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Irradiation pretreatment before fibroblast implantation in experimental PVR

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Abstract

Fibroblast injection into the vitreous body causes traction detachment in the rabbit's eye. Various working groups reported different results on the main causes of the development of experimental PVR. These contradictions encouraged us to investigate the main source of experimental PVR by irradiating the ocular tissues before fibroblast implantation thus suppressing cell proliferation originating from host tissue. Over a period of 3 weeks, 22 eyes received ten radiations in a total dosage of 3000 rad. After the last radiation, 250,000 fibroblasts were implanted into 22 eyes. In another 4 eyes, fibroblast implantation but no radiation was carried out. After 8 weeks, 59% of 22 eyes developed different stages of retinal detachment. Comparison with the group of unirradiated eyes, which developed retinal detachment in 85%, revealed no significant differences in the number of detachments.

Abbreviation: PVR – proliferative vitreoretinopathy

Introduction

Fibroblasts injected into the vitreous near the optic disc proliferate, form strands and cause retinal detachment in the eyes of rabbits. This model is worldwide used for testing different antiproliferative agents. Fastenberg et al. [1], for instance, demonstrated that preirradiated fibroblasts, which have lost the ability of cell division, cause lesser an effect when injected intravitreally. However, it was early observed by several other groups that strand formation and exudation also start from the vascularized part of the rabbit's retina so that injected cells and host tissues react together and develop a picture similar to PVR. Reactive proliferation of the host tissue cell was reported the main factor in experimental PVR [2–4].

These contradictions encouraged us to investigate by irradiating the ocular tissues before the fibroblast implantation, whether the fibroblast proliferation of implanted cells and the inflammatory reaction or the reactive proliferation of the surrounding host tissue were the source of PVR.

Material and methods

Eleven rabbits, 22 eyes, received radiation treatment of a total dosage of 3000 rad, divided into 10 single applications over a period of three weeks. The source of radiation was a betatron accelerator, fitted with a round tube of 4 cm in diameter, aimed at the rabbit's eye (field size 4 cm) in nearly right angle and emitting a single dose of 7.5 Me V beta electrons over a surface skin distance. The maximum concentration of the dose was received by the posterior vitreous. Immediately after the last treatment all 11 animals were anesthetized with an intramuscular injection of 3 to 4 ml ketamine hydrochloride, their pupils were dilated with a topical solution of 0.5% tropicamid and 10% phenylephrine hydrochloride. 250,000 homologous fibroblasts of 90% vitality were injected intravitreally above the optic disc in both eyes under the control of the indirect ophthalmoscope. Another 2 rabbits, 4 eyes, served as controls and received only fibroblast implantation in the same manner as mentioned above. All eyes were observed every week through two months after cell implantation. All procedures involving animals conformed to the Austrian Law on the Use of Animals in Research.

Results

Two weeks after cell implantation 10 of 22 preirradiated eyes had a partial retinal detachment, one eye showed a total retinal detachment. Three of four eyes of the control group developed partial retinal detachment. Four weeks after cell implantation, partial retinal detachment could be observed in eight of 22 pretreated eyes and in five eyes the retina was completely detached. Two out of four eyes of the control group showed total retinal detachment, one eye showed partial retinal detachment, and one eye had developed a strand formation at the optic disc and a pucker at one medullary ray.

At the end of our observation period after eight weeks, six of 22 preirradiated eyes had developed a total retinal detachment (27%), another seven eyes (32%) showed traction detachment at one medullary ray, and nine had just a strand formation but the retina was not elevated. The control group remained the same compared to the fourth week. Two out of four eyes had a total retinal detachment (50%), one eye developed partial retinal detachment (25%), and one eye had just a strand from the optic disc into the vitreous and a pucker on a medullary ray.

In summary, 59% of the preirradiated eyes and 75% of the control group had a partial or total retinal detachment. Statistical evaluation revealed no significant differences between both groups. In light microscopical examinations, inflammatory cells, such as lymphocytes and a few macrophages were present in the vitreous cavity near the retina. Membranes containing pigmented cells extended from the optic disc and the retina towards the lens. Except for a swelling of the inner layers of the retina in the irradiated eyes, no differences to the control eyes could be observed.

Discussion

Radiation treatment damages the DNA and thus enables the single cell to proliferate. Binder et al. [5] proved that fractionized radiation therapy with 3000 rad successfully suppresses the PVR process in the fibroblast model when applied from the first day after cell implantation. With the application of the same dosage of fractionized radiation therapy before implantation of fibroblast, we tried to determine the role of resident host cell proliferation of the retina, vessels, and pigment epithelium in the development of experimental traction detachment.

The question about the importance of implanted fibroblasts and host tissue reaction was first raised by Fastenberg et al. [1]. He found less of an effect by implanting radiated homologous fibroblasts than implanting normal homologous fibroblasts and postulated that the proliferation of implanted cells is a major factor in the development of traction detachment in the fibroblast model. However, Hitchins et al. [4] showed that fibrovascular proliferation from the optic nerve head and the medullary rays made a major contribution to the formation of membranes when homologous fibroblasts were in-

jected. The authors also pointed out that the major role of injected homologous fibroblasts was to induce aggressive proliferative reaction in contrast to the reactions seen by implantation of autologous fibroblasts. D. Hatchell [3] confirmed that mainly a reactive proliferation of host tissue took place. By using the 3 H thymidine incorporation, she found a ten times higher radioactivity in the retina than in the vitreous on the third day after homologous cell implantation. We raised the question, if irradiation pretreatment of 3000 rad completely suppresses proliferation of host tissue cells. As far as we know from *in vitro* studies of Sternberg et al. [6], radiation treatment of 500 rad already reduces the proliferation of fibroblasts to 41% and the proliferation of pigment epithelial cells to 56% compared to the controls. It is also evident that an *in vivo* applied dosage of 3000 rad easily suppresses proliferation of these cells. Glial cells, however, are less radiosensitive. Chakravarty et al. [7] found that the outgrowth of PVR membranes irradiated with a cobalt applicator mainly consisted of glial cells. We therefore assume that glial cell proliferation was not suppressed effectively enough with the dosage used in our experiment.

In our study, radiation therapy prior to fibroblast implantation showed some effect, although traction detachment could not be suppressed significantly. These results also demonstrate that migration, contraction, and inflammatory response, which are not altered by radiation, play an important part in the experimental PVR process. We suggest that the development of traction detachment in the fibroblast model is due to three components, which are equal in their importance:

- Proliferation of implanted homologous fibroblasts
- Proliferation of pigment epithelial, glial, and endothelial cells of the host tissues
- The inflammatory response, invasion of blood derived cells and agents into the vitreous cavity.

From our results we conclude that in the clinical course a multifactorial event such as the PVR cannot be suppressed effectively with an isolated agent responding to only one of the original causes. We therefore think it is necessary to combine drugs with different ranges of action, which has to result in a dose reduction of the individual drug.

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