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Aflatoxin B1 Induced Hepatic Neoplasia in Great Lakes Coho Salmon

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There is considerable interest in the development of fish models for carcinogen bioassays and the study of chemically induced cancer in wild fish species. Laboratory carcinogenesis experiments with fish have generally used either rainbow trout (Salmo gairdneri), or small aquarium species such as guppies (Lebistes reticulatis), zebras (Brachydanio rerio), medaka (Oryzias latipes), etc. (Black 1984; Couch and Harshbarger 1985) Among salmonid species, rainbow trout have mainly been used for carcinogenesis research, in part due to the role played by this species in the discovery of the carcinogenic action of aflatoxin Bl (AFBl) (Halver 1967) .

Recently, apparatus and methodology for microinjection of salmonid fish embryos with chemical carcinogens has been described (Black et al. 1985). By this methodology, nanogram to microgram amounts of chemical are administered via transchorionic injection into the yolk sac of late eyed-stage embryos. Because eggs produced by Pacific salmon are relatively much larger than those of rainbow trout, they would provide an attractive subject for embryo microinjection. However, members of the Pacific salmon (genus Oncorhynchus), have not been used very frequently in carcinogenesis research, perhaps because previous reports have indicated that this group of fishes lacks sensitivity to AFBl (Hendricks 1982).

The Great Lakes are annually stocked with large numbers of coho salmon (Oncorhynchus kisutch). Sonstegard and Leatherland (1984) have previously recommended use of coho salmon as an indicator for monitering ecosystem health in the Great Lakes, because stockings throughout the Great Lakes are from a common genetic strain and in the lake environment they have a defined food source and life cycle.

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These considerations led us to test coho salmon for their sensitivity to the potent hepatocarcinogen, AFB1. The present report describes in preliminary form, the results of these experiments.

MATERIALS AND METHODS

Eyed stage coho salmon eggs were obtained from Lake Ontario stocks collected during the 1986 fall spawning at the N.Y.State Department of Environmental Conservation fish hatchery at Altmar, NY. The average diameter and weight of the eggs was approximately 6 mm. and 230 mg. respectively.

Using apparatus and methods previously described (Black et al. 1985), groups of 300 eggs/embryos were microinjected with either 50 or 100 ng./egg of AFB1 contained in a 1 ul. volume of dimethylsulfoxide (DMSO). Controls received an injection of DMSO only. Mortality was monitered for 60 days post-exposure. Following hatching, and yolk-sac absorbtion fish were raised using routine fish cultural practices. They were fed on a semi-purified, 60% protein, casein/geletin diet, based on an Oregon State University formula (Hendricks, 1982). Cumulative mortality was monitered through 60 days post-exposure. At 14 mos. post-exposure, survivors were sacrificed and examined grossly and microscopically for neoplasms. Neoplasms were tabulated on the basis of size as either foci (smaller than a 400X field) or nodules (larger than a 400X field).

RESULTS AND DISCUSSION

The highest dose of AFB1 was acutely toxic and less than 10 fry survived 60 days post-exposure. Significant toxicity was also evident at the 50 ng. dose. Uninjected controls exhibited 43% mortality when observed over the same time period. When corrected for this relatively high mortality associated with this lot of eggs, mortalities were 34% and 17% for 50 ng. AFB exposed and DMSO injected controls, respectively.

Although neoplasms varied widely in size, most were similar in their histologic and cytologic appearance. No attempt was made in the present report to subclassify neoplasms on the basis of differences in histology. Most were very well differentiated and of hepatocellular origin. Most exhibited a trabecular growth pattern and were basophilic (H&E stain), although less so than the hyperbasophilic hepatic neoplasms usually observed in comparably AFB1 exposed rainbow trout. One fish exhibited a poorly differentiated, anaplastic neoplasm. Several exhibited focal bile duct proliferation sufficient to suspect early neoplasia.

Table 1. Results of embryonic exposure to AFB1 administered by direct yolk-sac microinjection.

DOSE	NUMBER	% : FOCI	NODULES	BOTH	NORMAL
50 ng	38	42	11	21	21
100 ng	no survivors				
DMSO	64	0	0	0	100

AFB1 has long been recognized as a potent liver carcinogen in the rainbow trout. Most experiments involving embryo exposure of rainbow trout to AFB1 have used aqueous exposure to low concentrations of this compound ie. 0.5 - 1.0 ppm. for 1 hr. or less (Hendricks 1982). Availability of the egg/embryo yolk-sac microinjection technique has enabled direct comparison of sensitivity between the coho and the rainbow trout. In our laboratory, Mt. Shasta strain rainbow trout embryos injected with as little as 1 ng. of AFB develop a low frequency of liver neoplasms. At higher doses of 10 ng. per egg, a tumor frequency of approximately 30% has been observed at 1 year post-exposure (Black, unpublished). This observation compares closely to results produced in Kamloops strain rainbow trout, using a perivitelline injection method, ie. 35% at an exposure of 13 ng. (Metcalfe and Sonstegard 1985). Rainbow trout eggs are considerably smaller than eggs of coho salmon. Eggs from 3 yr. old rainbow trout usually weigh between 80 to 90 mg., whereas coho eggs usually exceed 200 mg. in weight. In the present study, an overall tumor frequency of approximately 79 percent was observed. If allowance is made for the larger size and weight of the coho egg, we conclude on the basis of the tumorogenic response observed in the present experiments, that the Great Lakes (Lake Ontario) coho salmon used in these experiments were highly sensitive to AFB1.

Salmonid fish from the Great Lakes, especially those from Lake Ontario, are known to be contaminated with numerous organic chemicals of anthropogenic origin (Hesselberg and Seelye 1977; Clark et al. 1980). Some of the contaminant compounds identified in these fish eg. polychlorinated biphenyls (PCBs), dioxins, etc., may be acting as modulators of the carcinogenic effects observed in our experiments. Although concurrent administration of PCBs will partially inhibit the

carcinogenic effects of AFB1 in rainbow trout, prior exposure of gravid female trout to PCBs, greatly enhances the carcinogenic effect (Hendricks 1982) produced by AFB1. The latter situation is consistent with exposure received by the maternal parent in the Lake Ontario environment.

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