in the pathogenesis of the long QT syndrome. Indeed, in most inflammatory conditions, marked increases in IL1β, IL6, TNFα, in particular, are seen and correlate with increased QT prolongation even in patients without disease.

In long QT syndrome, there is delayed repolarization secondary to abnormalities in the potassium, sodium and calcium ion channels in myocardial cells. This may be caused by a genetic abnormality, by co-existing cardiac diseases, by hypokalemia or hypomagnesemia, or by one of numerous pharmaceutical agents, including commonly used antihistamines, sedatives, antibiotics, antiarrhythmics and psychiatric drugs. It is generally considered that a 50 msec increase in QT in patients doubles the risk for all-cause mortality, the main cause of which is Torsades de Pointes.

In this research project, we hypothesized that if QTc prolongation was associated with inflammatory cytokines activation, QTc prolongation may be mitigated by anti-inflammatory molecules. Lipopolysaccaride (LPS) and Kdo2-Lipid A, two components of the membrane of e. Coli, were used to induce a massive inflammatory cytokines release while monitoring the ECG of anesthetized animals. In a second series of experiments, anti-inflammatory compounds were tested for their ability to prevent this prolongation in QTc in the animals. Finally, a quantification of the cytokines was conducted to correlate inflammatory components with QTc prolongation.

Methods

All experimentation was conducted in accordance with the guidelines on laboratory animal use of the Canadian Council against Animal Cruelty (CCAC).

Purpose of the study:
This research program aimed at quantifying the relationship between inflammatory cytokines and QT prolongation through the use of LPS and Kdo2-Lipid-A intravenous challenges and plasma cytokine measurements, in the presence and absence of anti-inflammatory drugs and new innovative molecules.

Test system:
Male Hartley guinea pigs (350 - 400; Charles River) were used in these studies. The same program was started with rats in 2014. It was found that, while rats exhibited the anticipated response to the pro-inflammatory challenge, their electrocardiographic response was more variable and difficult to read, by virtue of exhibiting a much smaller T-wave. Since the guinea pig is an established preclinical CV and particularly ECG animal model, the experiments described below were rapidly modified to be applied to the guinea pig.

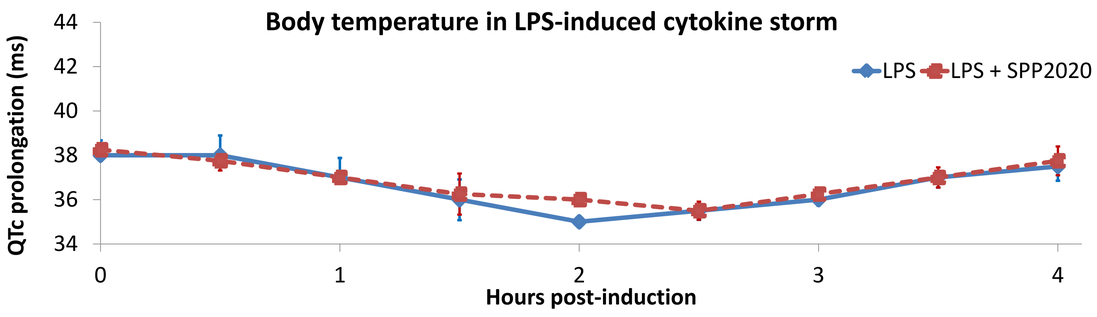
Experimentation:
LPS and Kdo2-Lipid-A were used to induce cytokine release in guinea-pigs with concomitant ECG monitoring and blood draws, followed by Q-ELISA measurement of cytokine production. Male adult guinea pigs previously instrumented with subcutaneous thermometers received a single i.p. injection of 150 µg/kg LPS at time 0. The animals were lightly sedated with a mixture of 1.0 to 1.5% isoflurane USP in 95% O_2 and 5% CO_2. ECG leads were placed on the animals in a 3-lead configuration. Blood draws were made from the jugular vein. ECGs were analyzed predose, and at 1h, 2h, and 4 hours post-LPS or Kdo2-LipidA, with simultaneous blood draw. Blood was collected into citrated tubes, spun immediately, and the plasma was frozen at -20°C until ready to be used.

ECG intervals were read manually using electronic cursors, or using pattern-recognition software. The QRS, QT, S-T_{peak}, T_{peak}-T_{end} intervals were monitored continuously for 4 hours post-challenge.

Q-ELISA for TNFα, IL1β, and IL-6 were performed using the plasma collected as described above. The enzyme-linked immunosorbent assay (ELISA) is a specific and highly sensitive method for quantification of cytokines and other analytes in solution. The assay involved a guinea-pig-specific monoclonal antibody (mAb) raised against each cytokine, which was used to coat a 96-well plate. A secondary mAb, used for detection, was labeled with horseradish peroxidase, which produced an intense red-hued color if activated. After addition of a substrate, a red-colored reaction developed that was directly proportional to the amount of cytokine bound. The concentration of cytokine in the sample was determined by comparison with a standard curve of known cytokine concentrations.

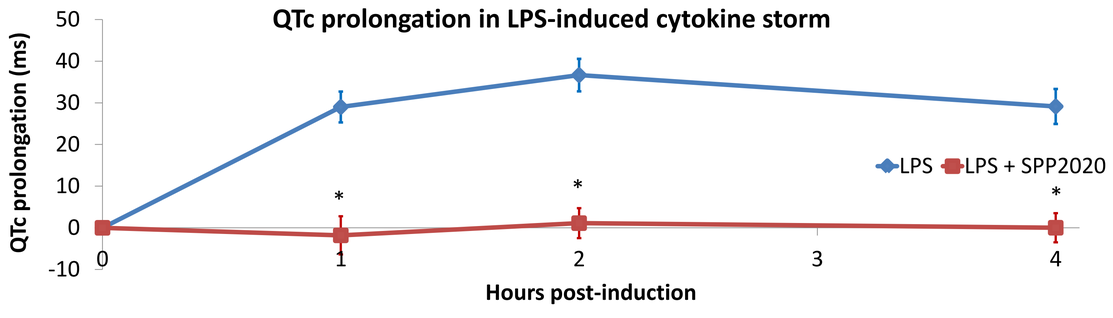
Drugs and treatments:
- LPS: Lipopolysaccaride is the major component of the outer membrane of Gram-negative bacteria.
- Kdo2-Lipid –A (KLA) is a nearly homogeneous Lipopolysaccaride (LPS) substructure with endotoxin activity
- SPP2020 is an anti-inflammatory compound in preclinical development. It has been shown to have anti-inflammatory effects in animal models of inflammation.

Figure 1. Body temperature measurements in LPS-challenged animals.



Intraperitoneal challenge with 150 µg/kg LPS caused an initial hypothermia in guinea pigs, which reversed over 3 hours and became hyperthermia in the animals (data not shown for 24h temperature monitoring only). Preceding the LPS challenge with 9 mg/kg SPP2020 did not prevent the changes in body temperature.

Figure 2. QTc prolongation in LPS-induced cytokine storm



QTc prolongations of 29 ms (1h), 37 ms (2h), and 29 ms (4h) were measured after LPS-challenge with 300 µg/kg in adult male guinea pigs. The correlation between inflammation and QTc prolongation is clear and long-lasting, despite the reported appearance and disappearance of various inflammatory cytokines over the 4 hours of monitoring.

Preceding the LPS-challenge with an oral dose of SPP2020 (9mg/kg) before the exact same challenge with 300 µg/kg LPS in naive animals failed to produce any QTc prolongation whatsoever. These surprising results were largely unexplained by the expected mechanism of action of SPP2020, and anti-inflammatory compound with no affinity for hERG or other structures generally associated with QTc prolongation.

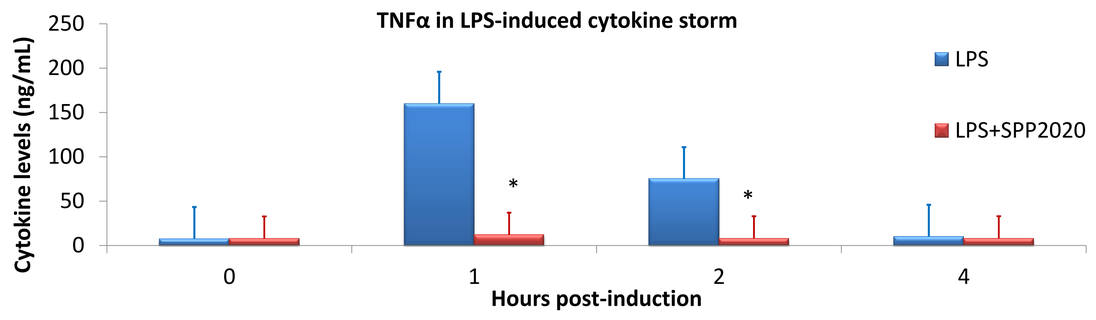


Figure 3. Plasma levels of TNFα in guinea pigs challenged with LPS. Sample size: n = 5
Four(4) blood draws were made from lightly sedated guinea pigs at the same time as ECGs were acquired: Pre-LPS (0h post-induction), 1, 2, and 4 hours. TNFα levels increased 21-fold following LPS injection, but quickly fell back to pre-LPS levels (within less than 4 hours). Pre-treatment with 9 mg/kg SPP2020 prevented the rise in TNFα over the 4 hours of monitoring.

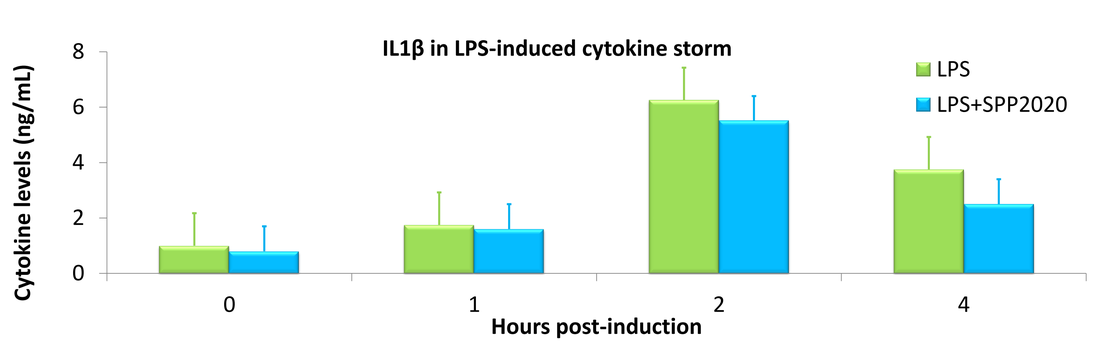


Figure 4. Plasma levels of IL1β in guinea pigs challenged with LPS. Sample size: n = 5
The same blood draw procedure as described in Figure 3. yielded plasma to assay IL1β levels. In contrast to TNFα, IL1β levels increased after 2 hours, to a maximal change of 6.25-fold baseline levels. The levels were essentially unchanged after 1 hour post-LPS. Also in contrast with TNFα data, SPP2020 had no significant effect on IL1β levels. It's interesting that SPP2020 prevented QTc prolongation despite its absence of effect on IL1β levels.

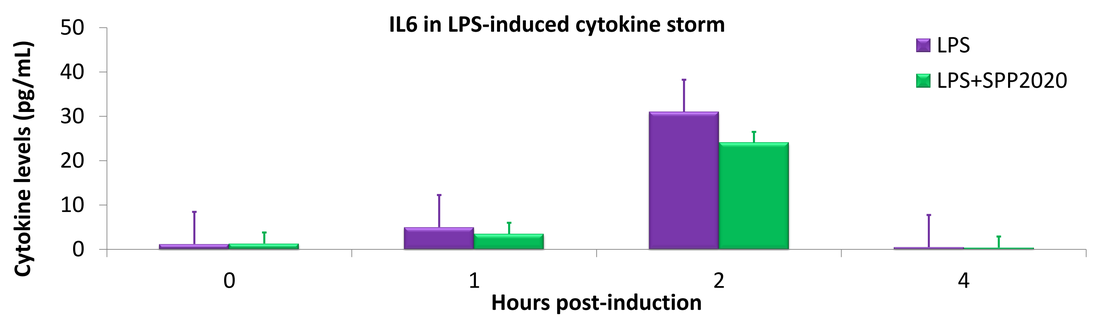


Figure 5. IL6 plasma levels in guinea pigs challenged with LPS. Sample size: n = 5 .
IL6 behaved very much like IL1β, insofar as levels remained low until 2 hours post-LPS. At that point, they peaked to almost 26-fold the baseline, pre-LPS levels. SPP2020 did not prevent the change in IL6 levels, suggesting that, like IL1β, may not be the main driver for QTc prolongation in guinea pigs.

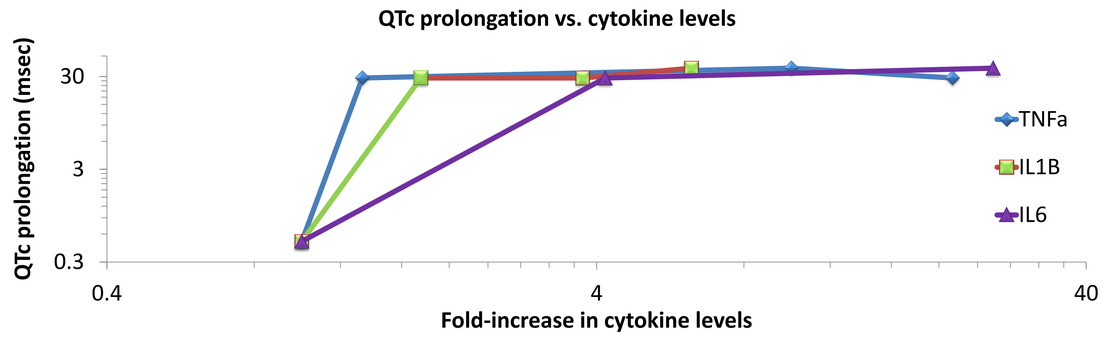


Figure 6. Correlation between cytokine levels and QTc prolongation. Sample size: n = 5 .
Log-log graph of the impact of cytokine levels on QTc prolongation. While we were hoping for a perfect correlation between one cytokine's levels and QTc prolongation in this model of LPS-induced cytokine storm in guinea pigs, the data suggests that an early increase in cytokine levels, up to 1-fold for TNFα and IL1β and 4-fold for IL6, is associated with prolonged QTc intervals. Beyond this, the relationship loses its causative character.

Discussion

Anti-inflammatory compounds are seldom associated with drug-induced QTc prolongation, at least nowhere to the extent that other classes of drugs have been implicated with changes in QTc. In parallel, inflammatory processes have been associated with QTc prolongation: patients with rheumatoid arthritis, psoriasis and other inflammatory conditions frequently exhibit prolonged QTc intervals, which correlate with increases in tumor necrosis factor alpha (TNFα), interleukin1 beta (IL1β) and IL6. Studies in experimental models have shown these cytokines act through stimulation of reactive oxygen species.

The mechanistic identification of reactive oxygen species was beyond the scope of this project. In LPS-induced inflammatory reactions in guinea pigs, TNFα, IL1β and IL6 all increased significantly, if transiently, and this change correlated with the peak of QTc prolongation. The transient nature of the peak in cytokine levels, the so-called "storm", makes it difficult to assess cytokine levels in long-term studies: the levels increase and return to baseline levels within 4 hours, roughly. The sudden increase in levels, however, gives rise to reactive oxygen species and changes in ceramide levels which are thought to change the fluidity of lipid rafts and alter ion channel activation/inactivation kinetics. This would contribute to prolonged QTc intervals. The correlation between cytokine levels suggest that the initial rise in plasma levels increases QTc duration, and is followed by cytokine-independent processes such as –for instance, and not limited to, ceramide accumulation and the ensuing changes in ion channel kinetics.

By preventing the peak in TNFα –but not IL1β or IL6-, SPP2020 completely abolished QTc prolongation in this LPS-induced model. This is not the first demonstration that QTc prolongation or Ikr/hERG currents can be salvaged: vitamin E and MnTRAP. Altogether, this data suggest a role of inflammatory cytokines in QTc prolongation, and the possibility of limiting this cardiac toxicity with anti-inflammatory compounds.

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