Clinicopathologic Features of Retinoblastoma After Primary Chemoreduction

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Background: Primary chemotherapy is a new treatment approach in retinoblastoma, aiming to avoid radiogenic adverse effects, such as second tumor–associated mortality, as observed following external beam irradiation.

Objective: To describe the clinical and histopathologic regression pattern after primary chemotherapy in retinoblastoma.

Methods: Five patients with sporadic bilateral retinoblastoma underwent planned enucleation of their functionally blind eye after 2, 3 (in 2 patients), 4, and 6 courses of primary chemotherapy with carboplatin, etoposide, cyclophosphamide, and vincristine. The eyes were examined histopathologically, using light microscopy and immunohistochemical analysis with proliferation markers.

Results: One patient had a type 1 (cottage cheese) regression and 4 patients had either a type 2 (fish flesh) or a type 3 (combined) regression pattern. Histopathologic examination revealed a complete tumor necrosis in 1 patient with type 1 regression after 3 courses of chemotherapy and in 1 patient with type 3 regression after 4 courses of chemotherapy. The remaining 3 patients with type 2 or type 3 regression had histologically still active proliferative tumor cells after 2, 3, and 6 courses of chemotherapy.

Conclusion: This article correlates histopathologically the clinically described efficacy of primary chemotherapy in the treatment of retinoblastoma, underlining, however, the necessity of careful observation and the use of ancillary treatment whenever there is no complete tumor regression.

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PATIENTS AND METHODS

Five patients with bilateral nonfamilial retinoblastoma are described who received primary chemotherapy for tumor reduction (chemoreduction) and enucleation of their more severely affected eye after 2, 3 (in 2 patients), 4, and 6 courses of chemotherapy. All patients were white; 3 were boys and 2 were girls. At the initial examination, the patients underwent complete ophthalmologic evaluation under general anesthesia, as well as computed tomographic examination of the orbits and magnetic resonance imaging of the orbits and brain. Laboratory analysis of cerebrospinal fluid and bone marrow aspirate was performed to rule out metastatic disease. Each eye was categorized according to the Reese-Ellsworth classification system. Table 1 shows the patient data at initial examination.

Chemotherapy was performed according to the treatment regimen as shown in Table 2 and repeated every 3 weeks. After course 3, the whole regimen was repeated, starting with course 1 at day 64. Table 3 shows the number of chemotherapy courses these children received before enucleation. Chemotherapy was performed for tumor size reduction (chemoreduction) to enable ancillary local treatment for the residual tumors. After each chemotherapy course, the children had a complete ophthalmologic examination to evaluate the response to treatment. If they had further tumor regression, treatment was continued. If it was judged that an eye was beyond salvage, enucleation was advised. Systemic chemotherapy was tolerated generally well by all patients. Typical adverse effects, such as mild reversible myelosuppression and alopecia, occurred in all patients.

The enucleated eyes were immersed in a 4% buffered formaldehyde solution. After macroscopic examination and photography, the eyes were transilluminated and cut in such a plane to include the bulk of the tumor in the main pupillary nerve block. Serial sections were obtained in several levels through the pupil-optic nerve block and the calottes. Sections (4 µm thick) were made and stained with hematoxylin-eosin and periodic acid–Schiff.

Immunohistochemical analysis was performed to evaluate the proliferative potential of tumor cells using the monoclonal antibodies (MoAb) PC-10, which binds to the proliferating cell nuclear antigen, and MIB-1, which binds to the Ki-67 antigen. The macrophage response was assessed by using the MoAb MIB-1. The alkaline phosphatase antialkaline phosphatase technique was performed with an antibody dilution of 1:10 for MIB-1, 1:50 for PC-10, and 1:100 for PG-M1. The MoAbs PC-10 and PG-M1, as well as the alkaline phosphatase antialkaline phosphatase kit, were obtained from DAKO Diagnostika GmbH, Hamburg, Germany; the MoAb MIB-1 was obtained from Dianova GmbH, Hamburg. The amount of proliferating cells was determined semiquantitatively by estimating the percentage of positive stained cells divided by the entire cell number as counted in 10 high-powered fields (×400 magnification) by 1 observer (N.E.B.). In all cases, positive controls were obtained by staining lymphomas with known proliferative activity. Negative controls were obtained by assessing nonaffected areas within the same section (eg, cornea, sclera, lens, choroid), as well as by omission of the primary MoAb.

<table>
<thead>
<tr>
<th>Table 1. Patient Data*</th>
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<tr>
<td><strong>Patient No./Sex/Age, mo</strong></td>
</tr>
<tr>
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<tr>
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<td>5/M/24</td>
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*All children were diagnosed as having nonfamilial bilateral retinoblastoma.

All patients described had nonfamilial bilateral retinoblastoma and Reese-Ellsworth stage V disease in the enucleated eyes. Three patients had vitreous seeding (Reese-Ellsworth stage Vb) at the initial evaluation, and all patients had subtotal or complete retinal detachment (Table 1). One patient had secondary glaucoma and heterochromia at the initial evaluation, and 1 patient had esotropia with retrohyaloid hemorrhage. Tumor height before treatment varied in the enucleated eyes from 6.0 to 12.0 mm (mean, 9.4 mm). In all patients, there was tumor reduction observed after various courses of chemotherapy, resulting in tumor height from 3.0 to 6.1 mm.

RESULTS

CLINICAL

All patients described had nonfamilial bilateral retinoblastoma and Reese-Ellsworth stage V disease in the enucleated eyes. Three patients had vitreous seeding (Reese-Ellsworth stage Vb) at the initial evaluation, and all patients had subtotal or complete retinal detachment (Table 1). One patient had secondary glaucoma and heterochromia at the initial evaluation, and 1 patient had esotropia with retrohyaloid hemorrhage. Tumor height before treatment varied in the enucleated eyes from 6.0 to 12.0 mm (mean, 9.4 mm). In all patients, there was tumor reduction observed after various courses of chemotherapy, resulting in tumor height from 3.0 to 6.1 mm.
reaction, resulting in type 3 tumor regression. Espe-
both showed some degree of cottage cheese–appearing
9.3 to 6.1 mm with a type 2 regression. Patients 4 and 5
served. However, the tumor was markedly reduced from
no sign of a cottage cheese–appearing reaction ob-
active tumor. Patient 1 had a small fish-flesh–appearing
tumor appeared to be in complete type 1 regression
on clinical evaluation. Patient 2, who was initially evaluated as having vitreous seeding,
the tumor appeared to be in complete type 1 regression
clinical evaluation. Patient 1 had a small fish-flesh–
tumor nodule near the optic nerve, resulting
in a type 3 regression pattern (Table 3).

In patients 3 and 4, enucleation was postponed af-
ter 3 and 4 courses of chemotherapy, respectively, because these patients had per-
sisting retinal detachment, indicating that they were
functionally blind. Nevertheless, in both eyes, a massive
tumor reduction was observed. Especially in patient 2,
who was initially evaluated as having vitreous seeding,
the tumor appeared to be in complete type 1 regression
on clinical evaluation. Patient 1 had a small fish-flesh–
tumor appearance. Microscopically, the tumor in this pa-
tient was calcified and necrotic and completely replaced
the optic nerve in conventional hematoxylin-eosin and pe-
lipid exudations forming cholesterol clefts. There were
clusters of necrotic retino-
migrated retinal pigment epithelial cells and macro-
phages were scattered in the gliotic tumor, along with
vital tumor cells present. Clusters of necrotic retino-
blastoma cells were present in the vitreous in areas of the
clinically described vitreous seeding before chemo-
therapy. These histopathologic features corresponded to
a type 1 regression.

In contrast to patient 2, patients 1, 4, and 5 had some
degree of fish-flesh–appearing reaction along with cottage-
cheese type regression (combined type 3 regression pat-
tern) (Figure 2, A). Patient 3 presented exclusively a
fish-flesh–appearing regression (type 2 regression pat-
tern) (Figure 2, B).

In patients 1 and 4, the bulk of the tumors had identi-
tical histological features as observed in patient 2 with
the exception that there were small foci of vital-
appearing retinoblastoma cells at the tumor base near the
optic nerve in conventional hematoxylin-eosin and pe-
periodic acid–Schiff stains. These foci had a moderate tu-
ner differentiation with the presence of numerous
Flexner-Wintersteiner rosettes and contained cells with
mainly round nuclei that had finely granular nuclear chro-
matic. These areas had, on macroscopic and clinical ob-

<table>
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<tr>
<th>Patient No.</th>
<th>Courses of Chemotherapy, No.</th>
<th>Resulting Regression Pattern</th>
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<th>Active Tumor Cells, %</th>
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<td>5</td>
<td>6</td>
<td>3</td>
<td>8.1</td>
<td>5.5</td>
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* Tumors measured by ultrasound before enucleation.
servation, a fish-flesh appearance. Whether these cells were truly vital and proliferating was evaluated by using immunohistochemical analysis with the proliferation markers PC-10 and MIB-1.

A common feature in all the patients who had some degree of cottage cheese regression and retinal detachment was the presence of small clusters of subretinal necrotic calcified retinoblastoma cells overlying the Bruch membrane.

In the fifth patient who also had a combined type 3 regression at initial evaluation, macroscopic examination revealed a residual retrohyaloid hemorrhage that partially obscured the posterior pole. Most of the residual tumor had a fish-flesh appearance, except at its apex that was cystic with small foci of cottage cheese–appearing regression. On microscopic examination, the tumor appeared mainly viable except for the cystic apex that contained foci of basophilic granular necrotic tumor corresponding to the small cottage-cheese–appearing areas. The tumor was composed mainly of pleomorphic undifferentiated retinoblastoma cells with large and hyperchromatic nuclei. Flexner-Wintersteiner rosettes were only rarely present, and mitotic figures were abundant. In multiple retinal areas, there was also a diffuse flat exophytic infiltration, but choroidal or optic nerve invasion could not be observed.

Patient 3 was the only one who had a pure fish-flesh–appearing type 2 regression pattern. On macroscopic examination, there was a diffuse multinodular thickening of the entire retina and a prominent endophytic retinoblastoma at the inferior nasal retinal quadrant that protruded into the vitreous with overlying retinal seeding (Figure 2, B). On microscopic examination, there was necrosis in the center of the main tumor, but calcification was not present (Figure 3, A). The tumor was well differentiated with the presence of Flexner-Wintersteiner rosettes and mainly composed of viable retinoblastoma cells with large and hyperchromatic nuclei. Mitotic figures were abundant. Over the tumor apex, there were clusters of vital-appearing pleomorphic retinoblastoma cells producing vitreous seeding. Infiltration of the tumor into the choroid could not be observed. The entire inner retinal circumference was covered by a multilayered sheath of retinoblastoma cells, accounting for retinal seeding. A marked invasion of the prelaminar part of the optic nerve was present at the optic disc with vital retinoblastoma cell clusters infiltrating around the central retinal artery and vein (Figure 3, B).

General pathologic features included the following: only patients 1 and 4 had circular anterior synechiae in the anterior segment. Peripheral superficial iris neovascularization and posterior synechiae producing a pupillary membrane were present only in patient 1, along
with pigment clumping and dispersion between the pli-
cae of the ciliary body. The retina in all cases had some
degree of diffuse reactive gliosis with cystoid edema. In
patients 1 and 2, who had the most pronounced retinal
detachment, there was marked photoreceptor outer seg-
ment atrophy. Segmental cystic optic nerve atrophy was
also a common feature. None of the patients had a lami-
nar or postlaminar optic nerve invasion.

IMMUNOHISTOCHEMICAL RESULTS

No proliferating tumor cells could be demonstrated using
the MoAbs PC-10 and MIB-1 in any tumor sections in pa-
tients 2 and 4 after 3 and 4 courses of chemotherapy, re-
spectively (Table 3). It was also notable that the apparent
vital tumor areas in conventionally hematoxylin-eosin–
stained sections in patient 2 did not show any reactivity
for proliferation markers even after repeated immunohis-
tochemical staining. In addition, negative controls did not
show any false-positive staining (Figure 4, A). Immunohis-
tochemical analysis with the macrophage marker PGM-1 showed an
accumulation of macrophages around vessels and around
clusters of calcification.

In patient 1, immunohistochemical analysis using the
MoAbs PC-10 and MIB-1 showed a proliferation rate of
10% within the vital area at the tumor base after 2
courses of chemotherapy (Figure 4, B). In patients 3 and 5 after 3 and 6 courses of chemotherapy, respectively,
immunohistochemical analysis with MoAbs PC-10 and

MIB-1 still showed a massive proliferation rate of 90%
within the vital areas of the tumor (Figure 4, C).

COMMENT

The clinical regression patterns of retinoblastoma after ra-
diotherapy are well documented and classified into 4 types.
Type 1 regression is characterized by rapid tumor reduc-
tion that takes on the appearance of cottage cheese. In type
2 regression, the tumor changes from pink to gray and loses
III.11 Any new approach to the management of retinoblastoma is mainly caused by the highly successful treatment of DNAtopoisomerase type II and is most effective against late S phase and early G2 phase cells.29 The vinca alkaloid vinblastine sulfate is lethal to mitotic cells by binding to tubulin and preventing the polymerization of tubulin subunits into microtubules, resulting in mitotic cells being arrested in metaphase from which cell death rapidly ensues.30 Cyclophosphamide is one of the main alkylating agents being used that exerts its toxicity through covalent binding to DNA, thus impeding DNA replication. An increased incidence of secondary primary tumors has been attributed to the use of cyclophosphamide in children with RB1 mutations; therefore, its use should probably be avoided when possible.31,32 Nevertheless, it is an effective chemotherapeutic drug in patients with advanced retinoblastoma13 and that is why it was used in these 5 children.

As has recently been published,20-22 it is possible to reduce chemotherapeutic treatment to a 3-drug regimen (carboplatin, etoposide, and vincristine) or even a 2-drug regimen (carboplatin and etoposide)30 thus minimizing toxic effects and possible second malignant neoplasms.

Enucleation was performed in our patients after 2, 3 (in 2 patients), 4, and 6 courses after commencement of chemotherapy in anticipation of a reduction in tumor size. All patients had advanced Reese-Ellsworth stage Va or Vb tumors in which optic nerve or choroidal invasion could not have been ruled out preoperatively with certainty.

Since it is difficult, if not impossible, to determine the viability of treated tumor cells based on conventional histological analysis alone, we used immunohistochemical analysis to determine the proliferative activity of retinoblastoma after various courses of primary chemotherapy. The MIB-1 antibody is a recently developed MoAb against a recombinant fragment of the Ki-67 antigen, which enables the assessment of cell-proliferating activity.34 Similarly, the PC-10 MoAb detects an epitope on the proliferating cell nuclear antigen.35 The concentration of proliferating cell nuclear antigen increases during the S phase of the cell cycle, thus providing an index for proliferative activity, which was determined semiquantitatively by estimating the percentage of positive stained cells divided by the entire cell number (Table 3).

In patients 2 and 4, after 3 and 4 courses of chemotherapy, respectively, we found a complete tumor regression without any proliferative activity. In patient 2, there was clinically a regression type 1 present that corresponded to a histologically confirmed complete tumor necrosis. In serial sectioning, no viable tumor cells could be observed. There was total tumor necrosis with residual calcification, retinal gliosis, and fibrosis present. In patient 4 after 4 courses of chemotherapy, there was a more complex, but highly interesting, finding. On conventional histological analysis, there were still areas of apparently viable tumor cells present at the tumor base, showing a Flexner-Wintersteiner rosette differentiation. Immunohistochemical analysis, however, demonstrated that the tumor cells in these areas had lost their proliferative activity. A false-negative result was ruled out by repeating the staining procedure 3 times with positive controls. Clinically, this tumor had a type 3 regression with a cottage cheese appearance in the areas of tumor necrosis and calcification. The area of apparently viable tumor on conventional histological analysis corresponded to a clinical fish-flesh appearance.

In patients 1, 3, and 5 after 2, 3, and 6 courses of chemotherapy, respectively, we found incomplete tumor regression with the presence of a still-viable tumor having maintained its proliferative activity. In patient 1, the re-
sult of incomplete tumor regression was anticipated, since it is unlikely that only 2 courses of chemotherapy can induce complete tumor necrosis. Enucleation was, however, encouraged in this early stage of therapy since there was massive tumor reduction. Only about 10% of the apparently viable tumor cells were actually in a proliferative state, allowing the conclusion that in this patient the chemotherapeutic regimen was highly effective. A completely different observation could be made for patients 3 and 5 after 3 and 6 courses of chemotherapy, respectively. Although there was a significant tumor reduction present in both patients, approximately 90% of the apparently viable tumor cells were in a proliferative state, even after 6 courses of chemotherapy in patient 5.

These results indicate the heterogeneity of retinoblastoma cells that results in different sensitivity to the same treatment. One of the major obstacles to the ultimate success of cancer chemotherapy is the ability of malignant cells to develop resistance to cytotoxic drugs. Unfortunately, the phenomenon of multidrug resistance is most commonly observed; this confers on the cells the ability to withstand exposure to lethal doses of many structurally unrelated agents. It has been shown that multidrug resistance is caused by the overexpression of a membrane-associated energy-dependent drug efflux pump, the P-glycoprotein. The P-glycoprotein is encoded by the mdr1 gene, and cells with multidrug resistance often show amplification of this gene. It has already been shown that the effect of P-glycoprotein is reversible with high concentrations of cyclosporine and might even be diminished in the future by using MoAbs against P-glycoprotein.18

There are no data showing which tumors can be classified as cured after chemotherapy alone as stated by some authors20,22 or whether there is a definite need for ancillary therapy despite the regression patterns of the intraocular tumors as stated by others.21 Similar to the effect of EBR, it has also been observed in primary chemotherapy that it is not equally effective in all patients. Therefore, chemotherapy alone is not a reliable treatment for all retinoblastomas. It has the potential to become a new first-line treatment approach if combined with adjuvant brachytherapy, photocoagulation, or cryocoagulation, hence avoiding the well-known potential of developing secondary cancers after EBR in children with bilateral retinoblastoma. Ophthalmic Paediatr Genet. 1987;8:289-302.


