Universidad Nacional Autónoma de México

CYTOCHROME P450 2J3 AND 2C11 REGULATION IN A LPS-INDUCED NEUROINFLAMMATION MODEL IN ASTROCYTES

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Introduction

The development and progression of several neurodegenerative and neuropsychiatric illnesses have been related to inflammatory processes in the Central Nervous System (CNS). The cytochrome P450 (CYP) epoxygenases and its metabolites, the epoxyeicosatrienoic acids (EETs), have been proposed as important therapeutic targets for the treatment of both systemic and organ specific inflammatory processes, due in part to its potent anti-inflammatory activity. However, little has been described about the regulation of these enzymes during inflammation in the CNS. It has been reported that the expression of some CYP can be modified by pro-inflammatory cytokines such as IL-6, IL-1b and TNF-a. Cytokine-mediated down regulation of some CYP has been related to NF-kB binding to the promoter region of its genes. Our goal is to elucidate whether an inflammatory process developed in astrocytes is able to modify CYP2J3 and 2C11 expression and the mechanism by which this process is carried out.

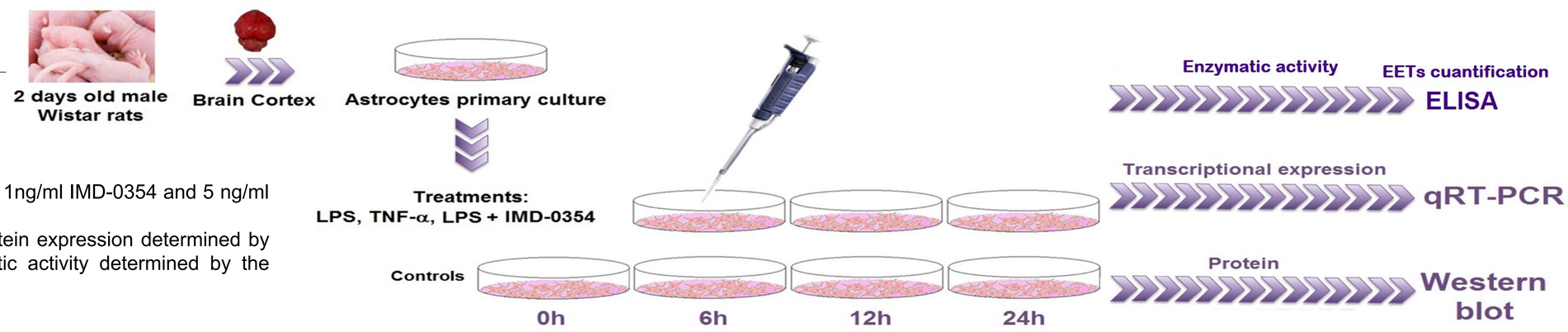
Objective

Determine whether an inflammatory process developed in astrocytes is able to modify CYP 2J3 and 2C11 expression, protein levels and activity. Additionally we want to describe the mechanism by which this process is carried out.

Hypothesis

CYP2J3 and 2C11 mRNA expression, protein levels and activity will be decreased during an inflammatory response in astrocytes. This reduction will be related to the production of pro-inflammatory cytokines and activation of NF-kB pathway.

Experimental strategy



•Primary astrocytes cultures.
•100 ng/ml LPS, 5 ng/ml TNF-α, 100 ng/ml LPS + 1ng/ml IMD-0354 and 5 ng/ml TNF-α + 1 ng/ml IMD-0354 treatments.
•mRNA expression determined by qRT-PCR, protein expression determined by immunofluorescence and Western Blot, enzymatic activity determined by the cuantification of 11,12-EET + 11,12-DHET.

Results

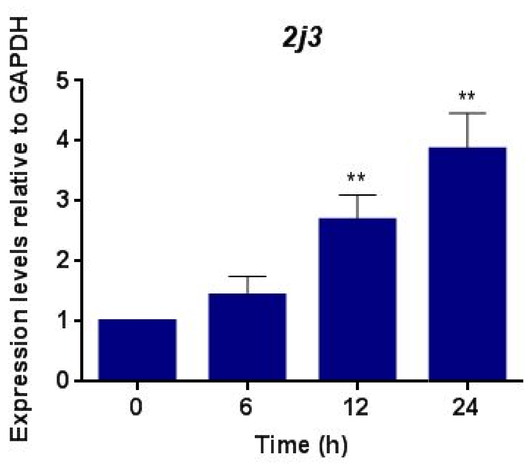


Fig. 1. Astrocytes *cyp2j3* and *cyp2c11* relative gene expression along time. Total mRNA was isolated at 6, 12 and 24 h without any treatment. *cyp2j3* and *cyp2c11* relative expression levels were determined by qRT-PCR. Expression levels are shown relative to GAPDH ± SD (n=3). * *t* student test (p ≤ 0.05, n=4); ** *t* student test (p ≤0.01, n=4).

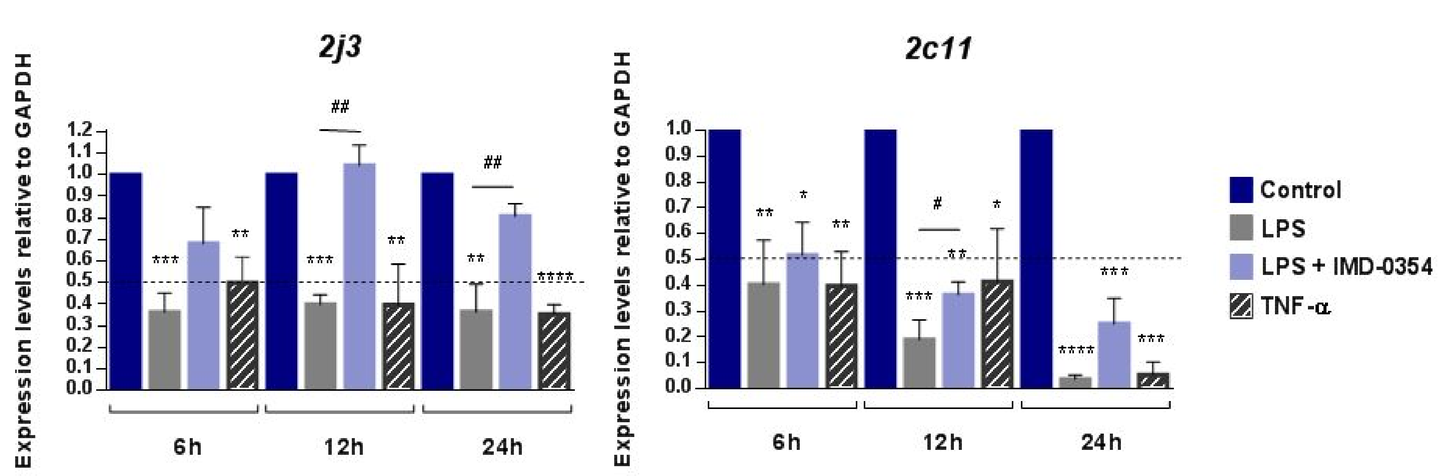


Fig. 2. Astrocytes *cyp2j3* and *cyp2c11* relative gene expression levels after 100 ng/ml LPS or 5 ng/ml TNF-α stimulation. Total mRNA was isolated at 6, 12 and 24 h after treatment. *cyp2j3* and *cyp2c11* relative expression levels were determined by qRT-PCR. Expression levels are shown relative to GAPDH ± SD (n=4). * *t* student test (p ≤ 0.05, n=4); ** *t* student test (p ≤0.01, n=4); *** *t* student test (p ≤ 0.001, n=4); **** *t* student test (p < 0.0001, n=4).

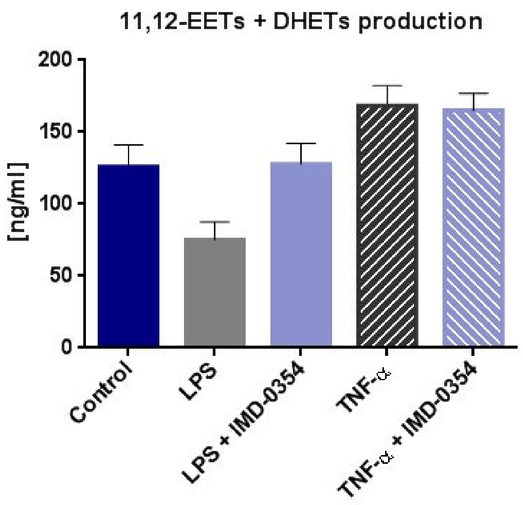


Fig. 3. Total epoxygenase activity determined as the production of 11,12-EET + 11,12-DHET after 100 ng/ml LPS or 5 ng/ml TNF-α stimulation. 1 ng/ml of IMD-0354 was administered as NFκB inhibitor.

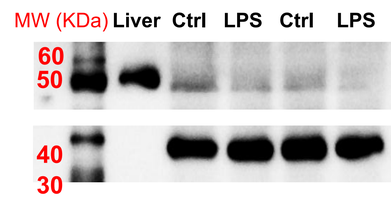


Fig. 4. Effect of 100 ng/ml LPS treatment in astrocytes CYP2J3 protein levels. Duplicate cultures were incubated with 100 ng/ml LPS or without it for 24 hours. Cells were washed three times with PBS and scraped for its recovery with extraction buffer. Each lane contains 60 μg of total protein from the recovered lysates.

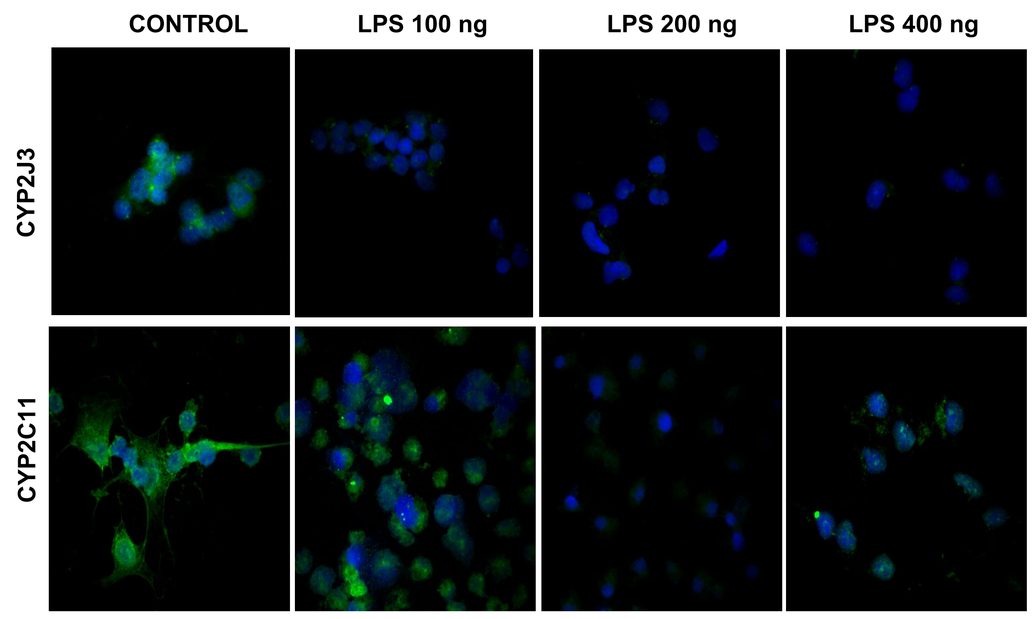


Fig. 5. . Immunofluorescent identification of rat brain CYP2J3 and CYP2C11 after 100, 200 and 400 ng/ml LPS treatment in a primary astrocytes culture. Blue: Nucleus stained with Hoechst. Green: CYP2J3 or CYP2C11.

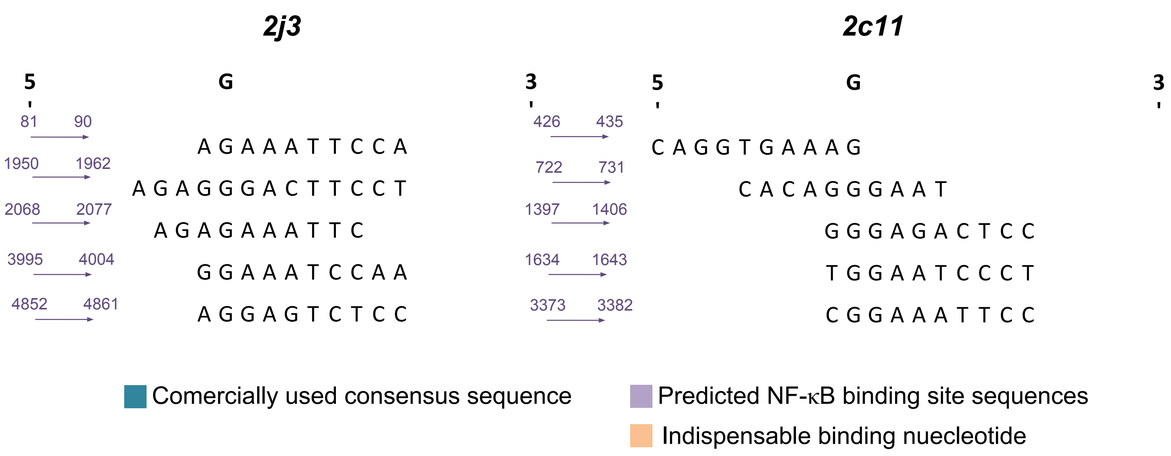


Fig. 6. NF-κB binding site predictions. Five-thousand base pair upstream start site for *cyp2j3* and *cyp2c11*. Predictions were calculated through AliBaba 2.1.

Conclusions

The inflammatory response triggered by the addition of LPS to astrocyte cultures was able to down-regulate *cyp2j3* and *cyp2c11* mRNA, CYP2J3 protein expression and total epoxygenase activity. LPS mediated down-regulation of CYP2J3 and CYP2C11 expression may be due in part to the production of pro-inflammatory cytokines like TNF-α, since this cytokine reproduced the LPS effects. Transcription factor NF-kB may play an important role in TNF-α mediated down-regulation of CYP2J3 since its inhibition by IMD-0354 reversed CYP down regulation.

References

•Anwar M.A., Zordoky B.N., Aboutabl M.E., El-Kadi A.O. (2010) Alteration of cardiac cytochrome P450-mediated arachidonic acid metabolism in response to lipopolysaccharide-induced acute systemic inflammation, Pharmacological research: the official journal of the Italian Pharmacological Society, 61 (5): 410-8.
•Nicholson T.E., Renton K.W. (2002) The role of cytokines in the depression of CYP1A activity using cultured astrocytes as an in vitro model of inflammation in the central nervous system, Drug Metabolism and Disposition, 30: 42-46.
•Phillis J.W., Horrocks L.A. & Farooqui A.A. (2006) Cyclooxygenases, lipoxygenases, and epoxygenases in CNS: their role and involvement in neurological disorders, Brain Research reviews, 52 (2): 201-43.