Neuropharmacology-Report 3

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Abstract:

Results:

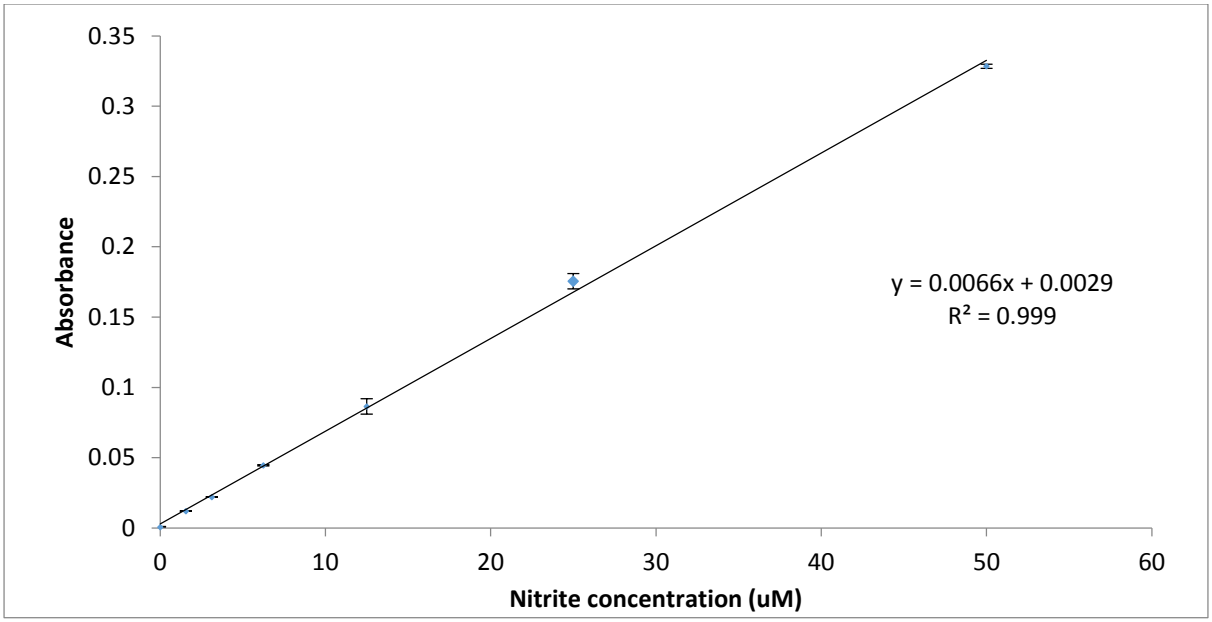
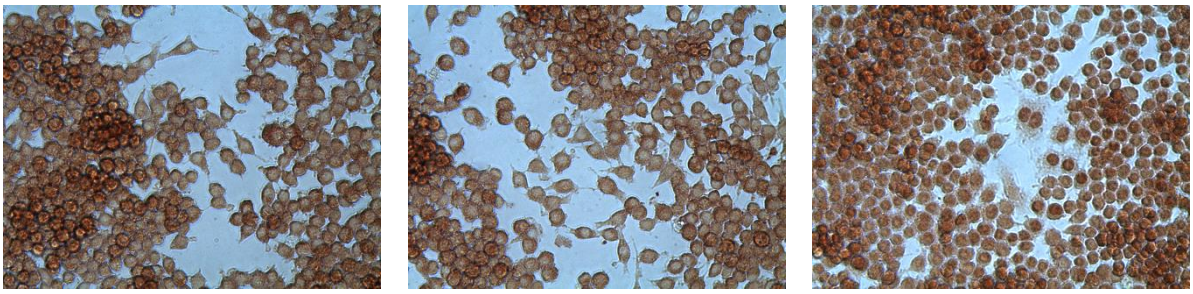


Fig 1. Nitrite calibration curve. Calculated from two sets of 6 calibration standards, diluted from 1Mm nitrite solution.

Table 1. Nitrite production by RAW 264.7 macrophage cells after treatment with LPS

Treatment time	Treatment	Average absorbance	Absorbance Standard deviation	Concentration of nitrite (uM)
15min	Control	0.016	0.004	1.25-2.62
	Vehicle	0.009	0.002	0.496-1.25
	LPS	0.011	0.002	0.856-1.60
24hr	Control	0.015	0.006	0.967-2.699
	Vehicle	0.008	0.004	0.213-1.43
	LPS	0.129	0.010	17.6-20.7

Concentration of nitrite given as a range arounds 1 SD from the mean. n=18. When RAW 264.7 macrophage cells are treated with LPS for 24 hours, the nitrite produced increases. This suggests that NO production takes some time to occur, or that the breakdown of it to nitrite takes longer than 15 minutes.



Control Vehicle LPS

Fig 2. Effect of 15 minute LPS treatment on RAW 264.7 macrophage cells. Control macrophage cells those treated with only the vehicle display a darker stain in the cytosol, while the LPS treated cells show a darker stain within the nucleus. A darker stain suggests a higher concentration of NFkB in that location.

Both the control and vehicle show a darker stain and therefore a higher concentration of NFkB in the cytosol. The LPS treated RAW 264.7 cells show a darker staining in the middle portion of the cell; the nucleus. This suggests that LPS treatment causes the translocation of NFkB from the cytosol to the central nucleus after only 15 minutes.

Discussion:

Nuclear factor kappa B (NFkB) is a protein which has been implicated in inflammatory responses around the body. Our results show that treating RAW 264.7 macrophage cells with LPS will cause the translocation of NFkB from the cytosol to the nucleus. We likely saw this because NFkB contributes to inflammation by inducing inflammatory gene expression (1). When inactivated, NFkB is bound to the inhibitory protein IκBα in the cytosol (NFKBA). Lipopolysaccharide (LPS) binds with TLR4 receptors, and through a signal transduction pathway seen in Fig 3, leads to the activation of the IκB kinase (IKK) enzyme, which disassociates IκBα from NFkB. NFkB is then translocated from the cytosol to the nucleus where it increases the expression of pro-inflammatory cytokines. This translocation explains why in our results we saw a darker stain in those cells which had been treated with LPS. In Fig 2 it is apparent that the stain is darker in the cytosol in the control and vehicle treated cells, while in the LPS treated cells the stain is darker in the nucleus. This suggests that most of the NFkB has been translocated into the nucleus after LPS treatment.

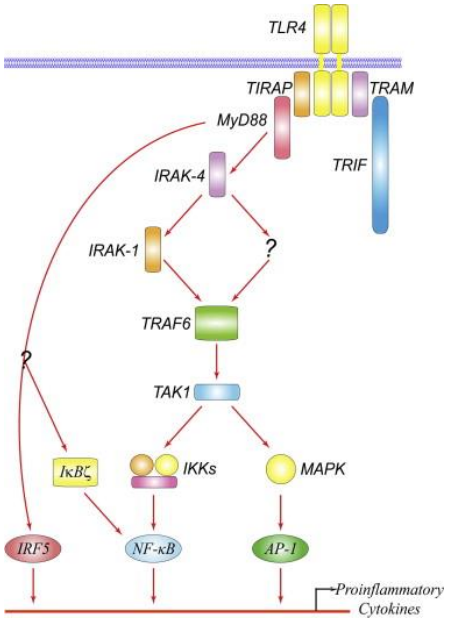


Fig 3. Signal transduction pathway showing activation of IκB (2)

Nitric oxide (NO) is another molecule which is important for inflammatory actions in the body. NO is produced by nitric oxide synthase (NOS) from L-arginine. A particular isoform of NOS, NOS II- inducible nitric oxide synthase (iNOS) is produced by macrophages. LPS is one endotoxin which can induce iNOS, leading to enhanced formation of NO in the macrophage(RNO). Increased levels of NO in activated glial cells have been associated with

neurodegeneration.
From our results it appears that the induction of iNOS takes more than 15min to occur, as seen in Table 1. The concentration of nitrite in the culture medium was not higher in LPS treated RAW 264.7 cells, compared to the control and vehicle cells. After treatment with LPS for 24 hours, there was a marked increase in nitrite levels. This fits with the previously mentioned mechanism, and further supports that LPS induces iNOS.

LPS likely leads to inflammation as it detects it as a foreign body. LPS is expressed by certain bacteria on their surface. Macrophages detect this LPS and through both the NFkB and NO mechanisms induce inflammation as part of the immune response.

NFkB and NO have been implicated in neurodegeneration. In one study performed on rats

Mechanism of NF-κB action. In this figure, the NF-κB heterodimer between Rel and p50 proteins is used as an example. While in an inactivated state, NF-κB is located in the cytosol complexed with the inhibitory protein IκBα. Through the intermediacy of integral membrane receptors, a variety of extracellular signals can activate the enzyme IκB kinase (IKK). IKK, in turn, phosphorylates the IκBα protein, which results in ubiquitination, dissociation of IκBα from NF-κB, and eventual degradation of IκBα by the proteosome. The activated NF-κB is then translocated into the nucleus where it binds to specific sequences of DNA called response elements (RE). The DNA/NF-κB complex then recruits other proteins such ascoactivators and RNA polymerase, which transcribe downstream DNA into mRNA, which, in turn, is translated into protein, which results in a change of cell function.[1][2][3]

References:

(NFKBA)http://link.springer.com.ezproxy.auckland.ac.nz/article/10.1385/CT:6:2:111#page-3

Roles of nitric oxide and superoxide in inflammation (RNO)