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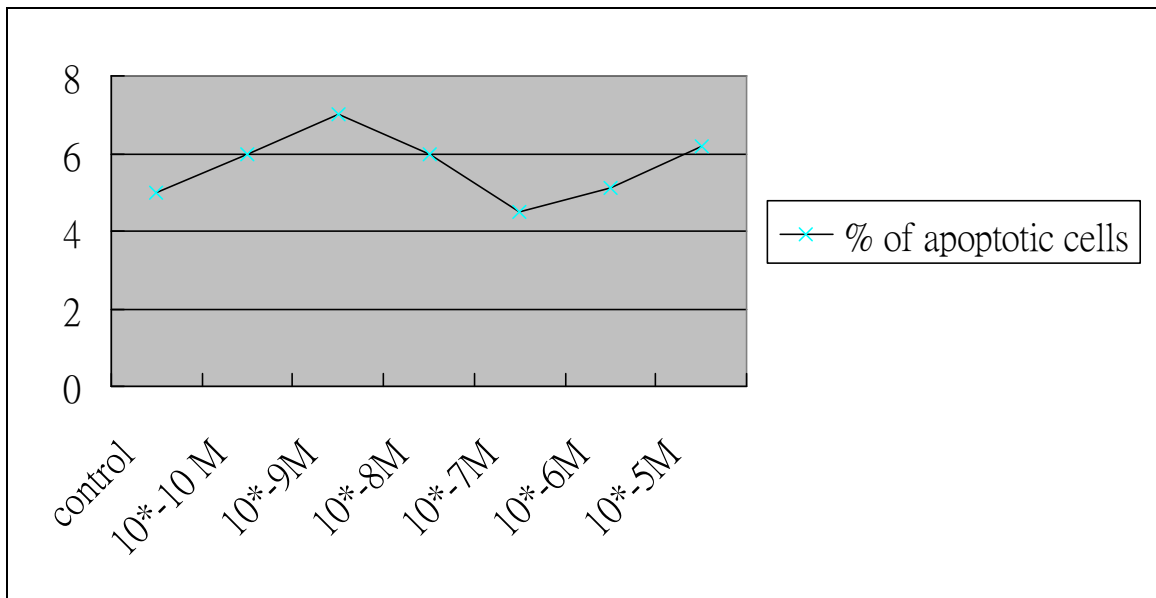
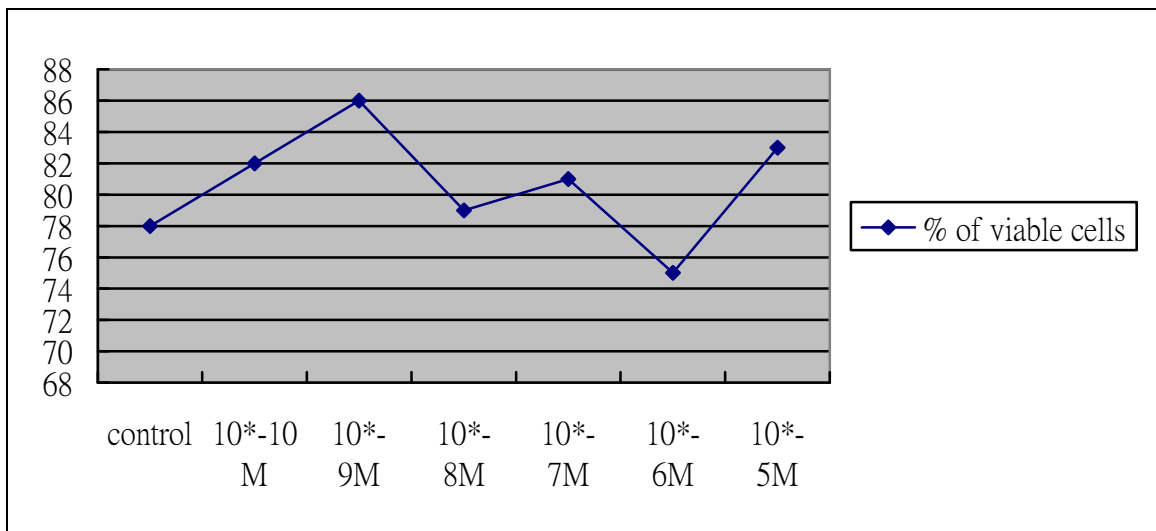
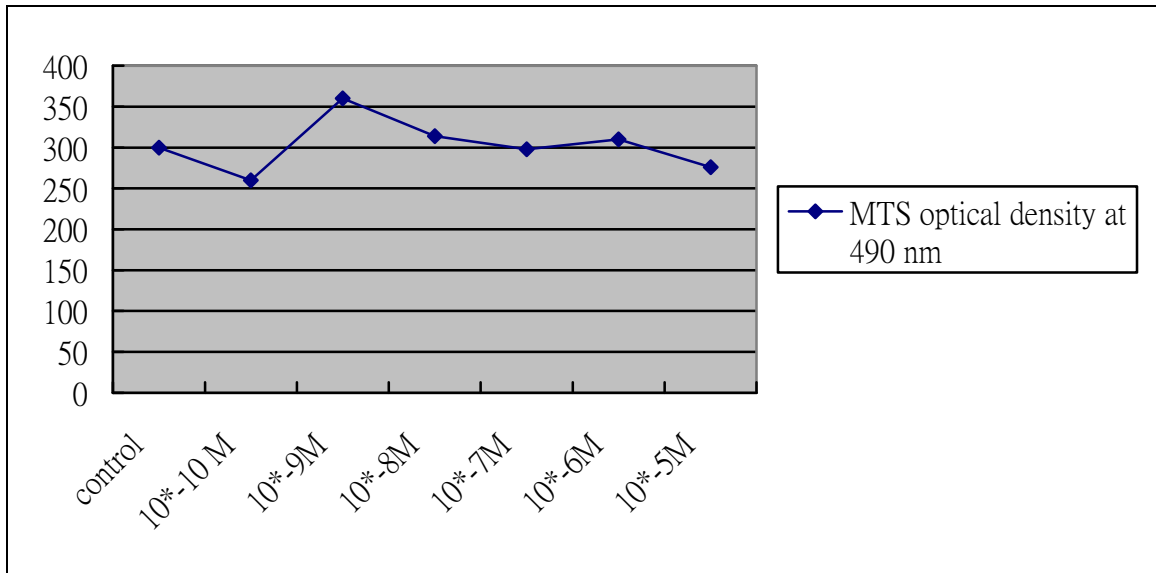
藥物對體外培養之鞏膜纖維  
細胞的作用

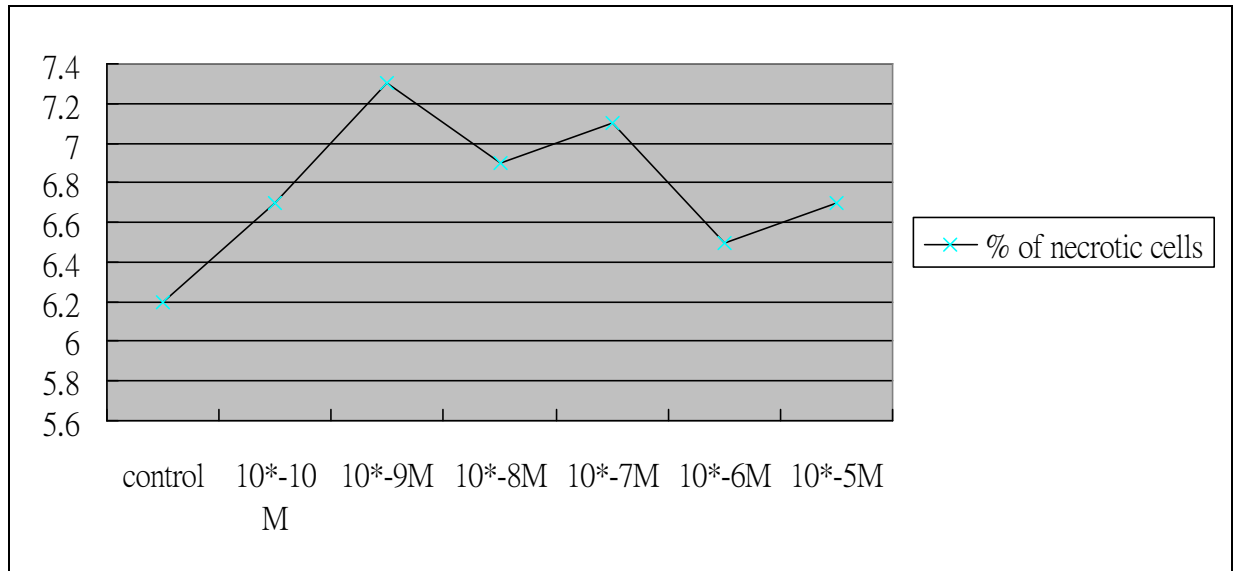
計劃主持人：台大醫院眼部主治醫師，陳偉勵

**PURPOSE**: To investigate whether cultured bovine scleral fibroblasts express the glucocorticoid receptor (GR) and to assess the influence of dexamethasone (DEX) on these cells.

**METHODS**: Bovine scleral fibroblasts were cultured in medium supplemented with various concentrations of DEX (ranging from  $10^{-10}$  to  $10^{-4}$  M). Cell proliferation was analyzed by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt (MTS) assay at 2, 4, and 6 days of culture. Some experiments were performed in the presence of mifepristone (RU38486), an antiglucocorticoid molecule. The early phase of apoptosis was studied by means of scleral fibroblast staining with a fluorescein conjugate of annexin V and propidium iodide, and cells were analyzed by flow cytometry. Glucocorticoid receptor mRNA was detected in keratocytes by means of reverse transcription-polymerase chain reaction (RT-PCR). Immunocytochemical staining of the cells was performed with a monoclonal anti-human GR.

**RESULTS**: RT-PCR and immunocytochemistry showed no result of GR (mRNA and protein) in cultured bovine scleral fibroblast. The reason may be due to improper primer, and short length of bovine GR checked from Genebank. However, there existed positive staining of dexamethasone on scleral fibroblast by immunocytochemical staining. As for the proliferative response of Dexamethasone, it has no **particular** increased or decreased scleral proliferation with concentrations ranging from  $10^{-9}$  to  $10^{-5}$  M. Dexamethasone's proproliferative effect was not inhibited by RU38486. However, DEX did induced some apoptosis of cultured bovine scleral fibroblasts at any concentration used.





### **CONCLUSIONS:**

These results can't indicate whether cultured bovine scleral fibroblast express the GR or not. The reason may be due to the improper antibodies, improper primers for RT-PCR, and no existence of GR on bovine scleral fibroblasts

The further directions of the study should be focus on

- (1) Use scleral ring of donor human cornea to do immunohistochemical staining, cell culture, RT-PCR and repeat the experiments
- (2) Find other primers for RTPCR
- (3) Repeat immunohistochemical staining
- (4) Try to culture rat scleral fibroblast, and repeat the whole experiments on rat specimens