Eye-Preservation Treatment of Retinoblastoma with Vitreous Seeding

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Retinoblastoma with vitreous seeding has been one of the most challenging conditions for eye-preservation therapy. Several modalities for treating vitreous seeding were reviewed in order to analyze the problems associated with them. External beam radiotherapy has been the most reliable method to treat vitreous seeding. However, recurrence after external beam radiotherapy needs other types of treatments to preserve the eyeballs. Due to the progress of investigations concerning retinoblastoma, chemotherapy has become the most promising method to cure not only recurrence but also primary tumors. Systemic chemotherapy can rarely cure vitreous seeding, but local chemotherapy using vitreous injections of melphalan can preserve about 50% of the eyeballs with vitreous seeding. Currently, animal experiments are being conducted to study the efficacy and safety of vitreous surgery combined with infusion of anticancer drugs for eradication of vitreous seeds and maintenance of visual function.

Key words: retinoblastoma – vitreous seeding – intravitreal injection – thiopeta – melphalan

INTRODUCTION

Vitreous seeding is a condition that is characterized by tumor cells floating within the vitreous cavity (Figs 1 and 2). These tumor cells frequently deposit and grow on the retina. In addition, implantation growth could possibly become larger and may even cause visual loss. Vitreous seeding is one of the most important limiting factors for successful eye-preservation therapy of retinoblastoma. Therefore, Reese and Ellsworth (1) grouped retinoblastoma with vitreous seeding to Vb, the group with unfavorable prognosis for eye-preservation. Some patients could be cured by external beam radiotherapy, and a limited number of vitreous seedings that were located only around the tumors were curable with brachytherapy. However, recurrence after external beam radiotherapy was difficult to cure by the second external beam radiotherapy because of the complications caused by a high radiation dose. Considering these circumstances, chemotherapy seems to be the only feasible measure to treat vitreous seeding. In this review, we describe and discuss the history of the strategies developed by many ophthalmic oncologists to cure vitreous seeding of retinoblastoma and thereby improve the success rates of eye-preservation therapy.

METHODS AND THEIR SUCCESS RATES IN EYE-PRESERVATION THERAPY FOR RETINOBLASTOMA WITH VITREOUS SEEDING

EXTERNAL BEAM RADIOThERAPY

This modality was first reported as an alternative to enucleation in 1903. Since then, it has been developed as a standard method of eye-preservation therapy for retinoblastoma. This is due to the high sensitivity of retinoblastoma to radiation and the refinement of hardware and software to overcome its complications. Concerning the treatment of vitreous seeding, Cassady et al. (2) reported a 7/41 (17%) success rate in eye preservation, with or without chemotherapy using triethylene melamine. Abramson et al. (3) reported the survival of eyes only of group Vb with seeding directly over the tumor. They recognized that all other group Vb cases with general seeding were enucleated, and apparently in the bilateral group V cases radiation merely delayed the inevitable enucleation. It can, therefore, be concluded that for bilateral group V retinoblastoma, a different approach is necessary for ocular survival.

BRACHYTHERAPY

Shields et al. (4) reported that eight patients with active localized vitreous seeds were successfully treated by I-125 plaque radiotherapy after failure of external beam radiotherapy. However, they did not describe the final results for either the
31 cases of primary treatment or the 42 cases with vitreous seeds among the 72 cases of secondary treatments. These eight cases with successful results may be regarded as special cases with localized vitreous seeding only around tumors.

Madreperla et al. (5) developed a customized plaque made from iridium-192/platinum wire to treat vitreous base seeding, which is postulated to occur as material shed from previously treated tumors and travels in the vitreous cavity to settle near the ora serrata. The poor response of these tumors to cryotherapy is similar to that of vitreous seeds overlying tumors elsewhere. Furthermore, the involvement of several clock hours (sometimes the entire inferior 180 degrees) makes treatment with conventional radioactive plaques difficult. Therefore, Madreperla et al. developed a customized plaque using an iridium-192 wire that was designed to treat vitreous base seeding.

Five patients were treated with a regimen using the iridium-192 plaque and systemic chemotherapy (vincristine, etoposide and carboplatin), and the eye was preserved in four of the five treated eyes with an average follow-up of 26.2 months. According to their report, the calculated dose from the iridium-192 plaque to the optic nerve and fovea was approximately 5% that of the dose at the sclera. They did not observe radiation-induced optic neuropathy or maculopathy in any of their patients. The lens received 10% to 20% of the scleral dose and no cataract was observed during follow-up.

**SYSTEMIC CHEMOTHERAPY**

Previous studies have shown that for eyes with advanced disease, chemotherapy is not efficacious in increasing eye preservation after primary radiation therapy. However, newer agents appeared to be effective in treating both extraocular disease and primary ocular tumors. Gallie et al. (6) reported that three recurrent tumors involving the ora serrata were treated with chemotherapy plus heat without radiation therapy and two patients achieved remission. However, it is difficult to determine the extent of vitreous base involvement from this report. In addition, one of the two patients had a follow-up for only 2.4 months.

Kingston et al. (7) reported 11 eyes of patients with bilateral retinoblastoma with vitreous seeding treated with chemotherapy (using carboplatin, etoposide and vincristine) and whole eye radiotherapy (40–44 Gy in 20–22 equivalent fractions). They successfully preserved 7 among 11 eyes with vitreous seeding (64%) in the median follow-up of 60 months. This is the most excellent result concerning eye-preservation therapy of vitreous seeding retinoblastoma.

Murphree et al. (8) reported the results of their chemotherapy plus local treatment in the management of 170 affected eyes of 136 consecutive patients with intraocular retinoblastoma. However, eye preservation was unsuccessful in all 18 eyes with vitreous seeds. Their results clearly show the importance of external beam radiotherapy as an eye-preservation therapy for advanced retinoblastoma (Figs 3 and 4).

**LOCAL CHEMOTHERAPY AND INTRAVITREAL INJECTION**

**A: THIOTEPA**

Ericson and Rosengren (9) at the Eye Clinic at Gothenburg pioneered using intravitreal injections of chemotherapeutic agents for eye-preservation therapy of retinoblastoma in 1960. Their idea was to achieve the highest possible concentration of the chemotherapeutic agent close to the tumor by using it intraocularly, while at the same time its concentration in the whole body was kept at the lowest possible level. They used thiotepe as the chemotherapeutic agent, because it caused less irritation compared to nitrogen mustard. A very fine cannula, with a diameter of 0.8 mm, was inserted for the injection through the posterior part of the ciliary body. While injecting 0.3–0.4 mm³ into the vitreous humor, an increase in pressure was observed,
which disappeared after 15–20 min. A concentration of this cytostatic drug, ranging between 3 and 4 mg/ml sol., could be easily injected into the vitreous humor without any serious irritative symptoms or other toxic damages. There was no effect on vision. Repeated injections of this agent resulted in slight opacities of the vitreous humor. However, these opacities disappeared after a couple of weeks by administering the injections at periodic intervals. The first patient treated with this method had previously received X-ray doses of about 35 Gy and nitrogen mustard (mustine) intravenously. Since further X-ray treatment was considered dangerous, injection of thiotepa into the eye was tried. The effect was significant, but temporary. They treated six eyes with intraocular thiotepa injection. In three of these cases, X-ray treatment was given simultaneously. This injection was given once a week and the patients received 1.0–1.5 mg of this agent on each occasion. The dosage recommended for general treatment was 10 mg per injection. The treatment was carried out for a period of 1.5–2.0 months, and the patients had received a total dose of 9.5 mg of thiotepa on its completion. In cases where a combination of X-ray irradiation and intraocular chemotherapy had been given, the tumor regressed faster, except in one patient who had massive recurrent tumors that required enucleation. They concluded that although the intraocular thiotepa injection cases were too small to allow any definite conclusions to be drawn, regression could, undoubtedly, be attained. However, probably it was not a permanent effect.

Figure 3. A large retinoblastoma with extensive vitreous seeding.

Figure 4. The fundus of the Fig. 3 case, 2 years after external beam radiotherapy with ocular hyperthermia. Only a small calcified scar is left without any vitreous seed.

Based on the positive outcome regarding the clinical trial in six cases, Ericson et al. tried systemic animal experiments on the intravitreal injection of chemotherapeutic agents using rabbits’ eyes (10). Different concentrations of several chemotherapeutic agents were intravitreally injected in rabbits’ eyes, with the aim of determining the upper concentration limit for different preparations. The injections, which were given just behind the limbus, presented no difficulties. It was possible to inject 0.3 cm³ into one eye of each animal. The other eye was untreated and served as a control. The experimental animals were ophthalmologically examined on alternate days over the course of 3–4 weeks. The toxic reaction in the eye was assessed by irritation in the anterior segment of the globe, the occurrence of aqueous flare, exudates in the vitreous, and retinal hemorrhages. Clouding of the lens was an inconstant finding and may be related to the damage at the time of injection, because the lens opacities occurred at the periphery and not in the posterior cortex. The retinal changes were inconsistent and could have occurred due to the trauma caused by the injection. Occasionally, the opacification of the media made it difficult to observe the changes satisfactorily. Assessment of the actual changes was more difficult in some cases due to the presence of injection-related infection. This may explain the clouding of the vitreous that was occasionally observed with low concentration of thiotepa. It has been possible to administer thiotepa at relatively high concentrations, up to 20 mg/ml, before clouding of the vitreous developed constantly. However, in cases where clouding developed, it was found that the vitreous cleared after 2 weeks. In addition to thiotepa, they tried other chemotherapeutic agents such as nitrogen mustard, cyclophosphamide and methotrexate. Nitrogen mustard proved to be the most toxic preparation with a concentration of 0.03–0.06 mg/ml. Cyclophosphamide is not the active form of an anticancer drug; therefore, it was not appropriate to inject it into the vitreous. At a relatively low concentration, methotrexate produced a flare in the anterior chamber and at concentrations of about 10 mg/ml, with one exception, caused membrane formation in the vitreous.

Ericson et al. examined functional changes caused by the intravitreal injection, with electroretinography (ERG). Seven rabbits were used for the trial and the equipment described by Karpe (11) was employed. Four eyes were injected with 0.2–0.3 ml of thiotepa at a concentration of 50 mg/ml. ERG was
performed before the injection and then repeated at weekly intervals of 2–4 weeks. In the majority of cases, ERG was performed on both the eyes; the uninjected eye served as the control. The assessment would, therefore, depend on a comparison between the two eyes. In fact, irreversible changes in the amplitude of the b wave were not seen in any eyes, and it was not possible to detect any definite ERG changes.

The Swedish tradition of vitreous injection of thiotepa initiated by Ericson et al. was revived later by Seregard et al. (12). They reported three cases of recurrent retinoblastoma after 45 Gy external beam radiotherapy with a cobalt-60 applicator (one case), the multicobalt Leksell gamma knife (one case), cryotherapy (two cases) and argon laser photocoagulation (one case). In all patients, multiple tumor recurrences with extensive vitreous seeding eventually suggested that the remaining eye had to be enucleated. Repeated injections of 2 mg thiotepa dissolved in 0.5 ml of balanced salt solution were administered intravitreally through a pars plana approach and a small amount was left subconjunctivally at the injection site. The injections were repeated twice in 2 weeks before a standard three port pars plana vitrectomy was performed. Following surgery, supplemental thiotepa injections were continued on a weekly basis until a total of 10 mg thiotepa (two cases) or 14 mg thiotepa (one case) was administered. No obvious clinical response was noted in any eye before vitrectomy. One of the patients had all the vitreous seeding removed, but retained one small tumor in the peripheral retina after vitrectomy. However, this residual growth turned gelatinous during the post-vitrectomy thiotepa injections. After cessation of the intraocular chemotherapy, this patient underwent an extraocular cataract extraction with an intraocular lens implant and experienced a 20/400 visual acuity at a 14 months follow-up. The other case had two additional vitrectomies because of the initial incomplete removal of tumor, and cytological examination of the vitreous washings confirmed the presence of retinoblastoma cells. After the final vitrectomy, only a few strands of tumor tissue remained adherent to the optic disc. These small remnants also turned gelatinous following the post-vitrectomy chemotherapy.

The patient’s visual acuity was 20/400 as a result of previous tumor growth in the macula in conjunction with a moderate subcapsular cataract. Another patient experienced more extensive retinal growth in addition to considerable vitreous deposits, and vitrectomy failed to remove the substantial parts. A few days after surgery, the patient suffered a major vitreous hemorrhage and 1 month later the eye was enucleated. Histopathological examination of the globe revealed that nearly all the tumor tissue was necrotic. However, the vitreous contained some retinoblastoma cells that appeared viable. No mitotic figures were present, but positive immunostaining for the proliferating cell nuclear antigen in a few tumor cells suggested that these cells maintained a proliferative potential. There were no signs of local recurrence or metastatic disease during follow-up periods of 14, 70 and 77 months after intravitreal chemotherapy.

Figure 5. Sensitivity of a retinoblastoma cell line (Y-79) to melphalan, showing the least concentration required to kill tumor cells.

Figure 6. Illustration showing the injection point of anticancer drug into the vitreous.

Figure 7. 30 G and 32 G needles for the intravitreous injection.
B: MELPHALAN

Inomata and Kaneko (13) investigated the sensitivity of retinoblastoma to 12 anticancer drugs to find a better regimen for eye-preservation therapy by using the colony assay on double agar layers reported by Hamburger and Salmon (14). They found that retinoblastoma was most sensitive to melphalan and that heating (42°C for 1 h) increased its sensitivity to all the drugs tested by various methods. Kaneko et al. (15) treated six patients with intraocular retinoblastoma that was recurrent after irradiation. This treatment included a 40 mg injection of melphalan into the ipsilateral intracarotid artery plus ocular hyperthermia (45°C, 1 h), using an applicator reported by Lagendijk (16). Two patients were cured (no recurrence for more than 10 years) with one treatment and although good useful vision was preserved, severe bone marrow suppression and hair loss were inevitable. To decrease the amount of melphalan, Mohri (17) developed a selective ophthalmic arterial injection, using a balloon catheter.

Ueda et al. (18) studied the effects of an intravitreal injection of melphalan on an ERG and on the retinal structure of albino rabbits to establish a non-toxic dose for its clinical use. ERG and the retinal structure remained unchanged after a 10 μg injection, but moderate change was observed after a 20 μg injection, and deterioration was observed after 90 μg injection. A 10 μg injection is equivalent to an intravitreal concentration of about 5.9 μg/ml, if homogeneously diffused in the rabbit vitreous. Since colony formation by retinoblastoma cells in vitro was completely suppressed by melphalan at a concentration of 4 μg/ml, it appears that an intravitreal injection of melphalan could be used as a non-surgical treatment for retinoblastoma. Kaneko (19) performed this intravitreal injection combined with ocular hyperthermia to treat vitreous seeding since ophthalmic arterial injection combined with ocular hyperthermia often did not cure vitreous seeding. The clinical result was remarkably good as shown in Figs 8–10 and this method is now routinely used at the National Cancer Center Hospital, Japan (Figs 5–9).

Recently in Europe and USA, systemic chemotherapy with carboplatin, vincristine and etoposide is routinely used as a primary treatment in eye-preservation therapy. Although chemotherapy by selective ophthalmic arterial injection is not an easy procedure, the quality of life of patients is much better than that with systemic chemotherapy. Concerning the clinical results from 1990 to 1998, 124 patients with unilateral retinoblastoma were primarily treated at the National Cancer Center Hospital (20). Ninety-four patients were treated by eye-preservation therapy and 30 cases were enucleated primarily. The 9-year survival rate for these 94 patients was 98% by the Kaplan–Meier method. Metastatic deaths occurred in two patients; one patient refused enucleation despite recurrence within the eyeball. Recurrence in the other eye could not be determined because the ophthalmological examination was impaired due to vitreous opacity. Although CT scans were performed, recurrence and extraocular extension were not detected. Enucleation revealed optic nerve invasion by retinoblastoma and extensive prophylactic treatment was not effective. The secondary enucleation was performed in 25 eyes and the 9-year success rate for eye-preservation was 69%. Most of these eyes were classified as Reese group V: According to Ellsworth (21), the success rate with conventional method in this group is 30%. Therefore, the success rate of eye-preservation therapy was more than twice as that cited by Ellsworth (Fig. 10).

EXPERIMENTAL TRIALS

One of the most difficult problems regarding the treatment of vitreous seeds is the blood–retinal barrier. The vitreous normally does not have its own vessels to supply nutrients. Therefore, this barrier prevents the transfer of anticancer drugs into the vitreous. Wilson et al. (22) investigated whether cryotherapy, which induces a serious effusion in the retina, increases the

**Figure 8.** Diffuse vitreous seeding recurrent after radiotherapy.

**Figure 9.** The seeding of retinoblastoma completely disappeared 15 months after the intravitreal injection of melphalan to the patient of Fig. 8.
access of systemic chemotherapy into the vitreous. The right eyes of 18 rabbits were treated with triple or single freeze-thaw cryotherapy at one or two locations, one day before administering intravenous carboplatin with or without cyclosporine. The left eyes were considered as controls and received no cryotherapy. The rabbits were killed 2 or 24 h after chemotherapy, and carboplatin concentrations were measured in the vitreous of each eye and in the blood. A significant increase was observed in intravitreal concentrations when cryotherapy was applied (P < 0.001) or high-dose cyclosporine was administered (P < 0.001) and if two locations were frozen as compared to one location (P < 0.02). Intravitreal carboplatin concentrations were always significantly greater after cryotherapy, either when the corresponding blood carboplatin concentrations were high (2 h after completing treatment) or when they had dropped to much lower levels (at 24 h). The triple freeze-thaw technique did not yield significantly better results than a single freeze-thaw technique. They concluded that cryotherapy administered 24 h before chemotherapy significantly increased the intravitreal penetration of carboplatin, and this strategy may enhance the capacity of chemotherapy to cure intraocular retinoblastoma, particularly avascular tumors such as vitreous seeds.

Another new approach is currently being investigated by Kishi et al. (23). They attempted vitreous surgery combined with perfusion of anticancer drugs into the vitreous to kill tumor cells during the procedure. If the leakage of tumor cells from the ports could be completely prevented during the vitreous surgery, this method might very efficiently eradicate not only extensive vitreous seeds but also retinal tumors. Furthermore, most of the vitreous body would be exchanged with the fluid containing anticancer drugs at a concentration high enough to kill tumor cells and preserve visual functions.

CONCLUSIONS

Vitreous seeds have been one of the most important limiting factors in the successful preservation of eyeballs with retinoblastoma. External beam radiotherapy has a long history and has proven to have successfully cured retinoblastomas. However, complications of radiotherapy and recurrence after radiotherapy need alternative modalities for treatments. Intravitreal injection of melphalan is not as dangerous or toxic as ocular oncologists of the previous generation had imagined. However, the long-term success rate of eye preservation was approximately 60%, which is not an ideal figure. Several new methods are being developed in order to improve the success rates of eye-preservation with useful visual functions. It is expected that newer and more effective anticancer drugs, which are less toxic to normal tissues, will become available for the treatment of retinoblastoma in the near future.

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References


