Metformin reduces NF-κB nuclear translocation under inflammatory stress in rat annulus fibrosus cells

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No Disclosures
Inflammation in Low Back Pain

Intervertebral disc degeneration (IDD) is a primary risk factor for lower back pain and is clinically associated with inflammation.

Inflammatory stress activates the NFkB pathway.

Recent studies in our lab have shown an association between metformin use and lower intensity of back pain in diabetic patients (unpublished date). However, it is unclear how metformin produces this effect.

Because NF-κB plays a central role in mediating cell response to inflammatory stress, we hypothesized that metformin suppresses inflammation in disc cells by downregulating the NF-kB signaling pathway.
Experimental Design

When activated, NFkB (detected by its subunit p65 immunofluorescence-red), translocates from cell cytoplasm to the nucleus.
NFkB quantification

- We tag the nucleus with blue DAPI stain and tag the NFkB with red stain.
- We used an in-house software to indicate the edges of the cell.
- The software uses the DAPI staining to determine the edges of the nucleus.
- Finally, it calculates the intensity of nuclear NFkB staining as a percentage of the total NFkB staining in the entire cell.

NFkB primary antibody= Rb anti-p65 subunit of NF-κB primary antibody (1:200 dilution, Cell Signal)
NFkB secondary antibody= Cy3 Goat anti-Rb IgG (1:500 dilution, Jackson ImmunoResearch)
Results

Control = minimal nuclear NFkB staining

Metformin = minimal nuclear NFkB staining

IL1 only = significant nuclear NFkB staining

IL1 + Metformin = reduced intensity and number of cells with nuclear NFkB staining.
Metformin treatment of cultured rat annulus fibrosus cells significantly reduced the nuclear translocation of NFkB after 4hr of IL1-beta treatment from 43.1% in case of IL1 beta treatment down to 26.2% in the case of metformin + IL1 beta treatment.

Other time points have similar trends but were not statistically significant.
Conclusion

Metformin suppresses IL-1-mediated inflammation in rat annulus fibrosus cells by inhibiting the nuclear translocation of the p65 subunit of NFkB, a key proinflammatory transcriptional factor.

Metformin’s anti-inflammatory action is mediated at least in part through inhibiting the NFkB signaling pathway. Thus, metformin could be an important therapeutic agent for treating intervertebral disc degeneration.
THANK YOU

• Petr Pancoska, PhD
• Gwen Sowa, MD PhD
• Nam Vo, PhD
• Ferguson Lab