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# Histopathologic findings after selective ophthalmic arterial injection of melphalan for retinoblastoma

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## Abstract:

**PURPOSE:** The aim is to describe histopathologic observations in eyes enucleated after selective ophthalmic arterial injection (SOAI) of melphalan for retinoblastoma (RB).

**STUDY DESIGN:** This is retrospective clinical study.

**PATIENTS AND METHODS:** Histopathologic analysis of 14 eyes (13 patients) from May 2008 through January 2015 at Chang Gung Memorial Hospital.

**RESULTS:** The eyes after SOAI were enucleated due to tumor viability ( $n = 7$ , 2 with vitreous hemorrhage), neovascular glaucoma ( $n = 4$ ), lens drop to vitreous with total hyphema and elevated intraocular pressure ( $n = 1$ ), retinal detachment (RD) progressed ( $n = 1$ ), and persistent RD with phthisis change ( $n = 1$ ). Almost all of the eyes showed vitreous seeding ( $n = 11$  eyes) before treatment. After the treatment of SOAI, the histopathological examination revealed complete regression in four eyes with one was clinically diagnosed as viable tumor and progression, one with RD progression, and two as neovascular glaucoma. Six eyes showed invasion into the optic nerves, reaching the lamina cribrosa in five eyes, and six eyes with invasion into the choroid were observed. All of the cases with lamina cribrosa involvement showed tumor progression before enucleation, four cases with lamina cribrosa involvement expired later.

**CONCLUSION:** Although some cases of RB can be controlled effectively with SOAI, but for refractory cases after SOAI, earlier decision of enucleation may be needed.

## Keywords:

Enucleation, histopathologic findings, ophthalmic artery injection, retinoblastoma

## Introduction

Retinoblastoma (RB) affects approximately 8.58/million children under the age of 5 years in Taiwan annually.<sup>[1]</sup> For decades, the management of RB included enucleation, external beam radiotherapy (EBRT), systemic chemotherapy, and local treatments such as laser photocoagulation, thermotherapy, plaque brachytherapy, and cryotherapy.

Twenty years ago, Yamane *et al.* in Japan pioneered a novel technique of delivering chemotherapeutic agents directly into the

ophthalmic artery.<sup>[2]</sup> In years, the technique became popularized worldwide, high globe salvage, and few local side effects were demonstrated in previous studies,<sup>[3-6]</sup> but few studies described histopathologic changes of the enucleated eyes after selective ophthalmic arterial injection (SOAI) for RB.<sup>[7-10]</sup> In our study, we reported the clinicopathological findings of 14 eyes of 13 patients enucleated after SOAI treatment failure.

## Patients and Methods

From May 2008 to January 2015, patients with ocular RB treated with SOAI and other

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modalities and ultimately received enucleation at Chang Gung Memorial Hospital were included in this study. The enucleation rate after SOAI was estimated to be about 40%. The study was approved by the Institutional Review Board (protocol No. 104–A191B) of Chang Gung Memorial Hospital, and the study followed the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants after a full explanation of the nature and possible consequences of the study were provided. Fourteen eyes of 13 patients initially were classified as International Classification of RB (ICRB) GrB (1 case), GrD (2 cases), GrE (11 cases). Causes for enucleation mostly were owing to disease progression, treatment failure or treatment-related complication. All specimens were submitted to the pathology laboratory following enucleation at Chang Gung Memorial Hospital. All eyes fixed in 10% formaldehyde and embedded in paraffin were dissected and examined histopathologically by pathologists with extensive experience specified in the histopathologic evaluation of RB.

## Results

Most eyes in this study initially presented as advanced disease, Reese-Ellsworth classification as Gr II ( $n = 2$ ), Gr V ( $n = 12$ ), and ICRB as GrB ( $n = 1$ ), GrD ( $n = 2$ ), GrE ( $n = 11$ ), while 11 eyes showed vitreous seeding and 12 eyes with subretinal seeding before treatment. Of all cases studied five were unilateral, eight were bilateral RB. Twelve cases received one eye enucleation, one case with both eye enucleated. Treatment numbers of SOAI ranged from 1 to 12 times. Four cases received external beam radiation therapy (EBRT) [Table 1].

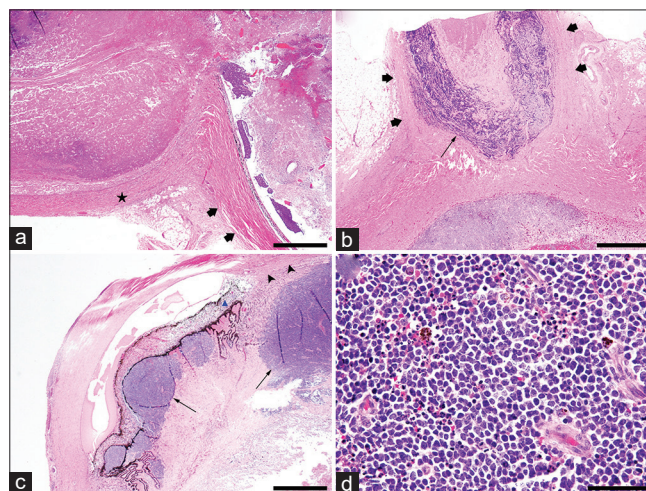
The analysis of the causes for enucleation after SOAI, seven eyes enucleated were due to progressive tumor growing, four eyes developed neovascular glaucoma, one eye showed extensive tumor necrosis after SOAI, with lens drop to vitreous, total hyphema and elevated intraocular pressure (IOP), one eye with progressive retinal detachment and another eye showed persistent retinal detachment with phthisis change [Table 2].

Following the combination treatment of SOAI with various therapies, the intraocular tumor demonstrated complete regression in four eyes. Of the four eyes with complete regression, two eyes were enucleated due to the development of neovascular glaucoma with uncontrollable high IOP, one eye with retinal detachment progression, the other one was clinically misdiagnosed as tumor recurrence. Microscopic finding of the four eyes all contained focal calcification, extensive gliosis and myxoid change with no viable tumor visible, the changes consistent with a complete therapeutic response [Figure 1a].

The microscopic finding of the other 10 eyes with incomplete regression all contained with variable amount of viable tumor cells and necrosis. The pattern of regression was classified as minimal, moderate, and extensive according to the viable tumor cells under microscopic examination. Of the five eyes retained with extensive viable tumor cells [Figure 1b-d], the treatment discontinued and eyes were sacrificed because the tumors failed to respond well clinically [Figure 2].

In the other five eyes, the histopathological changes disclosed remarkable treatment response but not yet achieved complete regression. The decision of ceasing further treatment were because of neovascular glaucoma developed in two eyes and one eye showed progression of vitreous seeding after twelve times repetitive SOAI and numerous intravitreal injection. One eye with lens drop to vitreous accompanied with massive vitreous hemorrhage and elevated IOP, and one eye showed persistent retinal detachment and phthisis change.

Focal or massive choroidal invasion were observed in six eyes. Six eyes showed invasion into the optic nerves, reaching the lamina cribrosa in five eyes, and one eye showed resection margin involved. The cases with lamina cribrosa involvement clinically were all observed with tumor progression before enucleation, 4 of the 5 cases with lamina cribrosa involvement expired later because of brain metastasis in spite of the adjuvant systemic chemotherapy after enucleation [Table 3].



**Figure 1:** The histopathologic findings. (a) The left eye of patient 4 enucleated due to progression of retinal detachment, vitreous hemorrhage, and elevated intraocular pressure shows almost totally tumor regressed (star: Sclera; short arrows: Optic nerve). The right eye of patient 6 shows (b) optic nerve (short arrows) and its margin extensively infiltrated by tumor cells (long arrow). (c) Retinoblastoma (long arrows) fills posterior chamber with ciliary body (blue triangle: Iris), choroid (arrowheads) and sclera invasion. (d) The malignant cells compose with small blue round cells with large hyperchromatic nuclei. (Scale bar: abc = 1.0 mm; d = 60  $\mu$ m) (H&E stain)

**Table 1: Characteristics of 14 enucleated eyes of retinoblastoma at the time of presentation**

Patient No./Age at diagnosis (months)/Sex	Laterality (OD/OS)	Reese-Ellsworth/ICRB grouping	Vitreous seeding	Subretinal seeding	No. of SOAI	EBRT
1/164/Male	U (OS)	Vb/D	+	-	4	+
2/50/Male	U (OS)	Vb/E	+	+	6	-
3/9/Male	B (OD)	Vb/E	+	+	9	-
4/13/Female	B (OS)	Vb/E	+	+	3	-
	B (OD)	Vb/E	+	+	3	-
5/22/Male	U (OS)	Vb/E	+	+	5	+
6/15/Female	B (OD)	IIa/B	-	-	1	+
7/45/Male	B (OS)	IIb/D	-	+	2	+
8/12/Male	B (OS)	Vb/E	+	+	6	-
9/12/Male	U (OD)	Va/E	-	+	1	-
10/15/Female	B (OS)	Vb/E	+	+	4	-
11/36/Female	U (OS)	Vb/E	+	+	5	-
12/4/Male	B (OS)	Vb/E	+	+	1	-
13/14/Male	B (OS)	Vb/E	+	+	12	-

B=Bilateral, EBRT=External beam radiotherapy, ICRB=International Classification of Retinoblastoma, OD/OS=Right/Left eye, SOAI=Selective ophthalmic arterial injection, U=Unilateral, +=Positive (presence), -=Negative (absence)

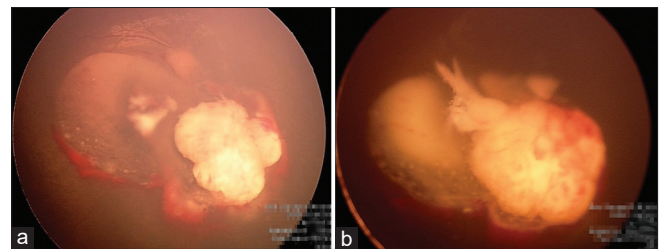
**Table 2: Causes for Enucleation of the 14 eyes**

Patient No./Age at diagnosis (months)/Sex	Causes for enucleation	Tumor regression
1/164/Male	NVG	Total
2/50/Male	NVG, VH	Total
3/9/Male	Misdiagnosed as RB recurrence around disc	Total
4/13/Female	Progression of RD, VH (OS)	Total
	RB progression, VH (OD)	Minimal
5/22/Male	RB progression	Minimal
6/15/Female	RB progression, VH	Minimal
7/45/Male	RB progression	Minimal
8/12/Male	RB progression	Minimal
9/12/Male	Lens drop, total hyphema, VH	Moderate
10/15/Female	VH, RD, phthisis	Moderate
11/36/Female	NVG, VH	Extensive
12/4/Male	NVG, phthisis	Extensive
13/14/Male	Progression of vitreous seeding	Extensive

NVG=Neovascular glaucoma, OD/OS=Right/Left eye, RB=Retinoblastoma, RD=Retinal detachment, VH=Vitreous hemorrhage

## Discussion

In these years, selective intra-arterial chemotherapy has been adopted by many ocular oncology centers as primary or as the main treatment modality of advanced intraocular RB and have successfully achieved the main goal of avoiding enucleation and radiotherapy in many centers.<sup>[5-7]</sup> After SOAI, about 71% of eyes with tumor regression and 67% of vitreous seeding with complete or partial regression were observed in previous study.<sup>[11]</sup> Side effects have been documented to be few and acceptable, the reported complications include conjunctiva congestion, periorbital swelling, ptosis, erythema, temporary eyelash loss, cranial nerve (third or sixth) palsy, and occasional retinal arterial occlusion. In previous studies, choroidal artery occlusion, retinal emboli formation, and intravascular birefringent foreign material following SOAI were also reported.<sup>[7-9]</sup>



**Figure 2:** The right eye of patient 6 with limited response to selective ophthalmic arterial injection. (a) After systemic chemotherapy, radiotherapy and local treatment (intravitreal injection with melphalan, laser therapy), the retinal tumor increased in size and height, with vitreous seeds. (b) After selective ophthalmic arterial injection and 1 month later, the retinal tumor grew to two times in size and approached the optic disc. The optic disc and macula were obscured by main tumor. Vitreous hemorrhage progressed. Selective ophthalmic arterial injection was performed again, but failed due to poor perfusion of ophthalmic artery with contrast stasis. Thus, enucleation was indicated

In our study, although in four eyes the malignant tumors were eradicated completely the globe preservation was unsuccessful due to the treatment related complications after SOAI. Two cases develop neovascular glaucoma, the other case with progressive retinal detachment, the repetitive SOAI either with or without EBRT probably would worsen ocular hypoxia and resulted in the development of neovascular glaucoma and persistent subretinal effusion, retinal detachment and eventually blinding and loss of the eye.<sup>[9,12-16]</sup>

Among the five eyes with extensive treatment response, three eyes were enucleated because of the complications following the SOAI procedure. The development of massive vitreous hemorrhage and high IOP not only obscuring the clarity of fundus, but also prevent the further treatment of the eye. Only two eyes were enucleated due to the notorious disease progression.

Kim *et al.* reported that SOAI is effective in treating advanced RB but appears to have limitations in

**Table 3: Histopathologic Findings in 14 Eyes With Retinoblastoma Following Treatment With Intra-arterial Chemotherapy**

Patient No./Age at diagnosis (months)/Sex	Retina	Choroid (Focal/ Massive)	Sclera	Optic nerve Prelaminar/Postlaminar	Mortality
1/164/Male	no residual tumor	-	-	-/-	No
2/50/Male	hemorrhagic necrosis, no viable tumor	-	-	-/-	No
3/9/Male	hemorrhage and granulation, no residual tumor	-	-	-/-	No
4/13/Female	gliosis, no malignancy (OS) residual RB (OD)	- M	-	-/ +/>+	Yes Yes
5/22/Male	necrosis, vitreous and subretinal seeding	F	-	+/>+	No
6/15/Female	residual RB	M	+	+/>+ a	Yes
7/45/Male	multicentric viable tumor	-	+	+/>+	Yes
8/12/Male	Necrosis with viable tumor	F	-	+/>+	Yes
9/12/Male	necrosis, focal subretinal seeding	F	-	-/-	No
10/15/Female	necrosis and calcification	F	-	+/>-	No
11/36/Female	residual RB	-	-	-/-	No
12/4/Male	minimal residual tumor	-	-	-/-	No
13/14/Male	Necrosis with viable tumor	-	-	-/-	No

<sup>a</sup>Resection margin involved, F=Focal, M=Massive, RB=Retinoblastoma, OD/OS=Right/Left eye, +/-=Positive/Negative for tumor cell

treating persistent vitreous seeding, the limitations are probably because of poor vitreous penetration and resistance of chemotherapeutic drug within the avascular vitreous cavity.<sup>[12]</sup> In our study, vitreous and subretinal seedings recurred commonly after multiple institution of SOAI, and the repetitive procedure of SOAI always lead to the treatment-related complication and resulted in failure of retaining the eyeball.

Some features of histopathological findings indicates high-risk for distant metastasis, massive invasion of the posterior uvea, and invasion of the optic nerve beyond the lamina cribrosa, more significantly, with the involvement of the surgical margin of resection.<sup>[10]</sup> In our study, six eyes showed invasion into the optic nerves, with five eyes reaching the lamina cribrosa, and one eye with resection margin involved, six eyes with invasion into the choroids. All of the five cases with lamina cribrosa involvement showed obvious tumor progression before enucleation, four cases died of brain metastasis. For advanced disease, before embarking the long journey of treatment with SOAI, the preexisting micrometastasis or massive posterior uveal invasion are not possible to be detected, clinicians only see the treatment response intraocularly and leaving the lesions of optic nerve and choroid hidden and undiscovered. Treating advanced cases ICRB Gr D or E, repetitive SOAI with prolonged treatment course sometimes could increase the risk of metastasis. Due to small number of patients, diversity of adjunctive treatment modalities, and treatment response variability in individual cases, multiple factors caused histopathological findings between cases different. Although RB can be controlled effectively with SOAI, for refractory cases after SOAI, earlier decision of enucleation may be needed.

## Conclusion

The analysis of the 14 enucleated eyes of RB after the procedure of SOAI, six eyes showed invasion into the optic nerves, with five eyes reaching the lamina cribrosa, and one eye with resection margin involved, six eyes with invasion into the choroids. With an attempt to avoid enucleation in the advanced disease eyes, multimodalities are used, but prolonged treatment course with SOAI probably will result in a dismal outcome and endanger the patients' survival. The repetitive procedure of SOAI also will result in progressive narrowing of the retinal and choroidal vasculature, and render the tumor eye more hypoxic. The ischemic eye sometimes will develop neovascular glaucoma and result in the loss of the eyeball. More precise evaluation before initiation of treatment and cautious observation continuously during treatment course are important. The management of RB remains a challenge for the advanced disease, clinicians, and parents need to judge the benefits and the potential risk of treatment carefully.

## Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Local Ethics Committee of the institute. Informed written consent was obtained from all patients before their enrollment in this study.

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Nil.

## Conflicts of interest

The authors declare that there are no conflicts of interests of this paper.

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