NEUROFIT SAS

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CNS & PNS preclinical Services

Unveiling the role of glial-derived cytokines and pro-inflammatory mediators in neurodegeneration: Insights from glia-neuron coculture model

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Introduction / Objective

- **Neuroinflammation and Neurodegenerative Disorders**
  - ✓ Neuroinflammation plays a major role in neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's dieaseas (PD), Multiple sclerosis (MS).

- **Putative role of Lipopolysaccharides (LPS) in Neurodegenerative diseases**
  - ✓ Elevated LPS levels observed in the brains of AD and the plasma of PD patients.
  - ✓ LPS through the glia's TLR-4 receptor activates the release of proinflammatory cytokines.
  - ✓ Thus, LPS is hypothesized as significant contributor to neuroinflammatory cascades underlying neurodegeneration.

- **Objective**
  - ✓ To delineate the role and temporal dynamics of glia-derived cytokines and pro-inflammatory mediators in neuronal death progression.

Experimental protocol

Primary coculture of glia-neurons from rat embryos (Wistar rats) in 96 well-plate in 3 independent cultures

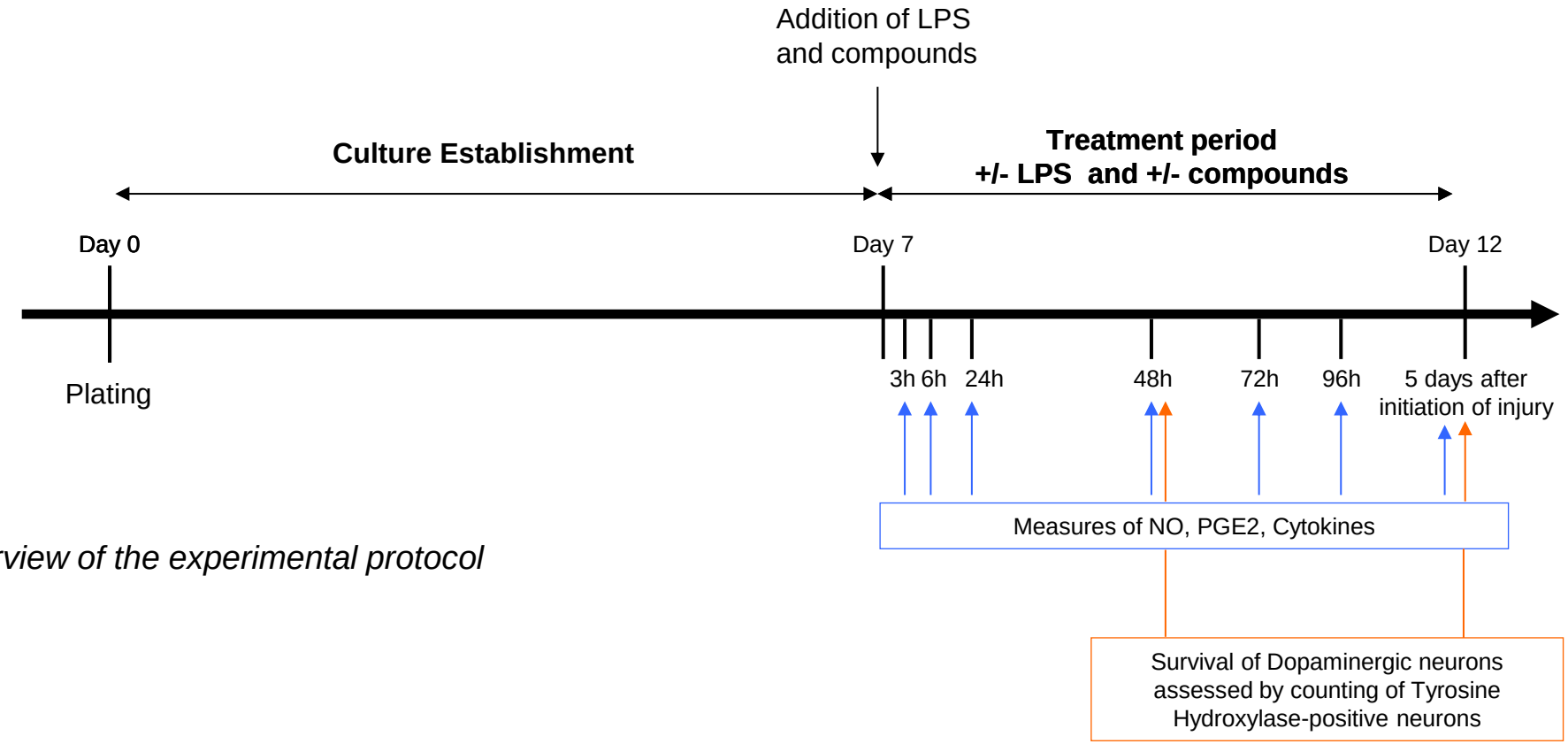


Fig. 1: Overview of the experimental protocol

Timecourse of immune response as assessed by the released mediators in the supernatant of the glia-neuron coculture following LPS stimulation

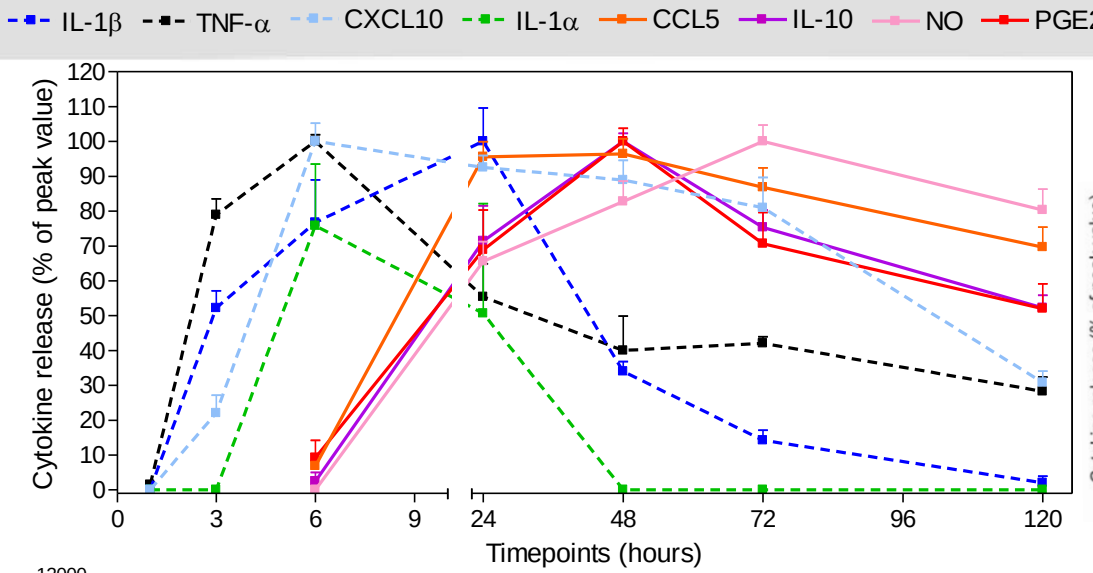
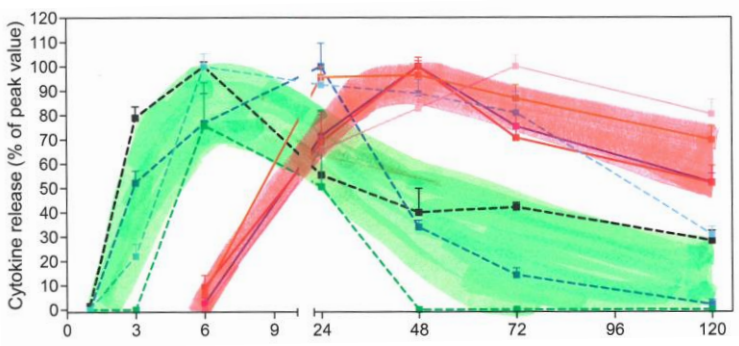
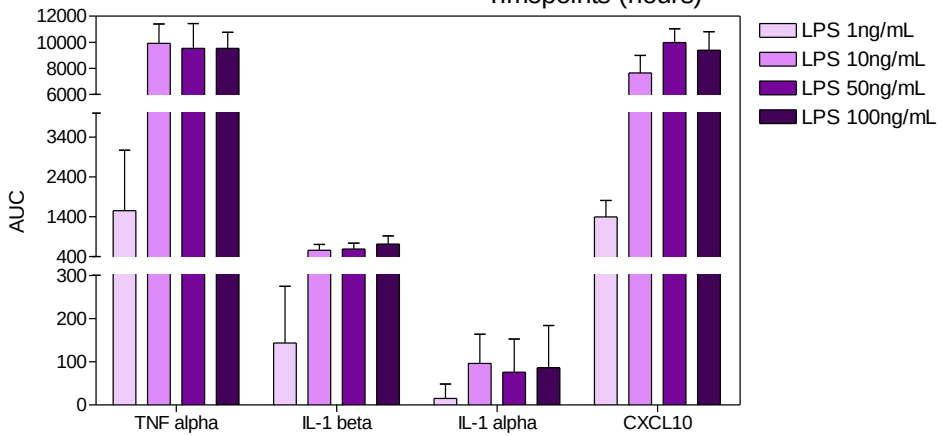


Fig. 2: Example of timecourse curves of various mediators released in the supernatant glia-neuron coculture following exposure to 50 ng/ml of LPS

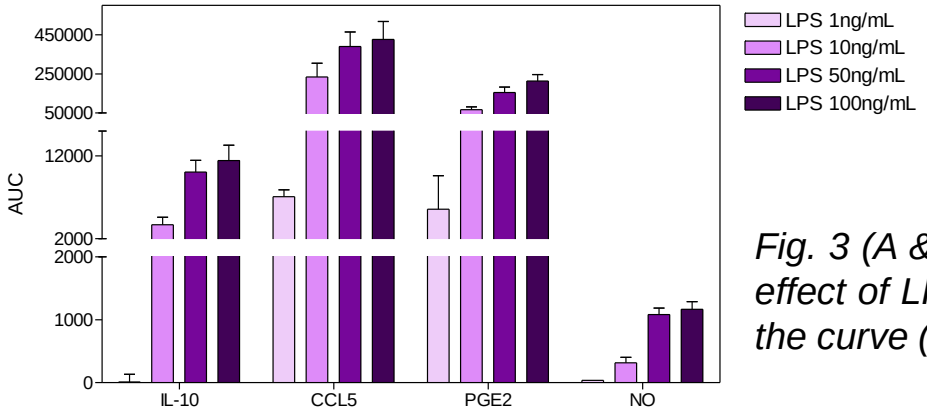


First wave of mediators
(IL-1β, TNF-α, CXCL10, IL-1α)

Second wave of mediators
(CCL5, IL-10, NO, PGE2)



First wave of mediators
(IL-1β, TNF-α, CXCL10, IL-1α)



Second wave of mediators
(CCL5, IL-10, NO, PGE2)

Fig. 3 (A & B): Dose-response of the immune effect of LPS as measured by the area under the curve (AUC) of the mediator timecourse

=> LPS induces a dose-dependent immune response characterized by a release in the supernatant of early (within an hour) and late (24h onwards) – phase mediators

Timecourse of Neuronal death induced by LPS in glia-neuron coculture
Role of glia-derived immune response

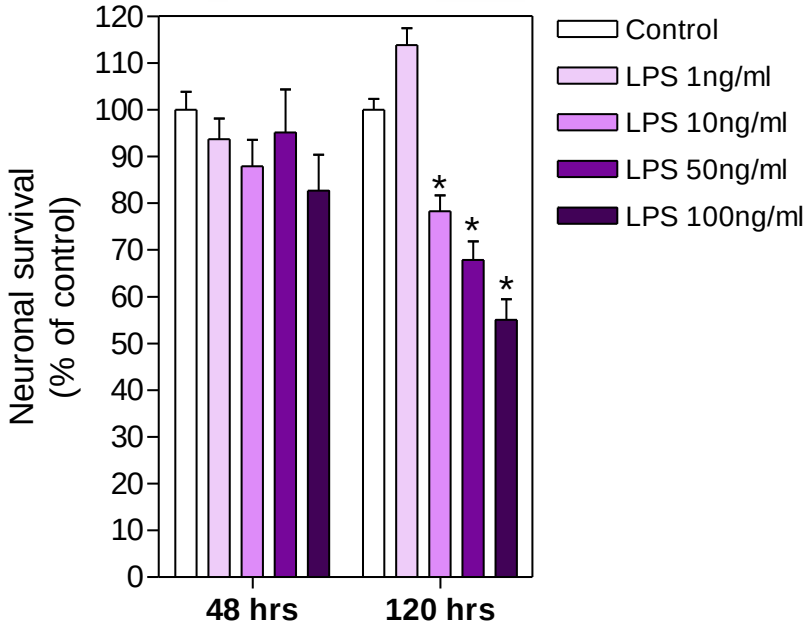


Fig. 4: LPS-induced neuronal death is absent at 48 hrs but significant at 120 hrs with a dose-dependent effect

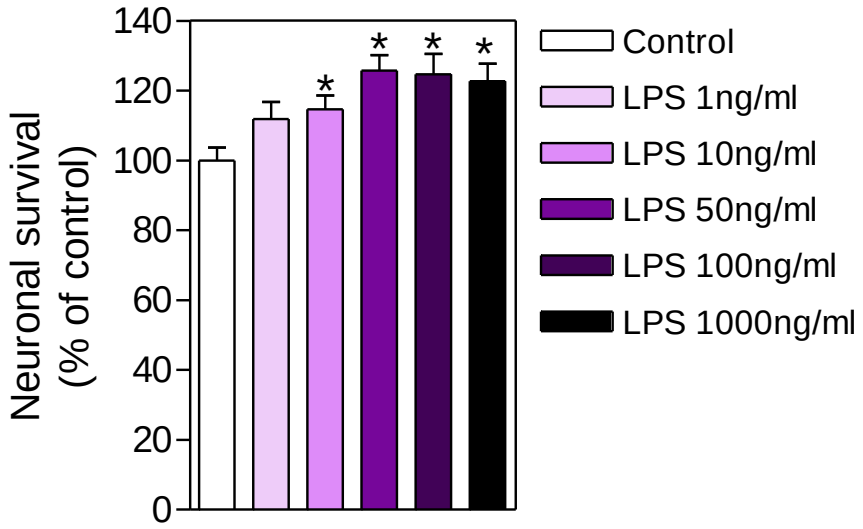


Fig. 5: LPS-induced Neuronal death does not occur in pure neuronal primary culture (i.e., absence of glia cells) even at high dose of LPS

⇒ Critical role of glia-derived immune response in the generation of neuronal injury.

⇒ Temporal separation of events: the delay between the onset of neuroinflammation and neuronal death suggests the involvement of a cascade of events that create a toxic environment which ultimately results in neuronal death days later.

⇒ Potential for Therapeutic Interventions: The separation between the initial inflammatory response and subsequent neuronal death suggests a potential therapeutic window.

Role of early cytokines environment in neuronal injury and death

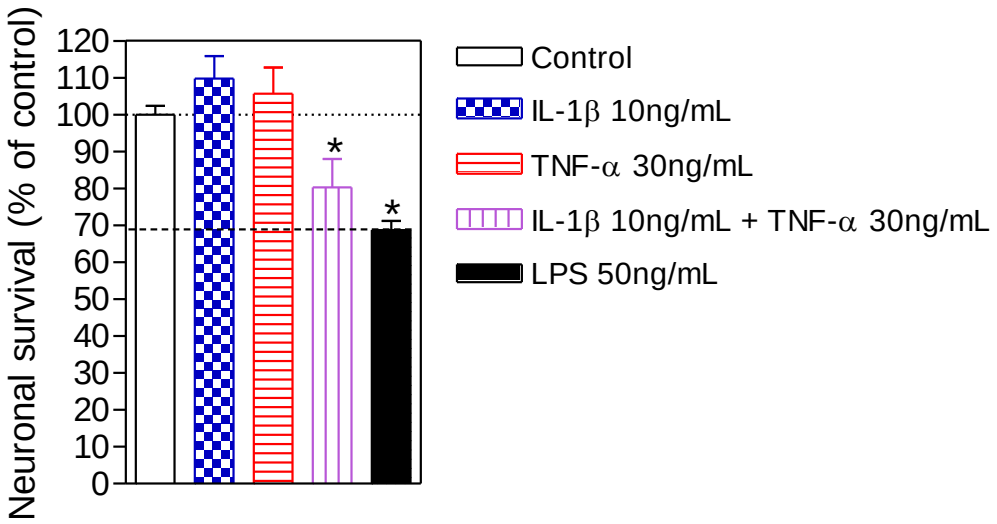


Fig. 6: IL-1β and TNF-α mixture, but not either cytokine alone, induces neuronal death in glia-neuron coculture

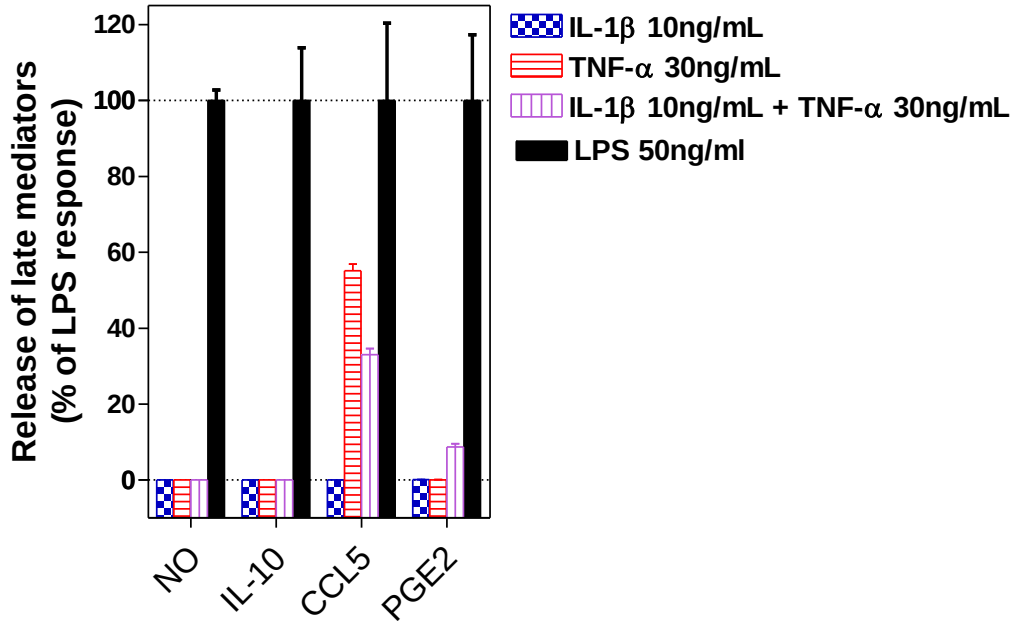


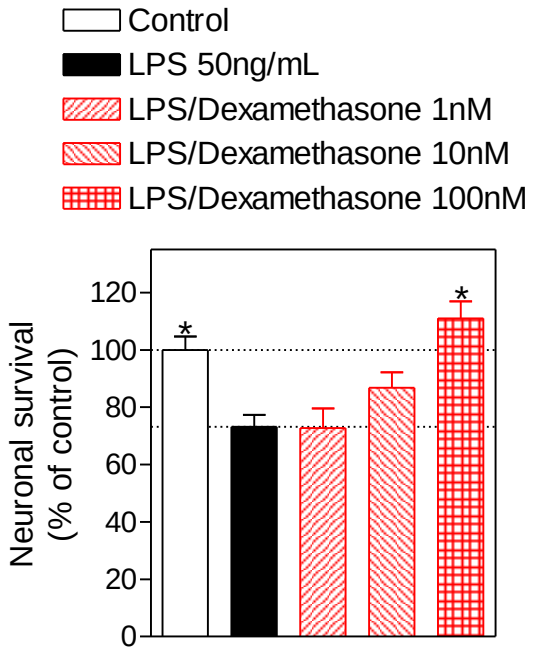
Fig. 7: The profile of late mediators (NO, IL-10, CCL5 and PGE2) stimulated by IL-1β and TNF-α mixture is slightly different from that induced by LPS in glia-neuron coculture

⇒ Early Release of IL-1β and TNF-α and synergistic neurotoxicity: Upon LPS stimulation, IL-1β and TNF-α are among the early cytokines released. While the individual presence of these cytokines might not be enough to cause neuronal death, their simultaneous presence can create a pro-inflammatory and neurotoxic environment responsible of the neuronal death.

⇒ Nevertheless, the profile of late mediators released by the coculture following stimulation with an IL-1β and TNF-α mixture is slightly different from that produced by LPS. The most notable difference is the absence of nitric oxide and IL-10 release, for example. This suggests that other early mediators are also needed in reproducing the full spectrum of LPS-induced neuroinflammation.

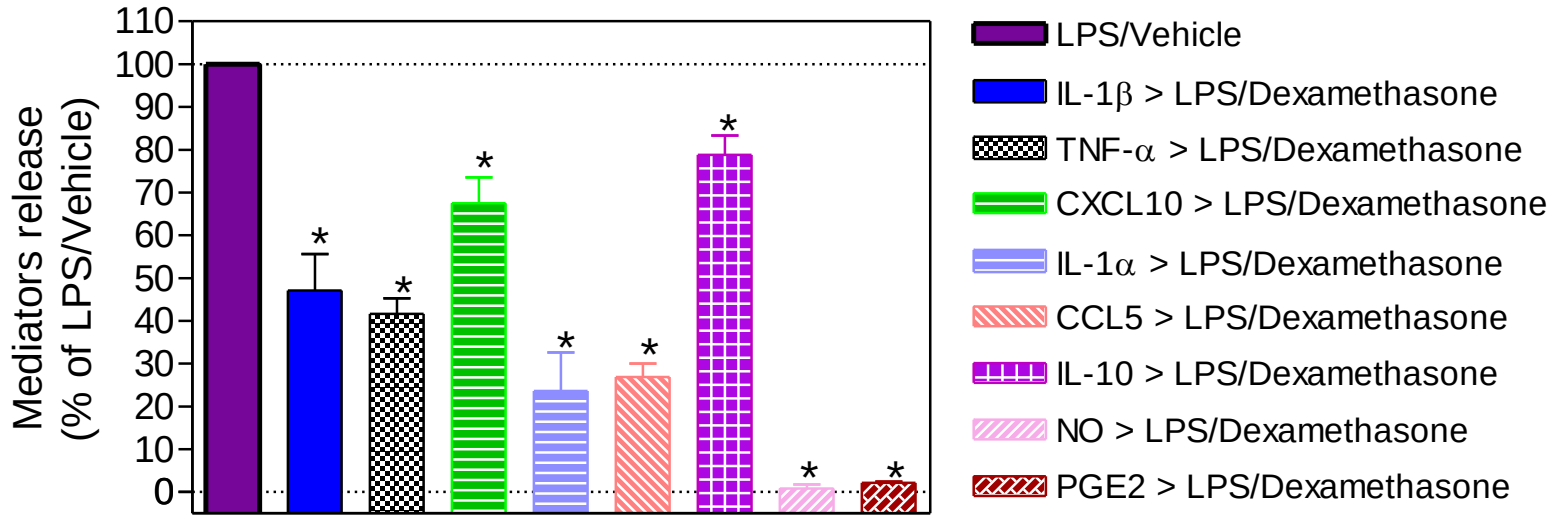
Pharmacological impact of immunosuppressive drug on neuronal injury and cytokines release

Fig. 8: Effect of dexamethasone on LPS-induced neuronal death



⇒ The anti-inflammatory drug dexamethasone protects in a dose-dependent manner LPS. Full prevention is obtained at the dose of 100 nM.

Fig. 9: Effect of dexamethasone on mediator level at the peak time release



⇒ Whilst 100 nM dexamethasone fully prevents LPS-induced neuronal death, it only partially inhibits cytokine release but fully suppresses the release of nitric oxide and PGE2. These results suggest that dexamethasone's neuroprotective effects are mediated through mechanisms beyond general cytokine suppression, likely involving the targeted inhibition of specific inflammatory mediators.

Conclusion

The present study elucidates the critical involvement of glial cells in orchestrating a dynamic cascade of mediators leading to that culminate in neuronal demise. It also demonstrates the synergistic effect of IL-1β and TNF-α in promoting neurodegeneration within the glia-neuron coculture system, underscoring the significance of interplay between inflammatory mediators. These findings offer valuable insights into the mechanisms underlying neuroinflammation and highlight the potential for targeted therapeutic interventions in neurodegenerative diseases.

LPS-induced neuroinflammation in the glia-neuron coculture represents a relevant model to study the pathogenesis of neurodegenerative diseases and serves as a valuable screening assay in the quest for effective therapeutic interventions.