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Elevated Concentrations of Serum α-Fetoprotein in Rats with Chemically Induced Liver Tumors[1]

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SUMMARY

The study was undertaken to determine whether aflatoxin B_1 (AFB_1)-induced liver tumors in rats produced α_1-fetoprotein (AFP) and whether the age of the animals would influence such an appearance, a finding suggested by data seen in man. Other liver carcinogens (*N*-hydroxy-*N*-2-fluorenylacetamide, *N*-2-fluorenylacetamide, and diethylnitrosamine) were tested for their ability to induce liver tumors producing AFP. The presence of AFP in the serum was determined by double diffusion in agarose and by comparison also by quantitative radioimmunoassay.

Using double diffusion, AFP was detected in the majority of tumor-bearing rats that had received either *N*-2-fluorenylacetamide or *N*-hydroxy-*N*-2-fluorenylacetamide. Sera of diethylnitrosamine-treated rats with liver tumors were all positive, whereas sera of rats bearing AFB_1-induced tumors were positive in only a few cases. However, all sera of tumor-bearing rats examined had elevated AFP levels by radioimmunoassay. Nonetheless, the average level of AFP in the sera of rats bearing AFB_1-induced tumors was considerably lower, compared to the sera of rats with tumors caused by diethylnitrosamine, *N*-2-fluorenylacetamide, or *N*-hydroxy-*N*-2-fluorenylacetamide. Rats started on AFB_1 when 6 weeks old had more mixed liver tumors with neoplastic hepatocytes and bile ducts and higher AFP levels than did rats started at 26 weeks of age. However, the histological grade of differentiation of induced tumors did not seem to influence the AFP level.

INTRODUCTION

Elevated serum concentration of AFP[7], a carcinoembryonic antigen, has been detected in animals with liver tumors from a variety of species, including humans (1, 2, 7, 8, 10, 12–17, 22, 24, 27, 36). Besides its occurrence in animals with chemically induced liver tumors, its early appearance has been demonstrated in experimental animals within weeks after treatment with certain hepatocarcinogens at moderate to high dosages (7, 8, 11, 15, 17, 18, 28, 40) and, more recently, even with apparently noncarcinogenic doses (5). Elevated levels of serum AFP have been found in a high percentage of animals bearing liver tumors induced by 3-methyl-4-dimethylaminoazobenzene, 4-dimethylaminoazobenzene, DENA, and dimethylnitrosamine. However, elevated serum AFP levels were not seen in animals with AFB_1-induced liver tumors (21, 36). This finding is surprising because this hepatocarcinogen is thought to play a role in the etiology of human liver cancer (3, 26, 34), in which the majority of patients have elevated serum concentrations of AFP (1, 9, 19, 37–39).

The present study was undertaken to determine whether AFB_1-induced liver tumors in rats produced AFP and whether the age of the animals influenced its appearance, a finding that has been suggested in man (4, 20, 39). In addition, other hepatocarcinogens were tested for their ability to induce liver tumors producing AFP.

MATERIALS AND METHODS

Rats. Male Fischer 344/CS rats were used in all experiments. At the start of the study, the rats were either 6 weeks or 6 months of age. The rats were housed in groups of 3 in hanging wire mesh cages and maintained on

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[7] The abbreviations used are: AFP, α_1-fetoprotein (α_1F in some current U. S. publications, and AFP adopted by the recent international assembly at the Nice, France meeting); DENA, diethylnitrosamine (also *N*-nitrosodiethylamine); AFB_1, aflatoxin B_1; FAA, *N*-2-fluorenylacetamide; NOHFAA, *N*-hydroxy-*N*-2-fluorenylacetamide (also called *N*-hydroxy-2-acetylaminofluorene or *N*-acetofluorenylhydroxamic acid); RIA, radioimmunoassay.

Wayne laboratory meal (4% fat) (Allied Mills, Inc., Chicago, Ill.).

Chemicals. DENA (Fisher Scientific Co., Silver Spring, Md.) was given the drinking water. The following were administered in the feed: FAA (Aldrich Chemical Co., Milwaukee, Wis.), NOHFAA (obtained through the courtesy of Dr. H. B. Wood, Jr., Drug Development Branch, National Cancer Institute, Bethesda, Md.), and AFB_1 (Makor, Jerusalem, Israel).

Antiserum. The monospecific AFP antiserum that was used was prepared and tested as described earlier (17). Confirmatory tests on identity and purity were conducted by immunoelectrophoresis.

Test for AFP. Animals were bled by orbital puncture and the sera were immediately separated and assayed by double diffusion in 1% agarose (25). Each sample well held about 1 to 2 μl of serum and the wells were separated by spaces of 5 mm.

In those cases where enough serum was available, a quantitative RIA was performed, according to the method previously reported (31-33).

Experimental Design. The plan of the tests conducted in 3 series of experiments is detailed in Table 1.

The sera in Experiment 1 were examined by double diffusion weekly during the 1st 10 weeks and every other week thereafter. The sera in Experiment 2 were examined every other week for 30 weeks and thereafter every 4 weeks. The sera from animals in Experiment 3 were examined every 4 weeks. At the termination of each experiment, control and test animals were bled by cardiac puncture. Necropsies were done on all animals and the livers and grossly abnormal tissues removed and fixed in 4% buffered formalin. Paraplast sections (5 μm) were stained with hematoxylin and eosin for histopathological examination.

RESULTS

The effects of equimolar concentrations of FAA and NOHFAA on the early induction of AFP have already been described (18). As detected by double diffusion, the elevated serum concentrations of AFP were transient in nature. All of the rats receiving 300 ppm FAA and 1 rat fed 200 ppm FAA died within 6 weeks on test. The results of the late appearance of AFP in the sera as well as the liver pathology of the rats surviving 22 weeks or more are given in Table 1. AFP was detected by double diffusion in the majority of the rats receiving 200 ppm FAA and 320 ppm NOHFAA, but only 1 rat showed a positive response in each group given 160 and 213 ppm NOHFAA. Detectable serum concentrations of AFP were usually found 2 to 4 weeks prior to death and remained high until sacrifice.

In all cases examined by RIA, elevated AFP levels were found in tumor-bearing rats, compared to the normal AFP serum levels for adult male Fischer 344/CS rats.

The liver tumors were usually moderately and well-differentiated hepatocellular carcinomas. Two of the 7 FAA-induced liver tumors and 1 of the 14 tumors caused by NOHFAA also had "mixed" patterns, displaying both neoplastic hepatocytes and neoplastic bile ducts with transitional elements.

For Experiments 2 and 3, only data for rats surviving

Table 1

Presence of AFP, measured by double diffusion and by RIA, in sera of male rats with liver tumors induced by 4 hepatocarcinogens

Experiment	Carcinogen	Dose (ppm)	No. of rats	Age in wk at start	Duration[a]		Pathology[b]				AFP		
					Treatment	Observation	HA	HN	HNA	Ca	Double diffusion +	Double diffusion −	RIA range (μg/ml)
1	FAA	150	5	6	15	52				4		5	42-73 (2)[c]
		200	5	6	15	52			1	3	3	1	70-980 (3)
		300	5	6	6	6					5		ND[d]
	NOHFAA	160	5	6	15	52				5	1	4	0.4-4.8 (3)
		213	5	6	15	52		1		4	1	4	590 (1)
		320	5	6	15	52				5	4	1	10-65 (3)
	Control		30			26-74							0.014-0.068 (30)
2	NOHFAA	160	20	6	15	36			1	15	12	4	0.4-450 (13)
	DENA	40	20	6	15	26				17	17	0	52-820 (6)
	AFB_1	0.2	25	6	74	74	1	2		18	0	21	0.1-77 (10)
		2.0	25	6	47	47			1	17	3	17	1.1-230 (9)
3	AFB_1	0.2	30	26	74	74	5	6	1	9	0	25	ND
		2.0	30	26	36	56	2	6	2	19	0	29	0.15-2.7 (6)
		4.0	30	26	20	56		3		18	3	18	0.1-18 (9)

[a] Treatment period is length of carcinogen administration. Observation period is total experimental time, including treatment; the difference, if any, is time maintained on control diet. All times are given in weeks.

[b] Liver lesions were graded as in Ref. 41. HA, hyperplastic area; HN, hyperplastic nodule; HNA, hyperplastic nodule with atypia; Ca, hepatocellular carcinoma.

[c] Numbers in parentheses, number of rats assayed.

[d] ND, not done.

treatment with DENA for 16 weeks or more, with AFB_1 for 28 weeks or more, and with NOHFAA for 30 weeks or more have been tabulated. Sera examined by double diffusion showed only a few cases of AFP positive sera in the AFB_1-fed rats with hepatocellular carcinoma. In contrast, the majority of the rats given NOHFAA and all the animals on DENA with liver cancer were positive. Liver tumors were present in all rats with positive sera.

All of the sera from treated animals examined by the RIA showed above normal levels of AFP. They included sera from 2 rats (1 on NOHFAA and 1 on 2.0 ppm AFB_1) exhibiting only hyperplastic nodules with atypical areas, which had AFP values of 0.4 and 45 μg/ml, respectively.

Chart 1 shows the individual determinations of AFP compared with the morphological differentiation of the tumors. When more than 1 tumor was observed, both tumors were classified, and if the level of differentiation was not the same, they were listed as combinations of the types. None of the DENA and only 1 of the NOHFAA tumors presented "mixed" patterns. However, this was a fairly common finding in the neoplasms from animals fed AFB_1 starting at 6 weeks of age but less so in animals begun on AFB_1 treatment at 26 weeks of age.

DISCUSSION

The results reported here show that all of the chemical hepatocarcinogens tested induced AFP-producing tumors. The highly sensitive RIA was able to quantitate elevated concentrations of AFP in all of the sera assayed. Although there was considerable variation, the levels in DENA-treated rats were generally high, while those from AFB_1-treated animals were low. Rats started on AFB_1 treatment at 6 weeks of age had higher serum levels of AFP than did those begun at 26 weeks of age. This age factor may correlate with that reported in man (4, 20, 39). A question arises whether the lower titer of AFP in the AFB_1-treated rats may be related to the "mixed" nature of the liver lesions produced, especially in the animals begun on treatment at 6 weeks of age.

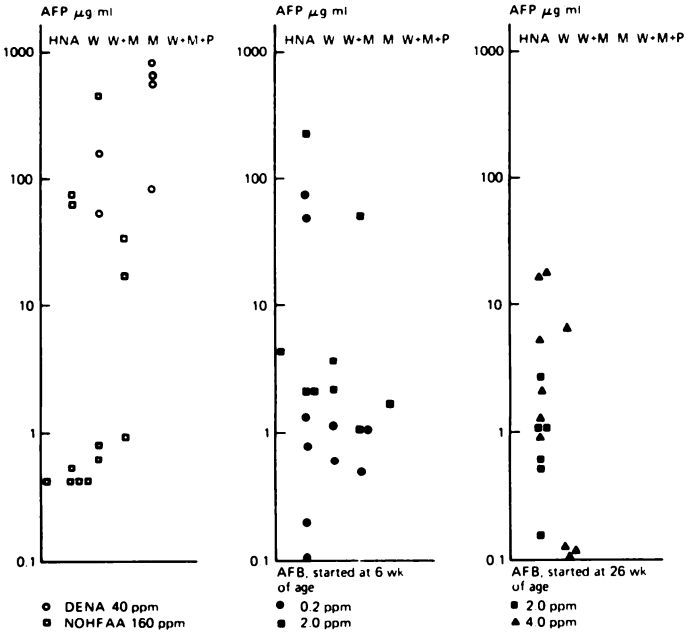

Chart 1. AFP levels in the sera of rats with liver tumors induced by DENA, NOHFAA, and AFB_1 in relation to histological criteria. *HNA*, hyperplastic nodule with atypia; *W*, *M*, and *P*, well, moderately, and poorly differentiated carcinoma, respectively; *W+M*, both types present; *W+M+P*, all 3 types present.

Although the double diffusion technique was capable of revealing elevated serum levels of AFP in 100% of the DENA-treated animals, this method detected AFP in the serum of only 75% of the NOHFAA-treated rats and in less than 6% of the AFB_1-treated rats. These findings confirm our results reported earlier that afb_1-induced tumors can produce AFP (16, 35). The low percentage of AFP-positive animals as detected by the double diffusion technique may explain the negative results reported earlier (21, 36). AFP was recently reported in the serum of a monkey bearing an AFB_1-induced hepatocellular carcinoma (6).

At equimolar concentrations, FAA was more toxic than NOHFAA and also seemed to induce liver tumors that were stronger producers of AFP. This finding is of interest especially with respect to previous reports that FAA is a more potent inducer of the early appearance of AFP (5, 18).

The histological grade of differentiation of the tumors seemed not to be related to the AFP levels, although the DENA-treated rats usually had high levels of AFP and less differentiated tumors. It is difficult to draw a conclusion in this regard because chemically induced liver tumors in rats may be multifocal and, therefore, it is not possible to specify which are producing AFP. High AFP levels could also be found in sera of rats bearing well-differentiated liver tumors. These results agree with reports indicating that morphological differentiation of liver carcinomas does not affect AFP production (14, 23, 29, 30). Two rats that exhibited only hyperplastic nodules had elevated serum levels of AFP detectable by RIA, confirming our earlier data (17). The latter observations may indicate that the appearance of elevated concentrations of AFP can be used as a predictor of a preneoplastic lesion. This might be determined by studying the pathogenesis of the liver lesions in conjunction with the onset and progression of elevated AFP levels.

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