89 年度計畫執行進度報告

細胞間素活化轉錄因子 NF-κB 引起眼球內炎性反應的研究

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Suppression of Proinflammatory Gene Expression in Bovine Retinal Pigment Epithelial Cells by NF-κB inhibitors

Introduction

The nuclear transcription factor NF-κB activates various pro-inflammatory genes expression and may play an essential role in ocular inflammation. In this study, we evaluated the inhibitory effect of several NF-κB inhibitors, including proteasome inhibitors, SN50 and prostaglandin, on proinflammatory genes expression in cultured bovine retinal pigment epithelial (RPE) cells.

Methods.

Cultured bovine RPE cells was pretreated with NF-κB inhibitors, including MG132, latacystin, SN50 and prostaglandin A1 and then stimulated by interleukin-1β (IL-1β), or tumor necrosis factor-α (TNF-α). Nuclear translocation of NF-κB was determined by immunofluorescent stain. Cytoplasmic I-κB protein was measured by western blotting. Nuclear extract binding to κB DNA motifs was measured by electrophoretic mobility shift assay. RT-PCR was used to determine mRNA expression of various proinflammatory cytokine and chemokine genes. Cytokine and chemokine protein levels in cell culture medium were measured by ELISA methods.

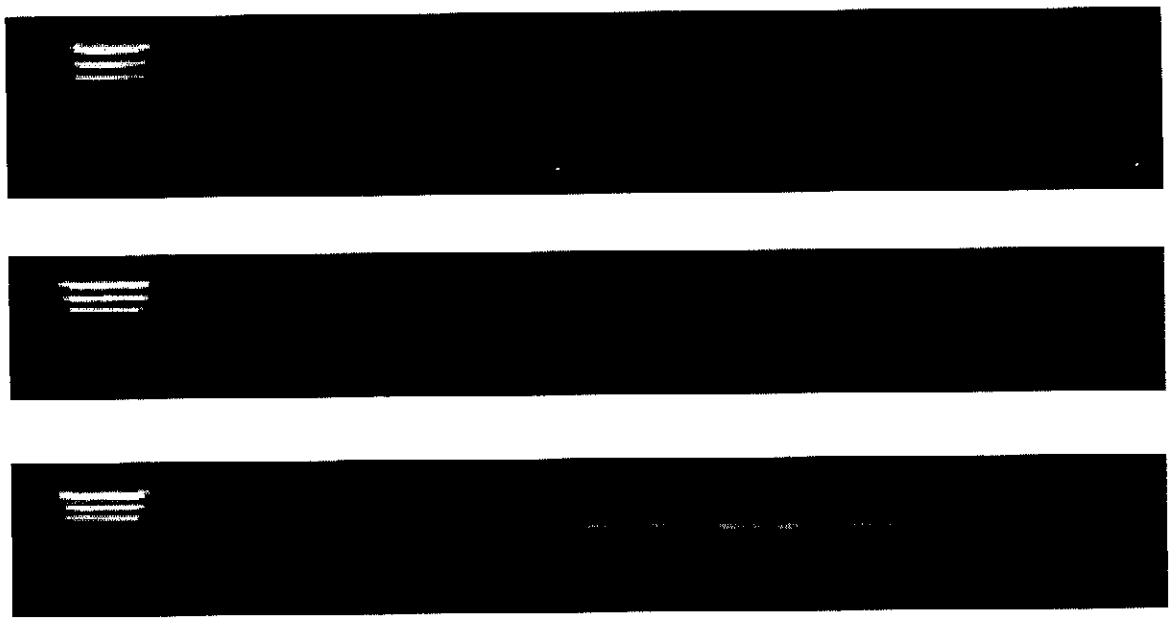
Results.

NF-κB inhibitors, including MG132, latacystin, SN50 and prostaglandin A1 all dose-dependently inhibited IL-1β or TNF-α induced proinflammatory genes expression.

Conclusion.

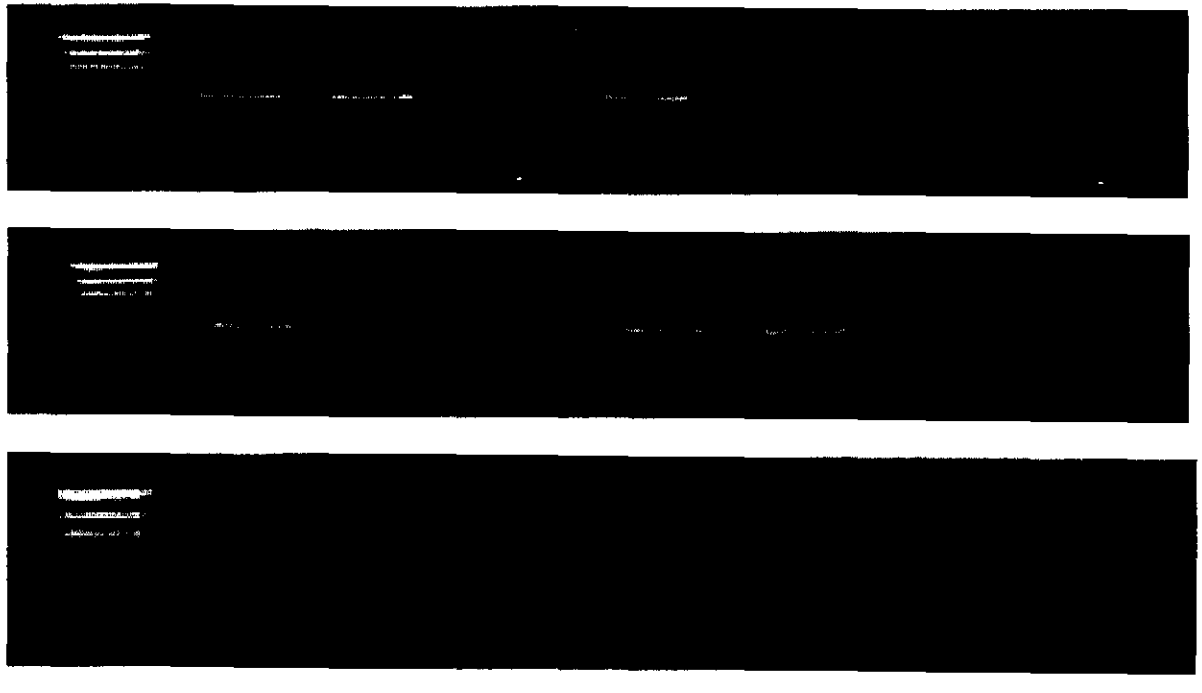
MG132, latacystin, SN50 and prostaglandin A1 suppress NF-κB dependent proinflammatory genes expression in RPE cells. Inhibition of NF-κB may be a useful strategy to treat ocular inflammatory disorders.

TNF-α	+	+	+	-	-	-
IL-1β	-	-	-	+	+	+
PGA1 50 μM	-	+	-	-	+	-
PGA1 200 μM	-	-	+	-	-	+

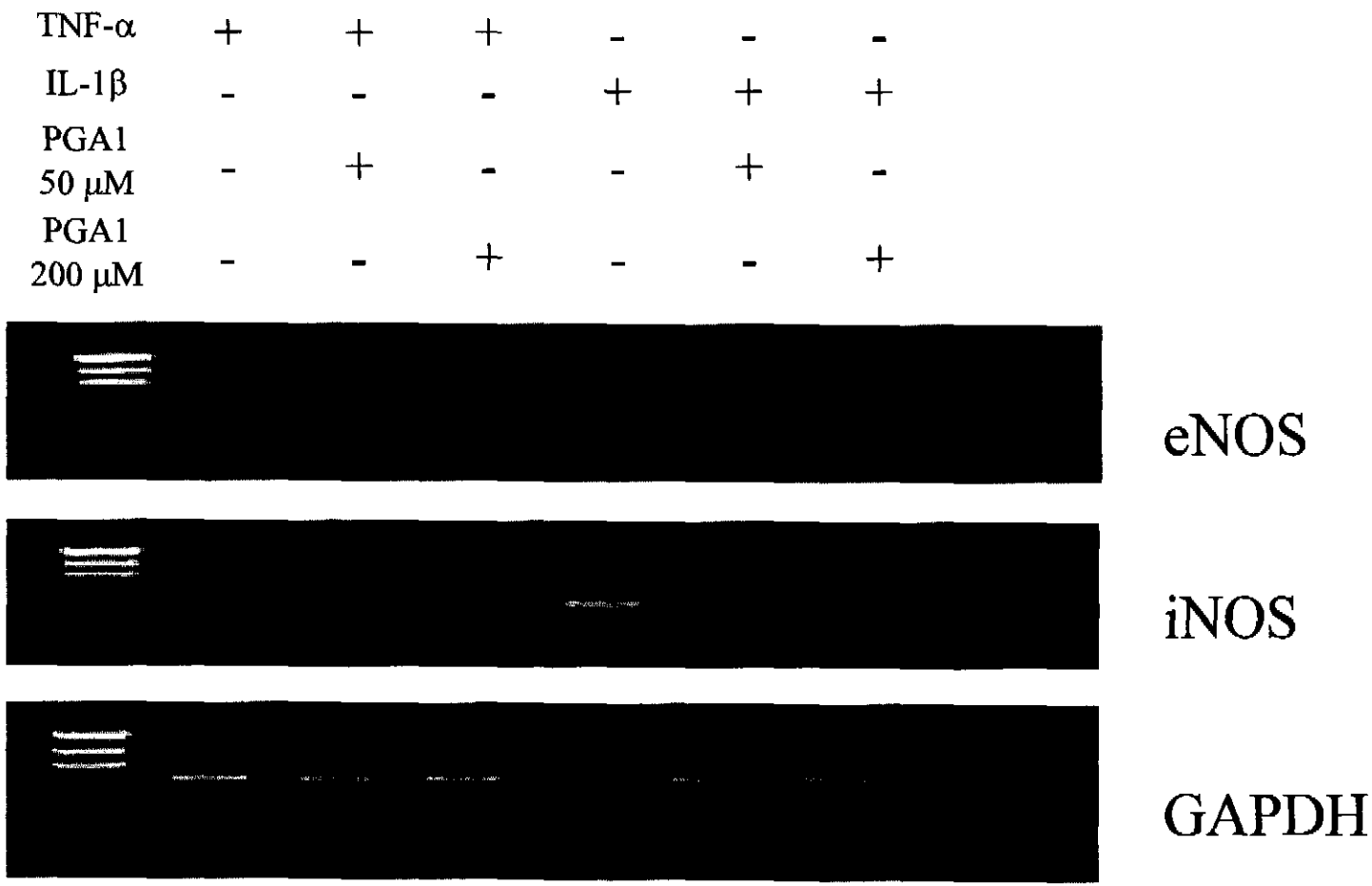


Prostaglandin A1 do not suppress TNF-α and IL-1β induced angiogenic cytokine genes expression. RPE cells do not express VEGF-C gene.

TNF-α	+	+	+	-	-	-
IL-1β	-	-	-	+	+	+
PGA1 50 μM	-	+	-	-	+	-
PGA1 200 μM	-	-	+	-	-	+

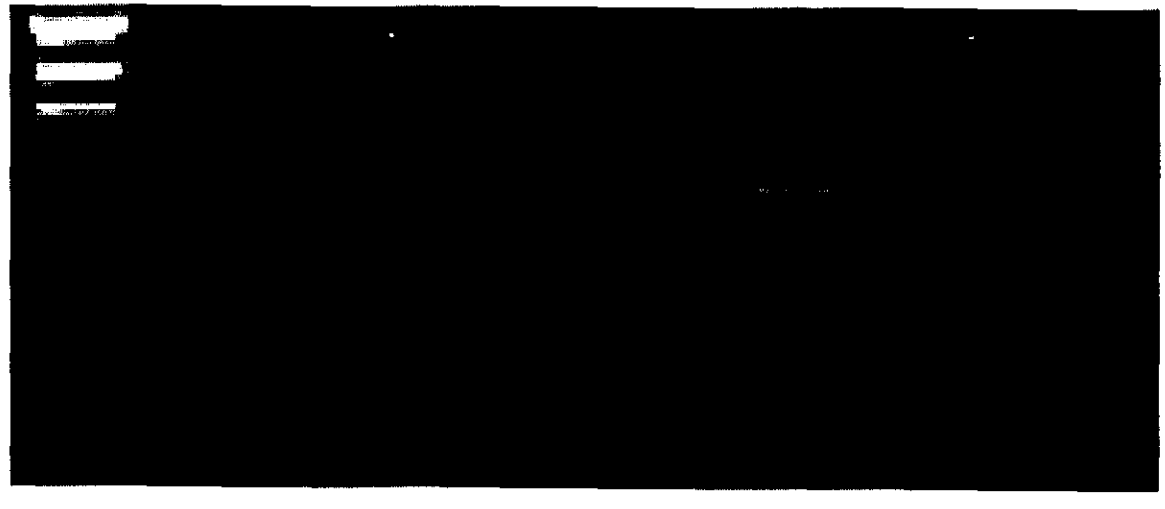


Prostaglandin A1 dose dependently suppress TNF-α and IL-1β induced proinflammatory genes expression.



Prostaglandin A1 dose dependently suppress TNF-α and IL-1β induced iNOS genes expression. PRE cells do not express eNOS gene

TNF-α	+	+	-	-
IL-1β	-	-	+	+
SN50 100 μM	-	+	-	+



iNOS

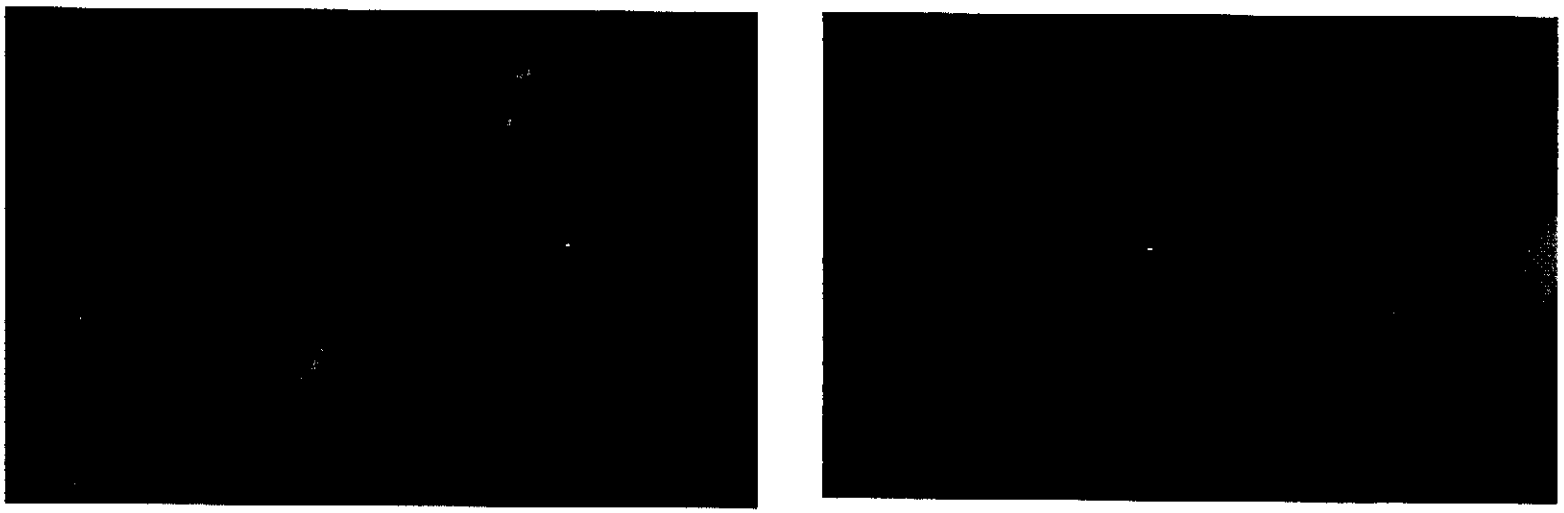
Suppression of iNOS gene expression by SN50 100 μM

Control

TNF-α 200 μg/ml

Pretreated by prostaglandin A1

(200 μM) + TNF-α 200 μg/ml



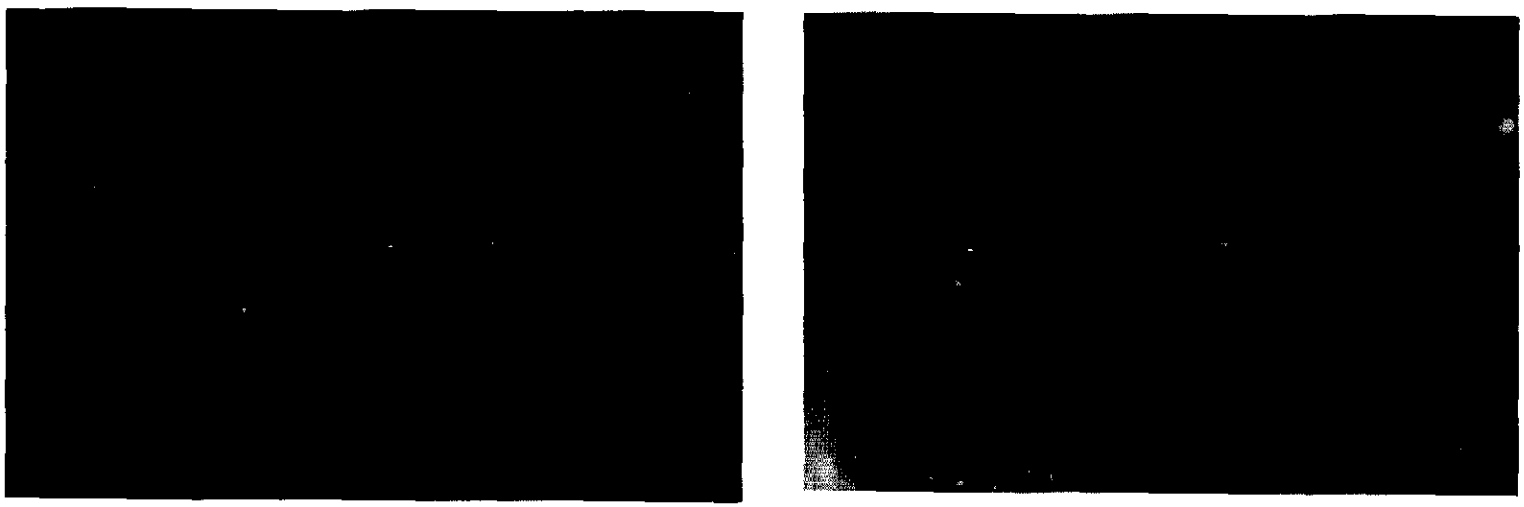
Prostaglandin A1 inhibits TNF-α induced nuclear translocation of NF-κB

Control

TNF-α 200 μg/ml

Pretreated by SN 50 (100 μM)

+ TNF-α 200 μg/ml



SN 50 inhibits TNF-α induced nuclear translocation of NF-κB

Effect of NF-κB inhibitor, Pyrrolidine Dithiocarbamate, on Experimental Autoimmune Anterior Uveitis

Purpose.

The nuclear transcription factor NF-κB plays an essential role in the upregulation of various pro-inflammatory genes. In this study, we evaluated the effect of pyrrolidine dithiocarbamate (PDTC), a potent NF-κB inhibitor, on Experimental Autoimmune Anterior Uveitis (EAAU), a clinically reverent rat model of human acute anterior uveitis.

Methods.

EAAU was induced in Lewis rat by injection of bovine melanin associated antigen (MAA) in the footpad. Intraperitoneal injection of PDTC (40mg/kg) was given daily in experimental animals (n=20). In the control group (n=20), PBS was injected intraperitoneally. Rats were observed daily for 4 weeks to evaluate the clinical course of EAAU. In a separated study, rats were sacrificed at various time points and iris/ciliary body and popliteal lymph node were harvested. Semiquantitative RT-PCR was used to determine mRNA expression of chemokine and their receptors.

Results.

The severity of ocular inflammation of EAAU in experimental group was significantly suppressed by PDTC as compared to control group. However, PDTC could not prevent the development of EAAU.

Conclusion.

NF-κB inhibitor, PDTC, did suppress the clinical course of EAAU. Factors other than NF-κB may involve in the pathogenesis of EAAU.

Effect of PDTC on the Clinical Presentation of EAAU