Inhibition of intraocular proliferation by homoharringtonine*
An experimental study

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Abstract. Homoharringtonine, an alkaloid indigenous to China, was studied for its effect on fibroblast growth in cell culture and on intraocular proliferation produced in rabbits by injecting homologous fibroblasts into the vitreous. The results demonstrate that homoharringtonine reduced the cell growth by 50% at a concentration of 0.005 mg/l in vitro, significantly inhibited vitreous proliferation, and prevented the occurrence of retinal detachment in vivo. Light and electron microscopy revealed no ocular toxicity in drug-treated eyes. Homoharringtonine may be of considerable value in the prevention and treatment of intraocular proliferation in patients.

Introduction
Proliferative vitreoretinopathy (PVR), a complication of many ocular diseases, including rhegmatogenous retinal detachment and penetrating eye injury, is a leading cause of vision loss [4-6]. Surgery alone cannot ensure long-term successful treatment of the condition. Because intraocular proliferation is the primary event in the sequence of proliferation, contraction, and collagen deposition leading to traction detachment, it is logical to seek a method of controlling cell proliferation to prevent PVR. Therefore, recently a number of researchers have studied many pharmacologic agents capable of inhibiting cell proliferation by using several animal models [2, 4, 5, 8-10].

Here we report the investigation of the effect of homoharringtonine on rabbit conjunctival fibroblast proliferation in vitro and its effect on intraocular proliferation induced by intravitreal fibroblasts transplantation.

Materials and methods
Cell culture
We excised conjunctival tissue from albino rabbit, cut it into 1-mm cubes, and then placed the cubes in a certain amount of 0.25% trypsin solution. The tissue was kept at 4°C for 12 h and then centrifuged (1000 rpm for 5 min), suspended, and cultured in Eagle’s minimal essential medium (MEM) containing 10% newborn calf serum, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 2.5 μg/ml amphotericin B. Cells were seeded into 100-ml tissue culture flasks and placed in an incubator at 37°C. The medium was changed twice weekly until the culture became a confluent monolayer. Then the cells were subcultured and reseeded. Only the confluent cells of the fifth to tenth generations were used for experiments.

Effects of homoharringtonine on cell proliferation in cell culture
Confluent cells were harvested, counted, and diluted to a concentration of 2.5 × 10⁶ cells/ml. The cells were seeded into 25-ml tissue culture flasks (5 × 10⁴ cells/per flask) and cultured in a 37°C incubator for 24 h. The medium was then replaced with media containing various concentrations (10⁻³, 10⁻², 10⁻¹, 1, 10, 30 mg/l) of homoharringtonine. The cells were reincubated under identical conditions until the control flasks (cultured in Engee'MEM free of homoharringtonine) became confluent, usually at 72 h. The viable cells were counted in a standard hemacytometer counting chamber by trypan blue exclusion. The degree of drug effect was determined by the ratio between the number of cells in the test flasks and that in the control flasks multiplied by 100. The result was plotted in a semilogarithmic fashion with the percentage of inhibition on the Y-axis and the log dose of the drug on the X-axis. The experiment was done in triplicate with highly reproducible counts. We then used the semilogarithmic dose-response curve to extrapolate the concentration at which 50% reduction of growth was achieved when compared with control flasks.

Effect of homoharringtonine on intraocular proliferation
A total of 35 albino rabbits of either sex, weighing 1.5-2.5 kg were used. We diluted the animals' pupils with topically applied 10% phenylephrine. Then the animals were given 2% lidocaine retrobulbarly and subconjunctivally. We made a conjunctival flap superiorly and cauterized the sclera ~3 mm posterior to the corneoscleral limbus with a red-hot pinhead. Two sites 5 mm apart were cauterized. Using an indirect ophthalmoscope, 2.5 × 10⁵ cells in 0.1 mm BSS were drawn up into a tuberculin syringe with a 5-gauge needle, and injected into the center of the vitreous cavity from one of the cauterized sites. Following the cell injection, either 0.1 ml BSS (control group) or 0.025 mg of homoharringtonine in 0.1 ml BSS (experimental group) was injected through the other cauterized site using the same technique.
We punctured the anterior chamber before the injections to prevent the rise of intraocular pressure and vitreous loss through the entrance sites. After the procedure, 4 mg gentamycin was injected subconjunctivally; antibiotic ointments and 1% atropine were applied topically. On days 1, 3, 7, 10 after the vitreous injection, each eye to be treated with homoharringtonine was administered 0.025 mg of the agent subconjunctivally. All eyes were followed up by indirect ophthalmoscopy, fundus drawings, and by photography in selected cases. Two investigators examined the eyes 1, 3, 7, 10, 14, 21 days after the vitreous injection. In this study, we used the following grading scheme described by Avni and colleagues [1]: 0, no intravitreal proliferation; 1, a fine fibrotic wisp not obscuring the fundus; 2, heavier fibrous proliferation without real band formation; 3, fibrous proliferation obscuring less than a quarter of the fundus; 4, fibrous proliferation obscuring a quarter to one-half of the fundus; 5, fibrous proliferation obscuring over half of the fundus. The results were collected at the end of the 3-week observation period and statistically analyzed using the Wilcoxon-Mann-Whitney method. The percentage of traction retinal detachment for each group was also calculated and statistically analyzed using the chi-square test ($\chi^2$ test).

**Results**

**Effect of homoharringtonine on cell proliferation in cell culture**

Homoharringtonine was an extremely potent dose-dependent inhibitor of proliferation of rabbit conjunctival fibroblasts. At a concentration of 30 mg/l, there was no cell growth at all (Fig. 1). Fifty percent inhibition of proliferation ($ID_{50}$) was achieved at a concentration of 0.005 mg/l as extrapolated from the semilogarithmic dose-response curve (Fig. 2).