

行政院國家科學委員會專題研究計畫 期中進度報告

以 NF- κ B 抑制劑及抗凝血蛇毒蛋白抑制眼球之新生血管形
成(2/3)

計畫類別：個別型計畫

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Inhibition of Bovine Retinal Capillary Endothelial Cell Mediated Angiogenesis by Disintegrin, A $\alpha v\beta 3$ Integrin Antagonist from Snake Venom

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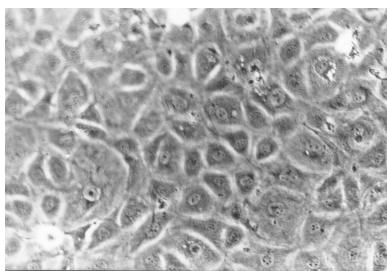
Introduction

Retinal angiogenesis occurs in a variety of disease, such as proliferative diabetic retinopathy, retinopathy of prematurity, and uveitis. A $\alpha v\beta 3$ integrin dependent vascular endothelial cell migration and proliferation may play an important role in the pathogenesis of new vessel formation. Disintegrin, a group of RGD-containing peptides from viper venom, is a strong antagonist of $\alpha v\beta 3$ integrin. In this study, the inhibitory effect of disintegrin on angiogenesis was evaluated in vitro by a bovine retinal capillary endothelial (BRCE) cell culture model.

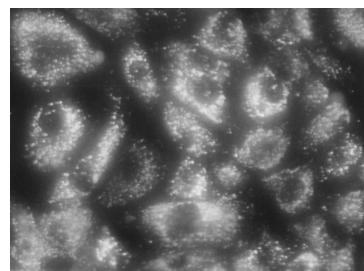
Materials and Methods

- A. *Bovine retinal capillary endothelial (BRCE) cell culture* A. Bovine eyes obtained from a local slaughterhouse
B. The retinas trapped on 53 μm nylon mesh, washed and suspended in a 10-fold volume of PBS.
C. Homogenization and resuspended in PBS and the microvessels collected onto a 83 μm nylon mesh.
D. Resuspended in PBS containing collagenase/dispase
E. Culture medium (M199 with 20% FCS).
F. Cultured in tissue culture flask at 37 ° C with 5% CO₂
G. Confirmed by staining with Dil-labeled low density lipoprotein (Dil-Ac-LDL)

BRCE



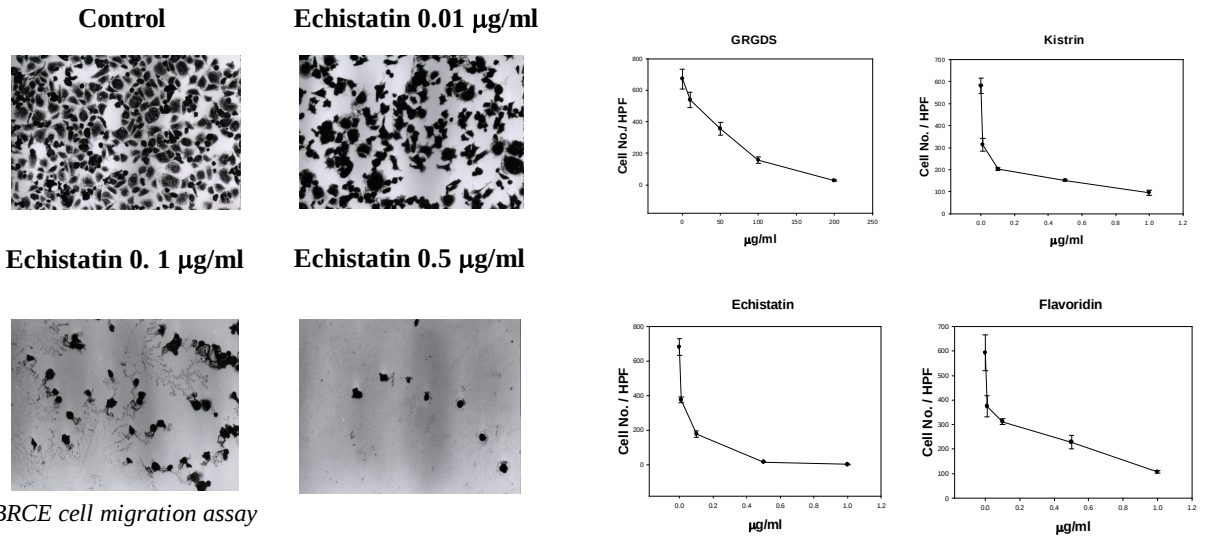
BRCE stained with Dil-Ac-LDL



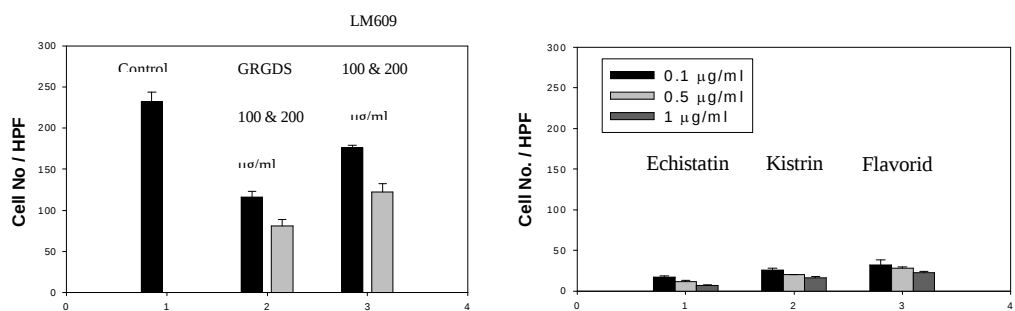
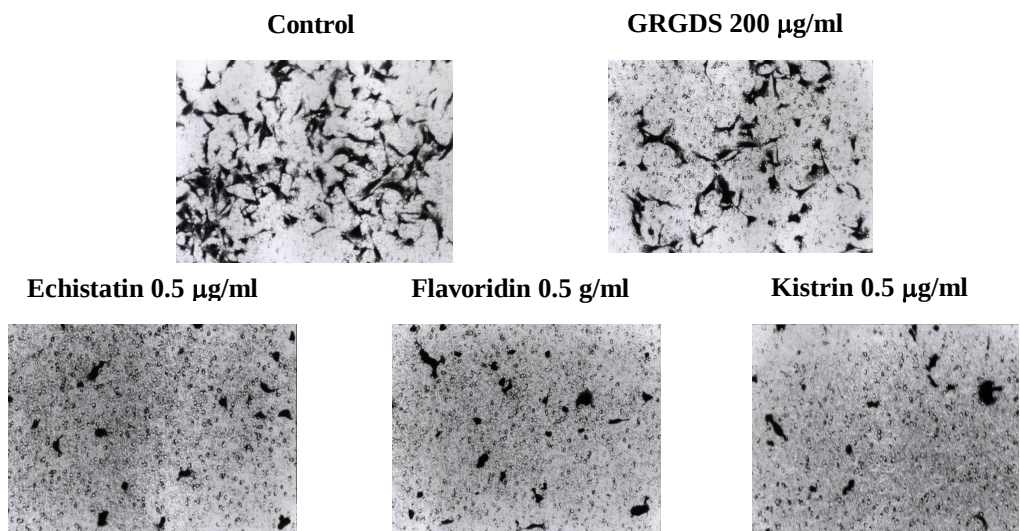
B. *BRCE cell adhesion assay* • 96 well plate coated with fibronectin and air dry

- 5 X 10⁵ BRCE cells/ml incubated with disintegrins for 30 min at 37 °C.
- Treated BRCE cells applied into FN-precoated plates and incubated for 90 min at 37 °C allowing to adhesion.
- Gentle washing and remove non-adherent cells
- Fixation and stain

- Count cell number under microscope

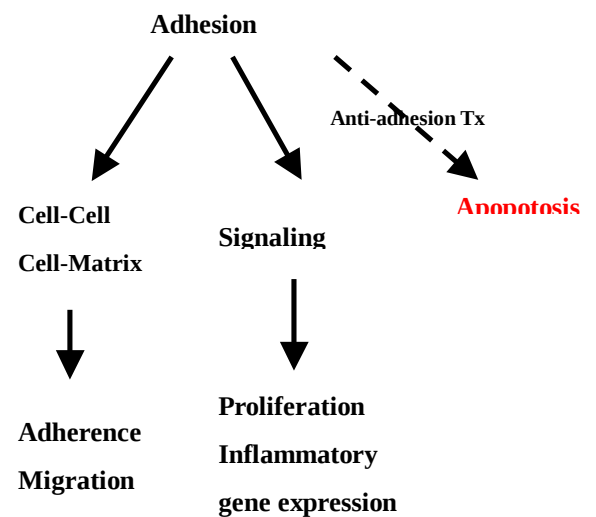
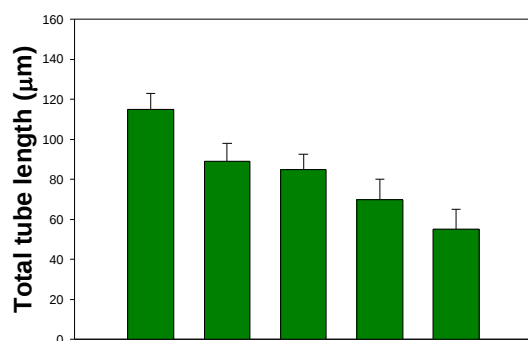
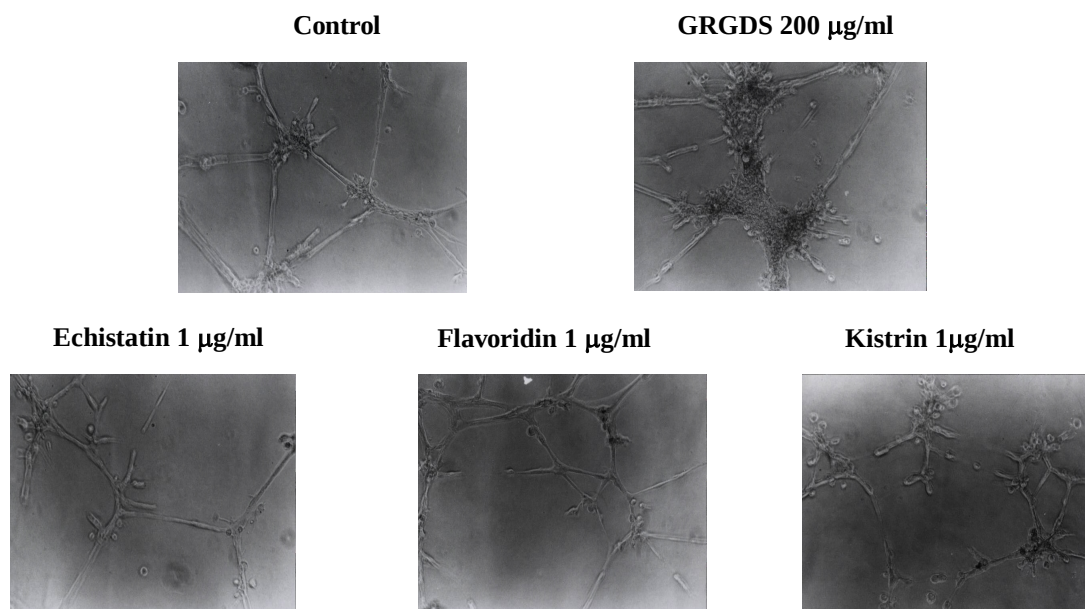


- Neuroprobe 96 well chemotaxis chamber filled with M199 with bFGF
- Polycarbonate filter with 8 μm pore size
- 5 X 10⁵ BRCE cells/ml incubated with disintegrins for 30 min at 37 °C.
- Treated BRCE cells applied into upper chamber incubated for 16 hrs at 37 °C
- Gentle washing and remove cells on filter
- Fixation and stain the filter
- Count cell number under microscope



D. BRCE cell capillary-like tube formation in Matrigel assay • Matrigel, a basement membrane matrix extracted from Engelbreth-Holm-Swarm mouse sarcoma

- 4 mg/ml matrigel with cold PBS and added to 96-well plates in each well. Plates will be stood at 37 ° C for 30 min to form a gel layer.
- 5 X 10⁵ BRCE cells/ml incubated with disintegrins for 30 min at 37 °C.
- Treated BRCE cells applied to matrigel plates and incubated for 16 hrs at 37 °C
- Photograph under microscope



Anti-adhesion therapy in ocular diseases

- Proliferative vitreoretinopathy
 - GRGDS peptide
 - Low MW heparin
 - Disintegrin
- AMD
 - Anti-ICAM Ab
 - Ab to cryptic angiogenic receptors in collagen • **Role of disintegrin**

Conclusion

- Disintegrins inhibits angiogenesis *in vitro* by blocking integrin $\alpha v \beta 3$ of endothelial cells.
- Disintegrin and their analogs may be a potential therapeutic agent for the treatment of ocular angiogenesis disorders