The effect of immunosuppressive and immunomodulatory drugs in a cellular model of brain inflammation: involvement of nitric oxide-mediated neuronal death

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Key points

- NO - dependent and independent pathways are involved in the neuronal death observed in LPS stimulated cocultures.
- Inhibition of NO production alone is not sufficient to prevent the neurodegeneration. The magnitude of change in production of NO-independent mediators counterbalances the potential beneficial effect of NO pathway inhibition during the inflammatory process.

Introduction

Neuroinflammation is now recognized as a critical process in different neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, stroke and multiple sclerosis. Microglia and astrocytes are key players in neuroinflammation since they release a wide variety of proinflammatory mediators, including nitric oxide (NO).
Glia-derived nitric oxide (NO) has been demonstrated to be a key effector responsible for the neurodegeneration following stimulation of a mixed culture of neurons, microglia, and astrocytes.[1]

([1] The Journal of Neuroscience, September 1, 2001, 21(17):6480–6491)

Objectives

- To study the correlation between suppression of glia-derived NO and the neuroprotection induced by immunosuppressive (dexamethasone) and immunomodulatory (doramapimod) drugs.
- To investigate the contribution of other inflammatory mediators such as TNF-α and IL-1β (NO-independent pathways) in the neuronal death.

Experimental design

Glia neuron culture from the mesencephalic brain of rat embryos (day 15 of gestation).

- Measure of death of dopaminergic neurons by immunostaining of Tyrosine Hydroxylase-positive neurons (TH-positive neurons)
- Measure of IL-1β and TNF-α release by ELISA
- Measure of NO production by Griess reaction

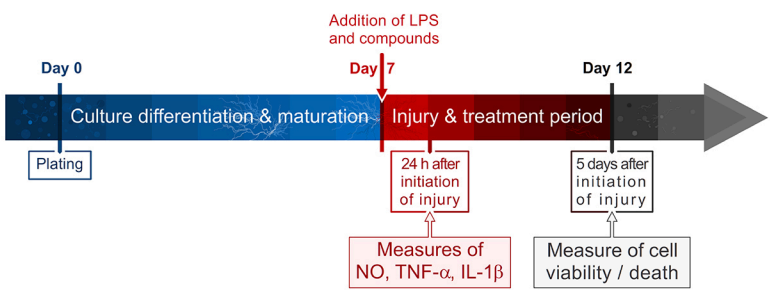
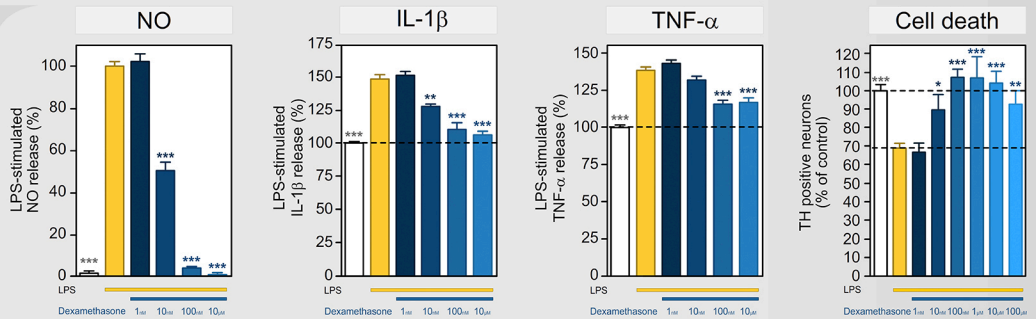


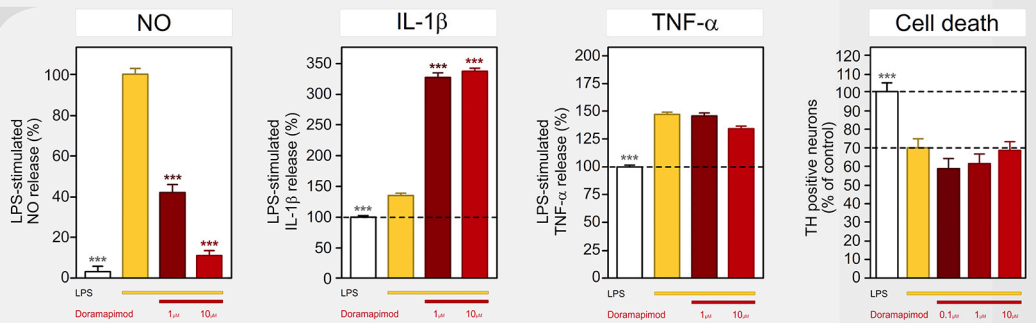
Figure 1: Dexamethasone (immunosuppressor)



Dexamethasone markedly reduced the release of NO as well as IL-1β and TNF-α.

Dexamethasone fully prevented LPS-induced neuronal death.

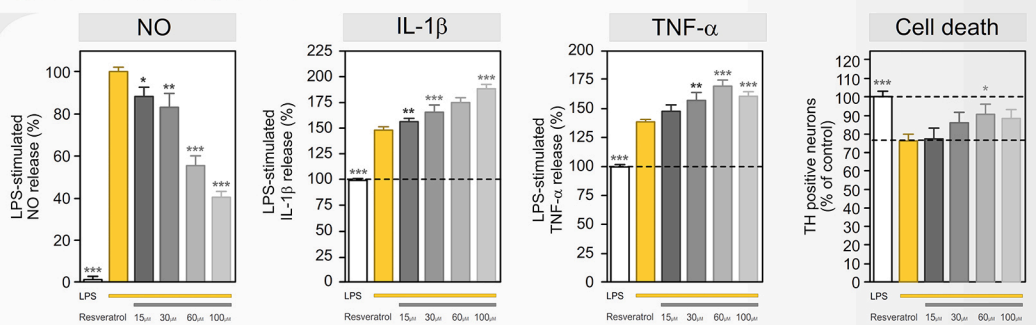
Figure 2: Doramapimod (immunomodulator)



Doramapimod fully suppressed NO production but dramatically stimulated the release of IL-1β (up to 2.5 times higher than under the control LPS condition).

Doramapimod did not prevent LPS-induced neuronal death.

Figure 3: Resveratrol (antioxidant)



Resveratrol markedly reduced NO production but dramatically stimulated the release of both IL-1β and TNF-α (1.3 times higher than under the control LPS condition).

Resveratrol partially, but significantly, prevented LPS-induced neuronal death.