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ORIGINAL ARTICLES

Effect of β radiation on proliferating human Tenon's capsule fibroblasts

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Abstract

The effects of different doses of β radiation from a strontium-90 source on the proliferation of human Tenon's capsule fibroblasts were studied. The cultured fibroblasts were exposed to doses of 100, 250, 500, 750, 1000, 1500, and 3000 rads, and cell numbers were counted at days 3, 7, and 14. Treatment inhibited the proliferation of the fibroblasts. At seven days the cells exposed to 3000 rads showed a decrease relative to the starting cell numbers, and at 14 days the cells exposed to 1500 and 3000 rads showed a decrease in cell numbers. The doses of radiation which inhibited cell proliferation more than 50% (at day 7 and 14) and yet did not cause a decrease in the cell population were 500, 750, and 1000 rads. β Radiation reduces the proliferation of human Tenon's capsule fibroblasts, and at higher doses this effect may be more pronounced one and two weeks after irradiation.

Filtering surgery is a highly successful procedure in elderly Caucasian patients.[1] It lowers intraocular pressure more than medical treatment[2] and may be superior in terms of preservation of visual function.[3] However, some groups of patients have a high risk of failure from filtering surgery. They include patients who have had previous conjunctival surgery or trabeculectomy,[4] aphakes,[5] patients with neovascular glaucoma, children,[6] and Afro-Caribbeans.[7] The use of postoperative subconjunctival injections of the antiproliferative agent 5-fluorouracil given over two weeks postoperatively has enhanced the success rate of trabeculectomy but has been associated with side effects such as corneal erosions and opacification, thin leaking blebs, and hypnotonic eyes.[8,9] In addition the regimen of injections, even in its simplified forms, is still inconvenient and poses practical problems of delivery in children and in rural areas of Africa where trabeculectomy may be the only feasible treatment.

The failure of filtering surgery in animals[10,11] and humans[12,13] is associated with scarring at the level of the subconjunctival and episcleral tissues. This is associated with cellular proliferation, particularly fibroblast proliferation.[11,13,14] Ionising radiation has been used to inhibit ocular cellular proliferation, particularly after pterygium resection.[15,16] Although there have been studies on the efficacy of postoperative β radiation after filtering surgery,[17-20] there have been no large controlled studies, and the reports of efficacy have been variable. In this unit 750 rads of β radiation from a strontium-90 source have been used after filtering surgery in children for the last eight years. The dose of 750 rads was chosen because of long experience with this dose on pterygia.

The effects of β radiation on filtering surgery in rabbits have been studied, and Miller *et al*[20] found that a single dose of 2500 rads prolonged bleb survival in the rabbit, which displays very aggressive healing. Nevarez *et al*[21] investigated the effect of β irradiation from a linear accelerator and a strontium-90 source on monkey Tenon's capsule fibroblasts in tissue culture over the course of a week. However, the wound healing in the monkey eye is also more aggressive than in the human.

We performed this study to ascertain the effects and dose responses of human Tenon's capsule fibroblasts to β radiation from the strontium-90 source used in our clinical practice.

Materials and methods

Pieces of redundant Tenon's capsule were obtained from a 2-year-old child during surgery. They were placed in 25 cm^2 tissue culture flasks and grown in Hams F10 (Gibco, Paisley, UK) supplemented with 10% fetal calf serum, amphotericin B (0·25 mg/l), penicillin (100×10^3 IU/l, and streptomycin (100 mg/l) and incubated in a humidified mixture of 5% CO_2 and air at 37°C. The cells were passaged 1:3 and used at fouth passage. The cells were seeded at a density of 2500 cells/well in four wells of 96 well plates. Three groups of 96 well plates were used with eight plates in each group.

The wells were 55 cm apart to minimise the chance of any scattering of radiation to adjacent wells. The cells were allowed to incubate and settle for 24 hours. The medium was then replaced with Hams F-10 buffered with 25 mM HEPES and the culture plates were transported to the radiation therapy room. Each plate was kept out of the incubator for the same length of time. The centre of each well was then placed in contact with the centre of the strontium-90 applicator, which covered the entire surface area of the well. The calibration certificate issued with the probe was used to calculate the exposure time for each well. The dose rate for the applicator was 4·8 rads/s. Doses of 100, 250, 500,

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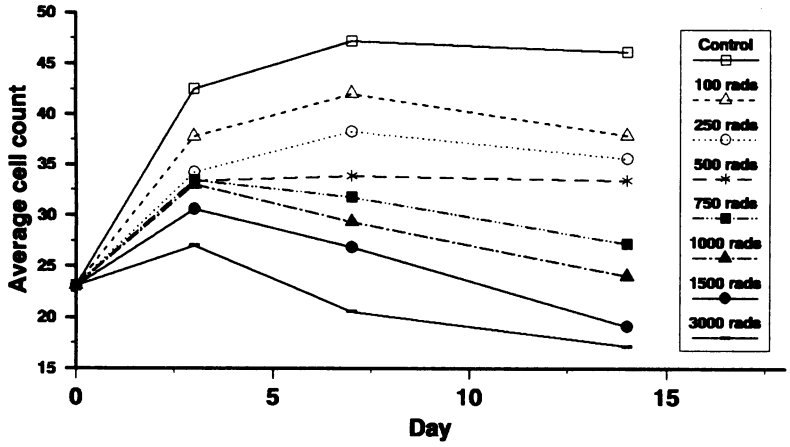

Figure 1 Average cell count at days 0, 3, 7, and 14 after treatment with different doses of radiation.

750, 1000, 1500, and 3000 rads delivered to the appropriate wells. After treatment the control and irradiated cells were returned to the laboratory, where they were fed with fresh F10.

Then at day 0, day 3, day 7, and day 14 the appropriate wells were rinsed gently with phosphate buffered saline, fixed with ethanol, and stained with haematoxylin. The number of cells in a field 0·75×0·55 mm were counted, and this was repeated in different areas of the well 10 times for each well (approximately 13% of the total well surface area). The average count for each well was then calculated, and the average and standard deviation of the four wells were then calculated and used for statistical analysis. This method was used because preliminary experiments in our laboratory had shown it to be more consistent and accurate than Coulter or haemocytometer readings when dealing with small numbers of cells.

The results were analysed by analysis of variance, and confidence intervals were derived for the different doses at each time interval. Regression analysis was also used to calculate the trend with increasing radiation dose for each day, by means of Minitab statistical software (Minitab Corporation, USA).

Results

There were no statistically significant differences between the groups before treatment. However, three days after treatment the control cells had proliferated significantly more than the treated cells, and this trend continued for days 7 and 14. At the highest two doses of 1500 and 3000 rads there was actually a fall in the total number of cells in comparison with the starting number of cells (Fig 1).

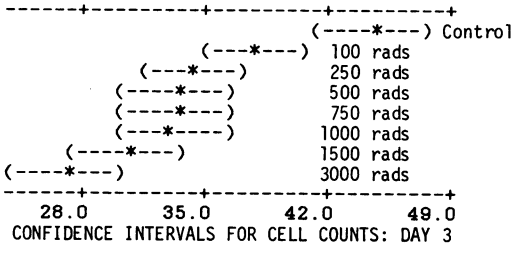

Figure 2 Individual 95% confidence intervals for mean based on pooled standard deviation.

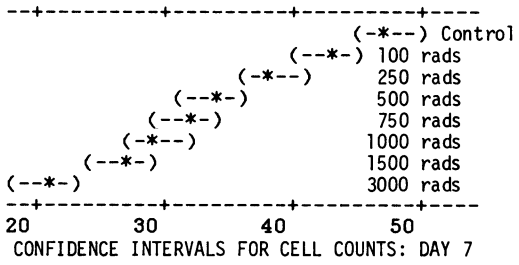

Figure 3 Individual 95% confidence intervals for mean based on pooled standard deviation.

At day 3 the untreated control cells had proliferated significantly more than the treated cells, but within the treatment groups only the 1500 and 3000 rad treated cell were different from the 100 rad group. For days 7 and 14 the spread within the groups increased, and at day 14 the groups could be divided into those treated with 100, 250, and 500 rads, those receiving 750 and 1000 rads, and those treated with 1500 and 3000 rads, which were statistically distinct from each other.

The statistical difference between the various radiation groups is represented graphically in Figs 2, 3, and 4 by one-way analysis of variance and plotted confidence intervals. If the confidence level bars do not overlap, the difference in count is statistically significant at the $p<0·05$ level.

Cell proliferation decreased relatively to the control when cells were treated with increasing radiation doses, and this effect was more pronounced at days 7 and 14 than on day 3. This is best illustrated by plotting the cell proliferation as a percentage of the control population (Fig 5). Regression analysis showed a decrease in proliferation with increasing radiation dose that was significant at the level of $p<0·0001$ for all three days. The change in cell proliferation for each dose of radiation at days 3, 7, and 14 was significant only at doses of 750 rads or more ($p<0·05$, analysis of variance).

The doses which inhibited proliferation more than 50% (ID_{50} level) and yet did not cause a fall in the overall number of cells were 500, 750, and 1000 rads. For the groups treated with 3000 rads at day 7 and those treated with 1500 and 3000 rads at day 14 there was an actual fall in the cell numbers below the number in the wells originally.

Morphologically the untreated fibroblasts maintained a relatively spindle shaped appearance. At increasing doses of radiation the cells became more polymorphic. At doses between 500 and 1000 rads there was more variation in cell size, with some large irregularly shaped cells.

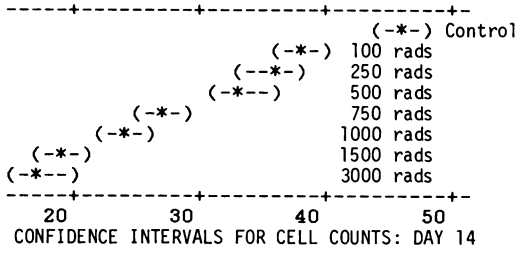

Figure 4 Individual 95% confidence intervals for mean based on pooled standard deviation.

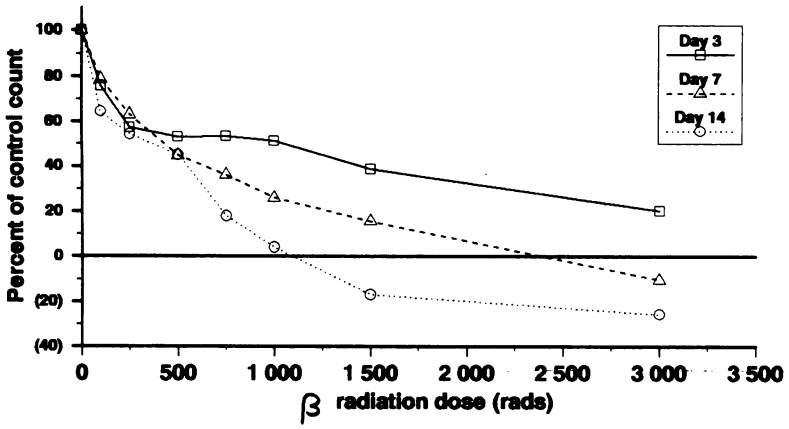

Figure 5 Fibroblast proliferation at days 3, 7, and 14 in relation to control.

At doses of 1500 and 3000 rads the cells were mostly small and irregular, with noticeably more cells floating free in the media.

Discussion

The inhibition of fibroblast proliferation is associated with an increased success rate following filtration surgery.[4] From the results of this study the use of β radiation clearly inhibits the proliferation of human Tenon's capsule fibroblasts. The optimum dose for retarding this fibroblast proliferation by more than 50% but not causing a decrease in the cell population over 14 days seems to be between 500 and 1000 rads. The effects of the different doses are more marked at day 14 and day 7 than at day 3, particularly at the higher doses of radiation from 750 rads upwards. At day 7 for the cells treated with 3000 rads there is actually a fall in the total number of cells, and this occurs at day 14 in the cells treated with 1500 and 3000 rads. This fall in cell number is probably due to severe damage and death in some fibroblasts, resulting in detachment of the cells from the tissue culture plate. This may be important clinically, as marked cell death may stimulate inflammation, which may result in further scarring.

This was unlike the study of Nevarez *et al*[21] using monkey Tenon's fibroblasts, where 3000 rads did not result in a significant decrease in the cellular population in comparison with 1500 rads at either day 3 or day 7. There was also a statistically significant recovery of the monkey cells treated with 100 rads between day 3 and day 7, unlike our experiment. This could be due to different responses of animal and human cells, but there are many other factors which must also be considered such as different culture media, cell passage number, and actual cell variations which may occur within species.

How long the trend of a prolonged effect on proliferation would continue is unknown. The long term effects of radiation on the resident fibroblast proliferation are important, as it is well known clinically that radiation may affect wound healing for at least several months after radiation treatment.[22,23] Failure of filtering surgery, though most common in the first few months, may persist several years after surgery,[13] and any long term effects on the local fibroblasts may be important. This aspect of antiproliferative treatment is being investigated further in our laboratory.

Experiments with tissue culture obviously have limitations. This experiment was performed on a monolayer of proliferating Tenon's fibroblasts, which may be more sensitive to radiation than non-proliferating cells in Tenon's capsule in the body. In the clinical situation the radiation is given directly postoperatively when the majority of fibroblasts are not actively proliferating and the radiation would be expected to have less effect on the cells.[24] However, the transcription of certain genes which are closely associated with the initiation of cell proliferation has been shown to increase markedly within 15 minutes of exposure to growth factors. These growth factors include platelet derived growth factor, which is released in abundance shortly after tissue is injured.[25] It is of interest that one study found immediately postoperative β irradiation to be more effective in preventing recurrence of pterygium than β irradiation given four days after surgery.[26]

Radiation has been given in divided doses after ocular surgery and is easily performed after filtration surgery in adults. However, divided doses of radiation are not easily given to young children. The optimum regimen for postoperative β irradiation is unknown, but this experimental model may be helpful in determining any fractionation of treatment.

The doses delivered to the cells in this model and in vivo are also not exactly the same. The cells in culture are monolayers with a layer of plastic between the cells and the strontium-90 applicator. In the in-vivo situation the fibroblasts are distributed throughout Tenon's capsule and in the sclera beneath. However, plastic is a good tissue model because β radiation is attenuated as in human tissue, and the thickness of the tissue culture plate approximates to the thickness of the conjunctiva and Tenon's capsule in children at the time of irradiation during surgery.

The size of the strontium-90 applicator may have some bearing on the clinical effect of the β radiation. The cell culture wells used in this experiment were smaller than the applicator and as such received a consistent dose of radiation. However, in clinical practice the β radiation is fairly localised to the region under the applicator. Fibroblast recruitment by cell migration may occur from regions of Tenon's capsule adjacent to the applicator, and peripheral scarring may restrict the area available for aqueous drainage. This is not the case in subconjunctival injections of 5-fluorouracil, when the drug achieves therapeutic concentrations throughout the conjunctiva.[27] Possibly a larger probe would further restrict any surrounding fibroblast recruitment.

The use of β radiation has advantages over 5-fluorouracil in that the radiation is relatively localised to the area of treatment. The strontium-90 applicator is portable (even with appropriate shielding and precautions) and can be used an infinite number of times without further cost until the dose rate has decayed so much as to make the length of treatment impracticable. This may be important in certain circumstances and in areas of the world where pharmacological

treatment is not practical. It is more convenient to deliver β radiation than to perform multiple subconjunctival injections after surgery. If further studies confirm its efficacy in similar doses to those used clinically at present, the lack of notable side effects may make it suitable for use in primary filtering surgery, not simply in high risk patients but in all patients undergoing filtering surgery. However, as with 5-fluorouracil, the optimum dosage and precise clinical role have not been fully established. Further studies in the laboratory and in the clinical setting are required.

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