Occurrence of Thymic Lymphoma in Carcinogenesis Bioassay Specimens of the Japanese Medaka (Oryzias latipes)


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Abstract

Multiple cases of thymic lymphoma were diagnosed in bioassay specimens of Japanese medaka (Oryzias latipes), a small fish species. Lymphoma in chemically exposed specimens was not clearly related to specific compounds, dose, or sex. Multiple cases of lymphoma occurred in three specimens held in one aquarium and in two specimens in two other aquaria, suggesting the possibility of horizontal transmission. Forty cases were observed in 9802 specimens (0.41%), with an incidence of 0.46% in exposed specimens (5690 total) and 0.34% in controls (4112 total). Lesions were dominated by small cells with large, basophilic nuclei and a thin cytoplasmic rim. Mitotic figures were frequently observed. Electron microscopy showed that the nuclei of lymphoma cells were highly invaginated but revealed no viral particles. Lymphomas appeared to originate in the thymus and subsequently infiltrate esophagopharyngeal regions, retroperitoneal spaces, cranial regions, heart, and abdominal viscera. Given the scattered occurrence of the lymphomas and the fact that they tend to occur in specimens in contact with one another, we suspect a viral etiology. Lymphoma appears to be the most frequently occurring spontaneous neoplasm in medaka and should be taken into account when assessing results of carcinogenesis studies.

Introduction

Neoplasms of hematopoietic origin, most frequently from lymphoid tissues, have been reported from several species of fish (1). Epizootics of lymphoma have occurred in muskellunge (Esox masquinongy) and northern pike (Esox lucius) (2–6). Lymphomas have also been reported from Atlantic salmon (Salmo salar) (7) and rainbow trout (Oncorhynchus mykiss) (8, 9). Several cases of lymphoma have recently been reported in the Japanese medaka (Oryzias latipes) (Footnote 3; Refs. 10–12). Other cases of lymphoma have occurred in various fish species that were exposed to chemical carcinogens, but a chemical-neoplasm relationship has not been confirmed (13–15).

Carcinogenicity tests using fish have been found to be responsive to a number of chemical carcinogens (16). Of several species of aquarium fish used so far, the Japanese medaka has been used to study the widest range of carcinogens, but a chemical-neoplasm relationship has not been confirmed (13–15).

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Age appeared to influence the frequency of occurrence of lymphoma (Table 2). Considering all cases, nearly every tissue was infiltrated by lymphocytic cells. In early stages, lymphoma cells infiltrated regions surrounding the thymus, esophagus, gill arches, and head musculature (Fig. 1). As the lesion advanced, lymphocytic cells infiltrated, in a time-dependent fashion, the kidney, orbit, cranium, general circulation, liver, androgen, gill arches, and head musculature (Fig. 2).

Materials and Methods

Animals. Data reported here are from approximately 9800 Japanese medaka that were from control groups or from groups exposed to a variety of test chemicals. Medaka 6 to 10 days old were exposed to the test chemicals for periods ranging from 6 h to 6 mo (Table 1). Brief exposures were made in static water, and long-term exposures were made in continuous flow through aquaria (20). Some of the continuous exposures were made intermittently over a 3-mo period in which fish were exposed 24 h per wk for 13 wk (21). Following exposure, fish were transferred to aquaria and were maintained in carcinogen-free water until sampled.

Pathology. Samples for histopathological examination were typically taken at 6, 9, and 12 mo post initial exposure. Moribund specimens exhibiting gross signs of lymphoma, including swellings in the head region or in areas adjacent to the operculum, were sampled when identified. For light microscopy, whole fish were fixed in Lillie’s fixative for 2 to 4 days, embedded in paraffin, sectioned parasagitally at 5.0 μm, and stained with hematoxylin and eosin. Moribund fish were treated similarly.

Samples of lymphomatous tissue from seven specimens were examined by transmission electron microscopy, as was a normal thymus. Tissues were fixed in 3.0% glutaraldehyde in 0.1 M sodium cacodylate buffer, postfixed in 1% osmium tetroxide, dehydrated in ethanol, and embedded in Embed 812 resin. Thin sections were mounted on uncoated grids which were stained with uranyl acetate and lead citrate and examined with a JEOL 100 SX electron microscope.

Statistical Methods. Differences in incidence of lymphoma occurring in control and chemically treated groups were analyzed using Fisher’s test (22). A result was considered significant when P < 0.05.

Results

Forty cases of lymphoma were diagnosed in 9802 specimens producing an overall incidence of 0.41%. Fourteen cases occurred in 4112 control specimens (0.34%), and 26 cases occurred in the 5690 (0.46%) specimens from chemically treated groups (Table 1). Two of the control lymphomas occurred in dimethylformamide solvent controls. Considering all cases, a significant difference between the incidence of lymphomas in pooled designated controls and chemically treated groups (P > 0.05) could not be discovered. Twenty-five lymphomas occurred in male specimens, and 15 occurred in females.

The highest incidence of lymphoma in chemically treated groups occurred in a test with DBE. Six lymphomas occurred in specimens from a pool of 403 fish, producing an incidence of 1.49%. When the incidence of these cases was compared with that of the historical controls, DBE exposure appeared to increase the prevalence of lymphoma (P < 0.05), although a dose-response relationship could not be established.

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Table 1  Compound-related incidences of lymphoma in the Japanese medaka

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration</th>
<th>Duration</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td>14/4112</td>
</tr>
<tr>
<td>30% Puget Sound microlayer</td>
<td></td>
<td></td>
<td>3/314</td>
</tr>
<tr>
<td>DMBA</td>
<td>1-182 ppb</td>
<td>96 h</td>
<td>2/102</td>
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<tr>
<td>DMBA/THM</td>
<td>70 ppb/0-8 ppm</td>
<td>6 h/3 mo</td>
<td>1/227</td>
</tr>
<tr>
<td>TCE/CCl</td>
<td>7 ppm/0-8 ppm</td>
<td>3 mo</td>
<td>1/440</td>
</tr>
<tr>
<td>CDMM/CCIc</td>
<td>0.1-6 ppm/0-3 ppm</td>
<td>3 mo</td>
<td>1/276</td>
</tr>
<tr>
<td>DCE</td>
<td>0.1 ppm-1 ppm</td>
<td>3 mo</td>
<td>6/403</td>
</tr>
<tr>
<td>THM mixf</td>
<td>4</td>
<td>3 mo</td>
<td>1/251</td>
</tr>
<tr>
<td>TBTO</td>
<td>4.5 ppb</td>
<td>3 mo</td>
<td>1/70</td>
</tr>
<tr>
<td>TCE</td>
<td>8-14 ppm</td>
<td>3 mo</td>
<td>1/191</td>
</tr>
<tr>
<td>AAF</td>
<td>0.7 ppm</td>
<td>7 days</td>
<td>1/116</td>
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<tr>
<td>BeP</td>
<td>5 ppb</td>
<td>6 h x 2</td>
<td>1/307</td>
</tr>
<tr>
<td>Other chemicals</td>
<td></td>
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<td>0/1960</td>
</tr>
</tbody>
</table>

* Pooled water and solvent (0.5 ml/liter of dimethylformamide) controls.
† Denotes the occurrence of two lymphomas in the same aquarium.
‡ Mix here denotes CHCl₃, CHBr, chlorodibromomethane, and CCl₄.
§ Other chemicals include THM, benzo(a)pyrene, DMBA, and cadmium chloride.

Table 2  Degree of lymphoma infiltration for control and chemically treated Japanese medaka with respect to age

<table>
<thead>
<tr>
<th>Age (wk)</th>
<th>Total no. of cases with varying degrees of infiltration*</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
<td>Control</td>
<td>18-29</td>
<td>4</td>
<td>25</td>
<td>75</td>
<td></td>
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<tr>
<td></td>
<td>30-39</td>
<td>5</td>
<td>20</td>
<td>20</td>
<td>60</td>
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<tr>
<td></td>
<td>≥40</td>
<td>5</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>19-29</td>
<td>5</td>
<td>40</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>30-39</td>
<td>9</td>
<td>11</td>
<td>11</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>≥40</td>
<td>12</td>
<td>8</td>
<td>17</td>
<td>8</td>
</tr>
</tbody>
</table>

* The degrees of lymphoma infiltration are as follows: 1, local invasion immediately surrounding the thymus; 2, esophageal, pharyngeal, and retroperitoneal; 3, intraorbital and intracranial; 4, leukemic, abdominal cavity, and intestinal lamina propria.

Discussion

Based on the location of lymphocytic cells in early stages of the lesion, lymphoma in these cases of medaka appeared to originate from the thymus, justifying the diagnosis of thymic lymphoma. Another possible site of origin is the kidney, but we
could not find lymphoma cells associated with the kidney in early stages of the lesion. Ultrastructurally and histologically the lesion resembles other reported cases of lymphoma in fish (8, 10–12). The deeply clefted appearance of lymphoma cell nuclei was also seen in lymphoma cells of the rainbow trout (8). Ghadially (23) noted that this feature was observed in the perifollicular zone of normal lymph nodes (of mammals), but was seen in larger numbers in lymphomas and leukemias. A similar condition was confirmed in the medaka. The clefted nucleus was sometimes observed in the lymphocytes of the normal medaka thymus, but it was more prominent in lymphoma cells. Ghadially also noted that these clefts appeared to be a result of proliferation of membrane or membranes of the nuclear envelope.

Lymphomas in esocids appear to have a viral etiology. Mulcahy (24) transmitted lymphoma to a pike by injecting a 0.22-μm filtrate from a tumor homogenate of lymphosarcomatous pike tissue. Papas et al. (25) reported C-type virus particles and reverse transcriptase associated with pike lymphoma tissue. Possible evidence for a viral etiology in the cases of lymphoma reported here comes from the occurrence of multiple cases in three separate aquaria. This occurrence of multiple cases might indicate a horizontal transmission by a virus. However, a viral etiology was not confirmed or disproved by electron microscopy.

The association between chemical exposure and the occurrence of lymphoma in fish has not been clearly established. One case in 3500 N-methyl-N-nitrosourea-treated platyfish/swordtail hybrids (13) does not represent a clear indication of a carcinogenically induced lymphoma. Two cases of lymphoma have been reported in N-methyl-N'-nitro-N-nitrosoguanidine-exposed channel catfish (Ictalurus punctatus) (15), and two cases were also reported in Poeciliopsis spp. exposed to DMBA (14). Whether these cases are treatment related or due to another cause is not clear, since sufficient background data on these types of lesions were not available for these species. One case of lymphoma occurred in a channel catfish which was not exposed to any known chemical carcinogen, indicating that this lesion can occur spontaneously (26). Another consideration is whether these lesions are frank lymphomas since reactive tissue or another lymphoproliferative disease might appear like a lymphoma.

The incidence of lymphoma in the DBE-treated fish was statistically significant, but lymphoma occurrence was not dose related. This presents a possible confounding result in the interpretation of the carcinogenic potency of DBE. Perhaps DBE promoted an otherwise spontaneous lymphoma or helped induce viral transformation.

The lack of a significant difference between the incidence of lymphoma in control and chemically treated medaka indicates that the neoplasm is probably spontaneous, and generally, chemical stress alone does not increase the occurrence. This supports the conclusions of other case reports of medaka lymphomas (Footnote 3; Refs. 10–12). Yet, the possibility of chemical induction of lymphomas in fish cannot be discounted.

In rodent bioassays there is a negative correlation between malignant lymphomas and hepatocellular proliferative lesions. A failure to recognize this correlation in rodents may distort the interpretation of carcinogenicity data for a particular test compound (27). In our studies, lymphoma does not appear to affect the incidence of hepatocellular lesions induced by carcinogens, but this may be due to the comparatively low incidence of lymphoma in medaka.

Data on spontaneous neoplastic lesions in medaka should provide useful information for evaluating results in carcinogenesis studies. Investigators should be aware of the historical incidence of lymphoma in control specimens of medaka when evaluating the occurrence of lymphoma in a carcinogen-treated fish. Without this sort of background data on the occurrence of neoplastic lesions in historical controls of a test organism, the random occurrence of a spontaneous or virally induced lesion might be misinterpreted to overestimate the carcinogenic potency of a chemical (18, 19).

Acknowledgments

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References


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