行政院國家科學委員會專題研究計畫 期中進度報告

MAPK 訊息傳導途徑在角膜傷口癒合所扮演的角色(2/3)
期中進度報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 95-2314-B-002-057-
執 行 期 間 ：95 年 08 月 01 日至 96 年 07 月 31 日
執 行 單 位 ：國立臺灣大學醫學院眼科

計 畫 主 持 人：胡芳蓉

處 理 方 式 ：期中報告不提供公開查詢

中 華 民 國 96 年 05 月 31 日

行政院國家科學委員會補助專題研究計畫 □成果報告

■期中進度報告

（計畫名稱）

MAPK 訊息傳導途徑在角膜傷口癒合所扮演的角色(2/3)

計畫類別：■ 個別型計畫　　□ 整合型計畫
計畫編號：NSC 94－2314－B －002 －057－
執行期間：　95 年 08 月 01 日 至　96 年 07 月 31 日

計畫主持人：胡芳蓉
共同主持人：陳偉勵
計畫參與人員： 陳美雲, 李靜雯

成果報告類型(依經費核定清單規定繳交)：■精簡報告　□完整報告

本成果報告包括以下應繳交之附件：
□赴國外出差或研習心得報告一份
□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年□二年後可公開查詢

執行單位：國立台灣大學醫學院眼科

中　華　民　國　　96 年　　05 月　　29 日

Abstrate

MAPK signal transduction pathway, an important TGF-beta associated signal transduction pathway, has been proved to play a very important role in wound healing in a lot of cell types and tissues. In this study, we compared the expression level of unphosphorylated-MAPK with phosphorylated-MAPKs such as p38, ERK, JNK in different areas of corneal endothelial cells in bovine and rabbit corneal cells. Primary cultures of bovine and rabbit corneal endothelial cells were used in this study. Cells were treated with different concentrations of ERK inhibitor (PD98059), p38 inhibitor (SB202190) and JNK inhibitor (SP600125). Immunohistochemistry was performed to demonstrate the phosphorylation of MAPK after injury. Confluent cells were scraped with a straight line injury and treated with different concentrations of PD98059, SB202190 and SP600125. The wound healing rate was evaluated after 24, 48 and 72 hours. After complete resurface of the scrape wound, immunohistochemistry with Ki67, MTS assay and cell density analysis were performed to evaluate the role of proliferation during wound healing. For analyzing the role of cell migration during wound healing, chemotaxis assay by modified Boyden chamber and time lapse microscopic video imaging were performed. Our data showed that phosphorylation of ERK was found from wound margin to submarginal cells after wounding by immunohistochemical analysis. PD98059 and SP600125 cause dose-dependent inhibition of the wound closing speed. The inhibition of wound healing becomes significant from 48 to 72 hours in cells treated with 10 and 50 uM of PD98059 and 10 uM of SP600125 ($p<0.05$). ERK inhibition suppressed cell proliferation in Ki67 positive cells and MTS assay. Delay in migration by Boyden chamber chemotaxis assay and time lapse microscopy was also found when treated with PD98059.

Introductions

It is well known that epithelial/endothelial wound can induce a multitude of cellular responses, such as propagation of calcium wave, activation of MAP kinases, and induction of gene expression around the wound. However, it is still not understood how these responses lead to coordinated migration of cell sheets as a unit especially in corneal endothelial cells. Stress-activated protein kinases such as p38 MAP kinase and c-Jun N-terminal protein kinase (JNK) modulate several responses to cell stress and injury, but their role in corneal endothelial cell migration and proliferation is not clear. To investigate the role of JNK1/2 in cellular proliferation and migration in wound healing process of corneal endothelial cells, primary culture of bovine and rabbit corneal endothelial cells were used in this study. We first examined the activation pattern of JNK1/2 in these cells after wounding. Cells were then divided into following groups: (1) control group (2) medium adding with 5uM of JNK inhibitor SP600125 (3) medium adding with 10uM of SP600125. For cell migration assay, confluent cells were treated with straight line scrape injury. The wound healing processes were evaluating 12 hours, 24, 36, 48 and 72 hours later. In addition, time lapse microscopy was used to evaluating the lamellipodia formation and cell spreading in different groups. For cell proliferation assay, Ki67 positive at wound edge and MTS assay were used.

Material and Methods

Reagents and antibodies. The chemical inhibitor of JNK, SP600125, was obtained from Calbiochem (San Diego, CA). The mouse anti-phosphorylated JNK (JNK1/2) monoclonal antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG were from Jackson Immunoresearch Laboratories (West Grove, PA).

Culture of bovine corneal endothelial cells. Primary cultured bovine corneal endothelial cells were used. Cells were cultured in DMEM supplemented with 10% fetal calf serum (Gibco BRL/Life Technologies, Rockville, MD). The cultures were

incubated at 37°C in 5% CO2. Secondary passage of cells were used.

Wounding Assay. Wound healing ability was measured using a monolayer-wounding protocol. Confluent cells were wounded with yellow tips. The observation time points were set as post-wounding 12, 24, 36, 48, 72 hours.

Immunocytochemical localization of JNK and the phosphorylated JNK. Cells were seeded onto glass slides and scratch wound will be performed with yellow tips. After various times after wounding, cells were washed with PBS, fixed in methanol at –20°C for 15 minutes, followed by permeablization with 1% Triton X-100. Cells were incubated in 2% fetal bovine serum in PBS and incubated with primary antibody at room temperature for 2 hours. Cells were then incubated for 1 hr with a 1:200 dilution of fluorescein isothiocyanate (FITC)-conjugated goat-anti-mouse antibody. Cells were then mounted in medium containing propidium iodide or DAPI.

Results and Discussions

Results

JNK inhibitors SP600125 can dose-dependently inhibit the wound healing process in the wounding assays. The difference of wound healing rate becomes significant from 10 hours to 72 hours in cells treated with 10uM of SP600125. However, there was no significant differences between control groups and cells treated with 5uM of SP600125. Cell proliferation also was no significances between control groups and experiment groups by ki67 staining and MTS assay.

Discussion

This study demonstrated that JNK plays an important role in coordinating the wound healing process in bovine corneal endothelial cells. Cellular proliferation, migration and spreading are all involved in this signal transduction pathway. To the best of our knowledge, this is the first study demonstrated the important role of JNK in the wound healing process of bovine corneal endothelial cells. Further studies focusing on cellular migration, including Boyden chamer analysis, quantification of lamellipodia formation, and cell spreading will be performed in the near future.

References

(1)Altan ZM, Fenteany G. c-Jun N-terminal kinase regulates lamellipodial protrusion and cell sheet migration during epithelial wound closure by a gene expression-independent mechanism. Biochem Biophys Res Commun. 2004; 332:56-67

(2) Matsubayashi Y, Ebisubya M, Honjoh S, Nishida E. ERK activation propagates in epithelial cell sheets and regulates their migration during wound healing. Curr Biol. 2004 ;14:731-5.

(3)Robinson MJ and Cobb MH. Mitogen-activated protein kinase pathways. Curr Opin Cell Bio 1997;9:180-6

附表及附圖

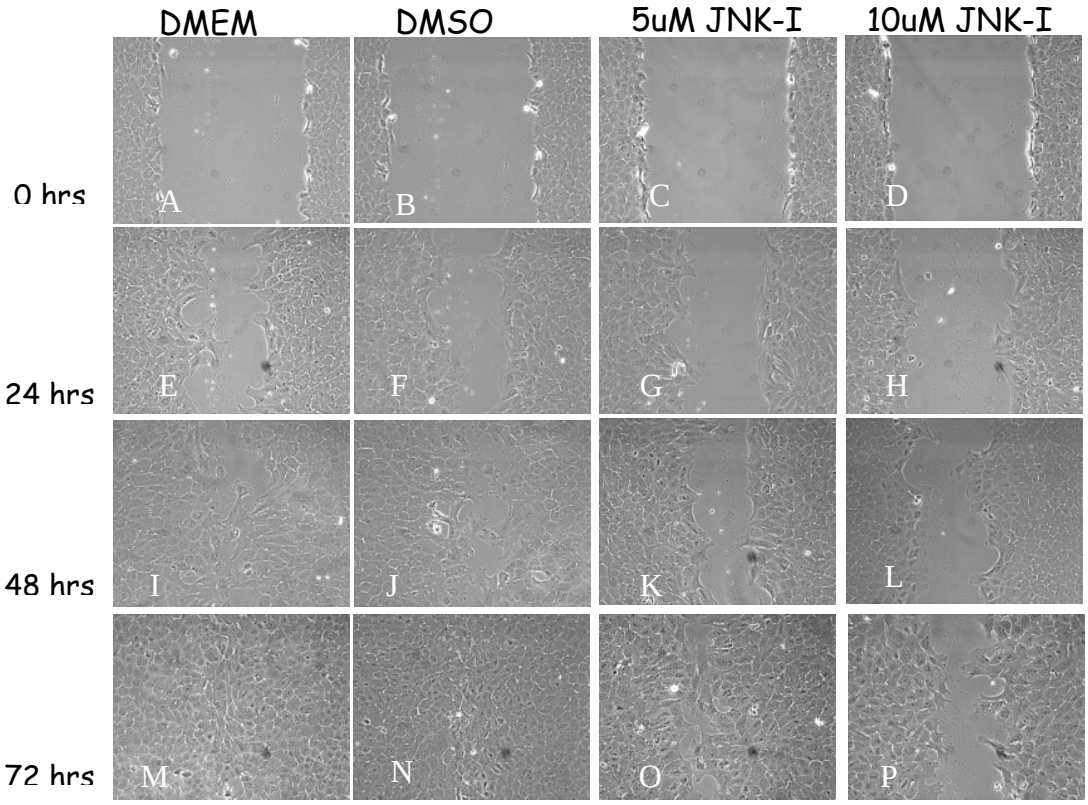

Figure 1. JNK1/2 activation involved in the wound healing process of bovine corneal endothelial cells. A-D: Immediately after wounding. E-H: 24 hours after wounding. I-L: 48 hours after wounding. M-P: 72 hours after wounding. A, E, I, M: Cells were cultured in media without inhibitors. B, F, J, N: Cells were cultured in media with 10 uM of DMSO. C, G, K, O: Cells were cultured in media with 5 uM of SP600125. D, H, L, P: Cells were cultured in media with 10 uM of SP600125.

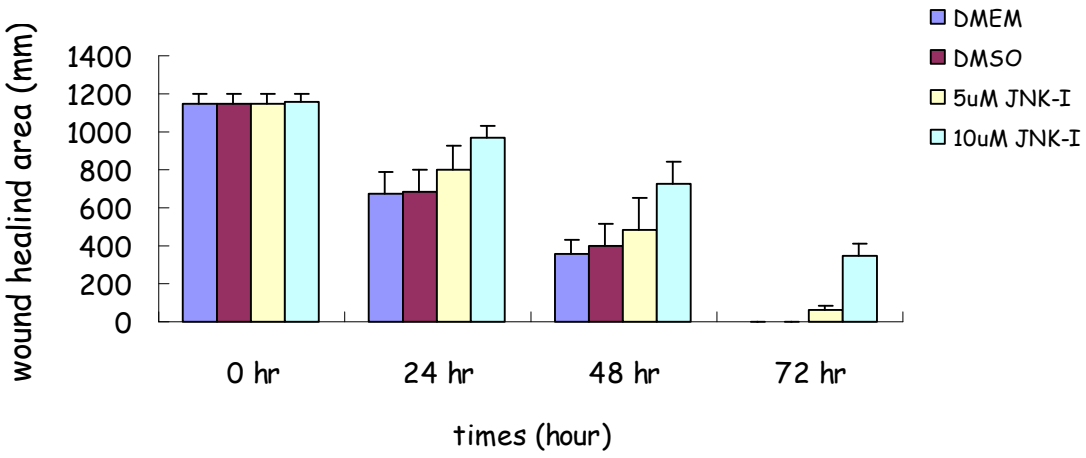

Figure 2. JNK1/2 activation involved in the wound healing process of bovine corneal endothelial cells.

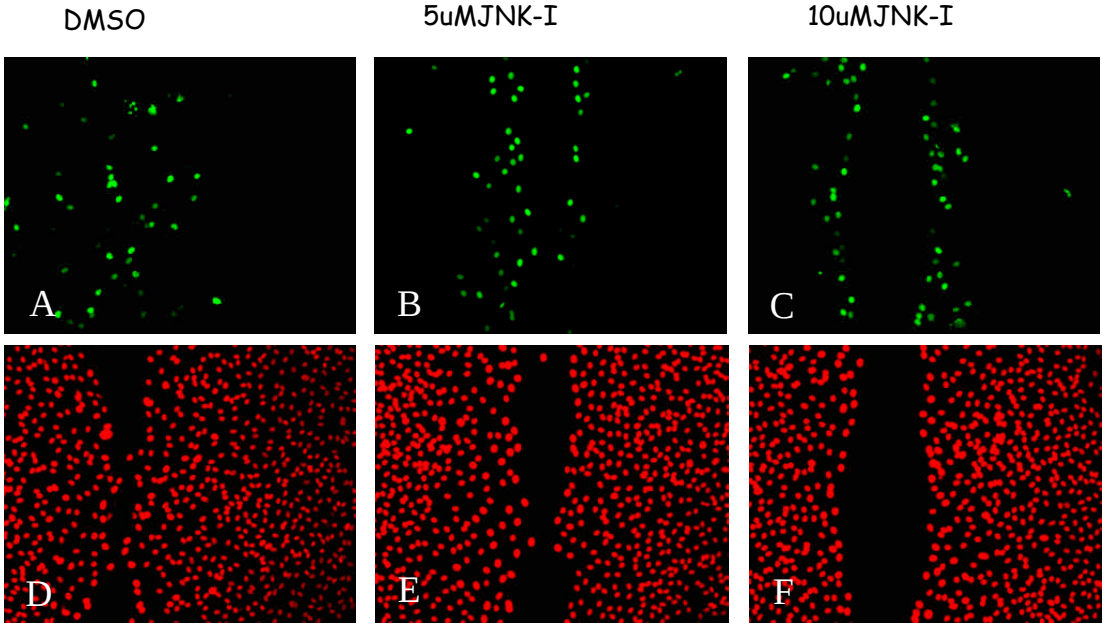

Figure 3: The proliferation of corneal endothelial cells in wound healing assay was stained by ki67. 5, 10uM of SP600125 were added in culture medium. The pictures were taken 24 hours after wounding. Green: Ki67, a marker for proliferation. Red: PI, a marker for nucleus.

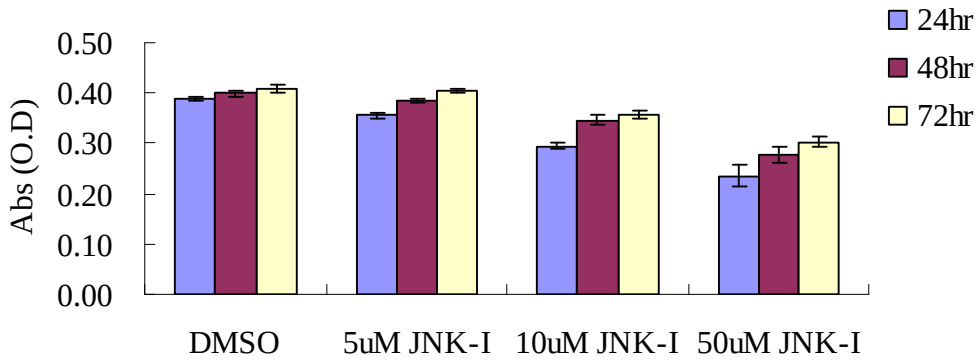

Figure 4: MTS assay and cell density analysis were performed to evaluate the role of proliferation during wound healing. 5, 10uM, 50uM of SP600125 were added in culture medium.