Tumor Res., 8, 71-74, 1973

Further inverstigation on AFP in rats subjected to chemical hepatocarcinogenesis

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We previously reported that AFP appeared in serum of rats in 2 phases during hepatocarcinogenesis with 4-dimethylaminoazobenzene (DAB)[1]. The serum levels of AFP measured by the Mancini method in the early stage, 6 weeks after the onset of feeding with DAB, were 20 to 60 μg/ml. On the other hand, the AFP levels after the development of hepatoma (20 weeks after) were about 1 mg/ml. The sensitivity of the Mancini method used in this previous experiment was 10 μg/ml or thereabouts.

Present study reports the mode of appearance of AFP more in detail using the radioimmunoassay established[2] in rats treated with various chemicals.

Treatments

As shown in Table 1, rats of Donryu strain were fed with a synthetic diet containing 0.02% or 0.06% DAB, 0.08% of FAA and 50 ppm of DMN all of which are hepatocarcinogenic agents. As an analog of DAB, 4'-Me-DAB was used for reference at 0.06% which is practically a non-carcinogenic compound.

CCl_4 and GalN were given to rats, these are simple hepatotoxic agents

Table 1. *Experimental groups*

Group	Initial body weight (grams)	Age (weeks)	No. of rats used	Treatment*	Observed period (weeks)
1	150~170	10	22	0.06% DAB	46
2	100~120	7	20	0.02% DAB	33
3	140~160	9	20	0.06% 4'-Me-DAB	27
4	90~110	6	25	50 ppm DMN	26
5	110~130	8	17	0.08% FPA	8
6	210~260	15	11	CCl_4	1
7	190~210	14	7	GalN	1

* Abbreviations used: DAB, 4-dimethylaminoazobenzene; 4'-Me-DAB, 4'-methyl-4-dimethylaminoazobenzene; FPA, N-2-fluorenylphthalamic acid; DMN, dimethylnitrosamine; GalN, D-galactosamine.

which cause acute liver cell injury. CCl_4 was administered as a single intraperitoneal injection of 0.1 ml of CCl_4 dissolved in 0.4 ml of olive oil. A total dose of galactosamine was administered in 6 separate intraperitoneal injections according to the method of Keppler *et al.*[3] at a rate of 150 mg/100 g/body weight.

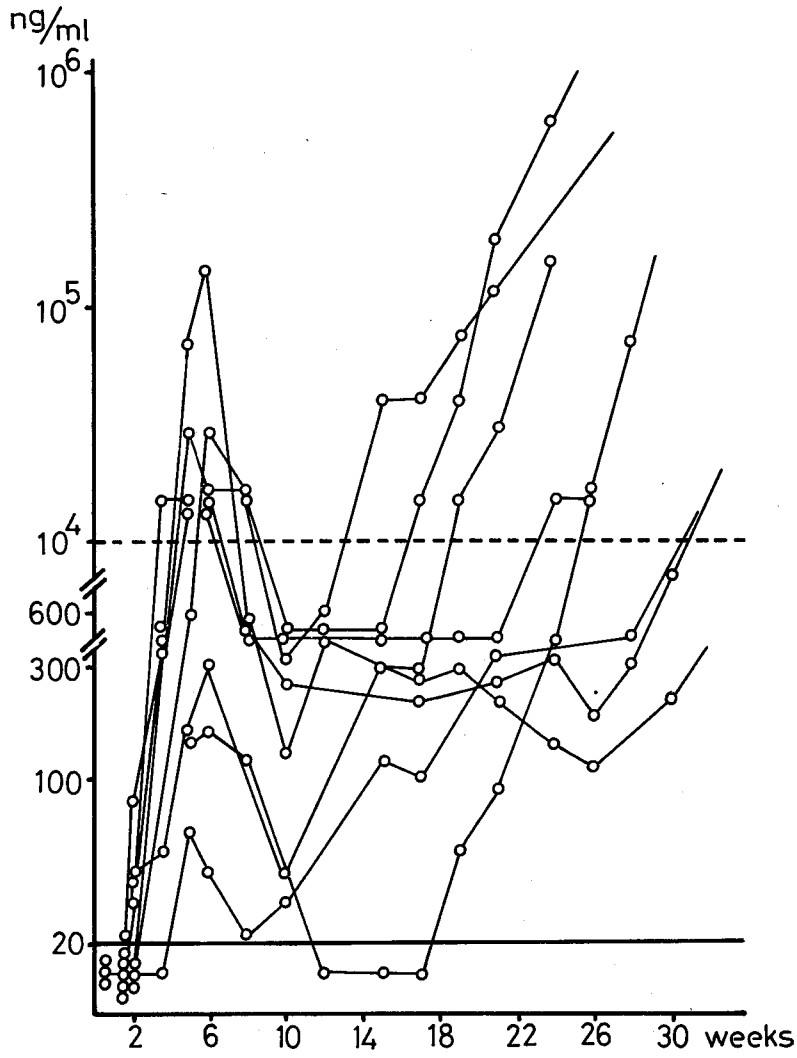

Fig. 1. Change of AFP concentration in sera of rats fed with 0.06% DAB. Each rat was shown by a continued line. Values above 20 ng/ml were taken as positive. Dotted line indicates a minimal concentration detectable by the Mancini method.

Appearance of AFP in rats treated with 0.06% DAB

AFP was already detected in the 2nd week after the commencement of feeding with 0.06% DAB (Fig. 1). After 6 weeks, the concentration of AFP gradually decreased but did not become negative (less than 20 ng/ml) except in one rat. All rats tested, revealed almost the same pattern in change of AFP concentration.

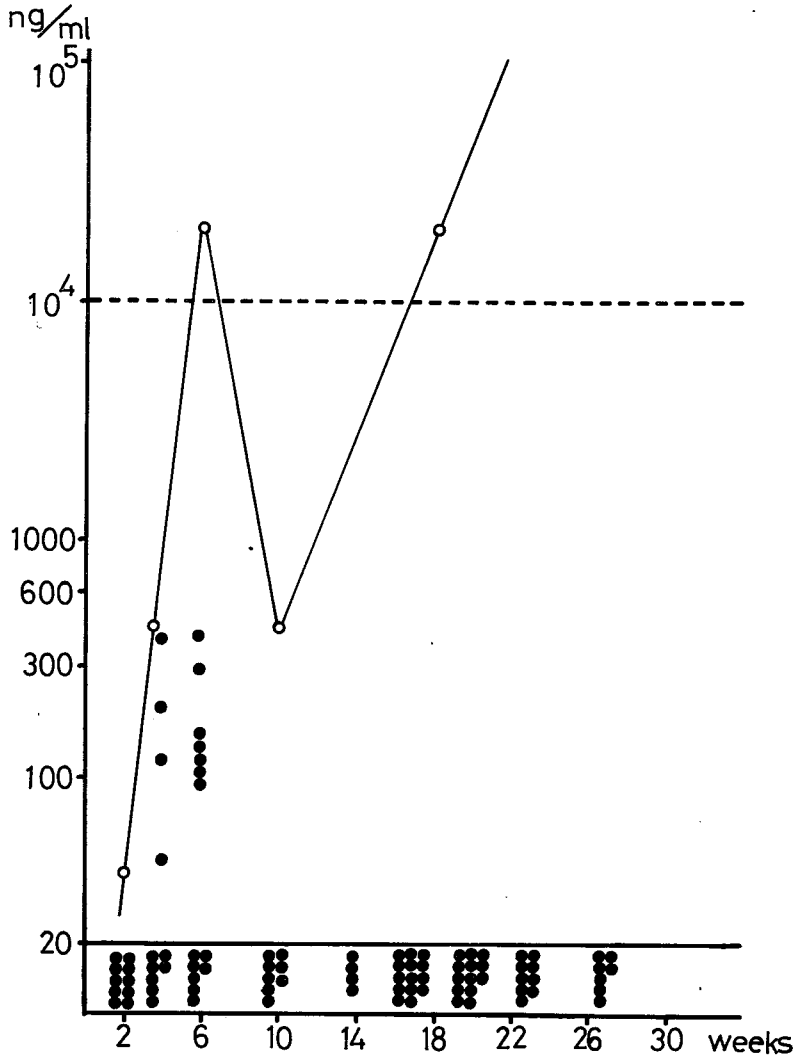

Fig. 2. Concentration of AFP in rats during the treatment with 4′-Me-DAB. The AFP values of each rats were shown with closed circles. A typical pattern of 0.06% DAB experiment is shown with open circles.

Appearance of AFP in rats treated with other chemicals

On the other hand, when rats were fed with 4′-Me-DAB which is a practically non-carcinogenic agent, AFP appeared only transiently in 50% of rats on the 4th to 6th week (Fig. 2). The concentrations were from 45 to 500 ng/ml. These values were significantly lower than the values observed in 6th week in rats fed with 0.06% DAB.

Compared to the rats treated with 4′-Me-DAB, the rats fed with 0.02% DAB and 50 ppm DMN showed a sporadic appearance of AFP, but the concentration did not exceed the level of 200 ng/ml. On the other hand, AFP was detected continuously in all cases fed with 0.08% FPA, and the concentration was only slightly below 300 ng/ml.

Effect of acute liver injury on the appearance of AFP in rat

The results of the experiment with CCl_4 or GalN intoxication are presented in Fig. 3. Four days after treatment, a transient appearance of AFP was observed in about 50% of rats in both cases although the highest value was under 500 ng/ml.

DISCUSSION

The transient appearance of AFP in acute liver cell injury resulting from CCl_4 or GalN intoxication suggests that regenerating liver cells synthesize a small amount of AFP. The same explanation may be applicable in the case of 4′-Me-DAB which is an analog of DAB but is practically non-carcinogenic.

Low levels of AFP production observed in the case of DAB at a low concentration of 0.02%, DMN and FPA may also be simply explained by the fact that regenerating liver cells synthesize AFP. On the other hand, AFP levels in the early stage of carcinogenesis with 0.06% DAB were more than 100 times higher than those observed in the experiments with non-carcinogenic agents. This fact suggests that the cells which are responsible for synthesizing AFP in the early stage of DAB carcinogenesis should be quite different from the cells in simple intoxication with non-carcinogenic agents.

REFERENCES

1. WATABE, H.: Early appearance of embryonic α-globulin in rat serum during carcinogenesis with 4-dimethylaminoazobenzene. *Cancer Res.*, **31**, 1192 (1971).
2. HIRAI, H., NISHI, S. and WATABE, H.: Radioimmunoassay of α-fetoprotein. *Protides Biological Fluids* (ed. H. Peeters) Pergamon Press, **20**, 579 (1973).
3. KEPPLER, D., LESCH, R., REUTTER, W. and DECKER, K.: Experimental hepatitis induced by D-galactosamine. *Exp. Mol. Path.*, **9**, 279 (1968).