Change of serum AFP levels in carbon tetrachloride induced hepatitis

Masaru ISHII and Hideo IKEHARA

The Second Department of Internal Medicine, School of Medicine, University of Kobe, Kobe. Japan

In 1971, we isolated highly purified AFP from the ascites of a patient with hepatoma and established a radioimmunoassay for the detection of human AFP based on the coprecipitation inhibition technique using the double antibody method. From the results of AFP tests for serum of patients with hepatitis using this assay, a certain relationship between the AFP dynamics and transition of transaminase activity levels in serum was found. Namely, a small amount of AFP appeared while the transaminase activity level increased and AFP levels reached a maximum at the beginning of the decreasing phase of transaminase activity, and thereafter the AFP levels showed a reduction almost concomitantly with the transaminase levels and finally became undetectable. However, no relationship between the appearance of AFP in serum and the regeneration of liver cells was found[2].

We also established a radioimmunoassay method for rat AFP by using AFP purified from rat fetus. The present study is concerned with the comparative dynamics of serum AFP and transaminase activity levels in rats with carbon tetrachloride induced hepatitis and the relationship between the AFP levels in serum and the regenerating liver.

A radioimmunoassay method of rat AFP was employed for the detection of AFP in serum of the following experimental rats. Details of the assay method are described below.

A radioimmunoassay for the detection of AFP in the sera of rats was based on the coprecipitation inhibition technique using the double antibody method in order to separate bound ^{125}I-AFP from free AFP. Highly purified AFP of a molecular weight of 69,000 was employed as the standard AFP and for the preparation of ^{125}I labeled AFP and monospecific AFP-antiserum. Favorable conditions for assay were investigated and are given as follows.

Labeled AFP was prepared by the method of Hunter and Grenwood. Namely, 20 μg of purified AFP was radioiodinated with I mCi of ^{125}I and then ^{125}I-AFP was separated from residual unreacted ^{125}I by applying on a Sephadex G-75 column. ^{125}I-AFP with a radioactivity of 10 to 20 mCi/mg of AFP was obtained. The assay system is described as follows. The

reaction mixture of 0.1 ml of sample, 0.1 ml of a 1 : 4,000 dilution of AFP-antiserum and 0.5 ml of diluent (pH 8.6, 0.05 M phosphate buffered saline containing 1% BSA) was preincubated at 4°C for 12 hr, after which 0.1 ml of ^{125}I–AFP (10,000 cpm) was added and incubated for another 36 hr. At the end of these periods, 0.1 ml of a 1 : 100 dilution of normal rabbit serum and 0.1 ml of a 1 : 10 dilution of anti-rabbit γ-globulin goat antiserum were added and further incubated for 24 hr. After incubation, centrifugation at 3,000 rpm for 30 min was carried out and the ^{125}I–AFP content of the precipitate was counted on a gamma counter.

The sensitivity of this assay allowed reproducible detection of 8 ng of AFP/ml of the serum. The standard curve obtained on serial dilution of AFP-positive serum produced a dose response line parallel to that of a standard curve prepared employing AFP standards. This assay was found to possess satisfactory specificity for AFP in rat serum.

Change of serum AFP levels in carbon tetrachloride induced hepatitis was studied.

A single dose of 0.1 ml of carbon tetrachloride per 100 g body weight was injected intraperitoneally into male Wistar rats of a body weight of 250–350 g. On day 1, 1.5, 2, 3, 4, 5 and 6 after the drug administration, blood samples were collected under anesthesia, to measure AFP and GOT in serum and thereafter the animals were sacrificed for correlative studies of liver histology with change of AFP levels in the sera. The sections

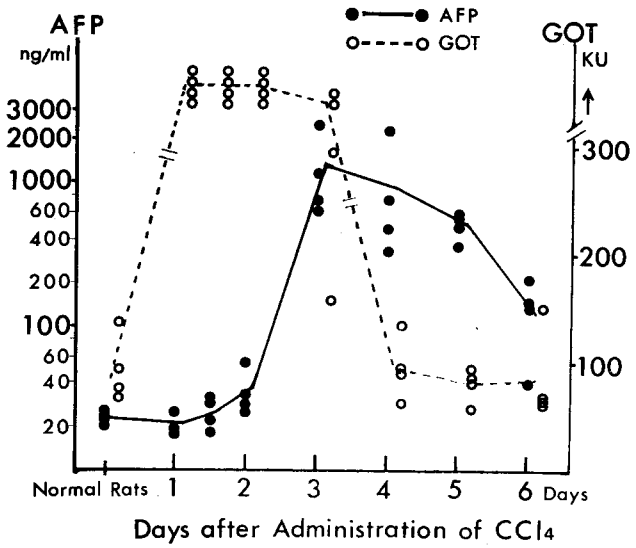

Fig. 1. Changes of serum-AFP and serum-GOT levels in CCl_4 induced rat hepatitis.

of liver were stained with hematoxylin and eosin. Changes of AFP and GOT levels in the sera are shown in Fig. 1. The AFP in the sera of the normal control rats was commonly detected at the levels of 18 to 24 ng/ml of serum, while the presence of AFP in the sera of normal human subjects could not even be demonstrated by use of radioimmunoassay for the detection of human AFP. As shown in Fig. 1, a close relationship between AFP and transaminase activty (GOT) levels was found. The changes in both parameters in rat with CCl_4 induced hepatitis followed exactly those of patients with viral hepatitis. A small increase of AFP levels was found on day 1.5 and 2 (AFP value, 25 and 35 ng/ml on an average, respectively) of CCl_4 induced hepatitis, while GOT levels revealed markedly high values. The specimen of liver on day 1 and 1.5 showed a typical zonal central necrosis of liver cells around the vein. The AFP levels on day 3 at the beginning of the GOT decreasing phase rose to maximum values (1,215 ng/ml on an average) simultaneously with the appearance of the marked zonal regenerating liver cells around the central vein from low levels on day 2. Thereafter, the AFP levels fell gradually to a relatively low value (731 ng/ml at day 4, 498 ng/ml at day 5 and 138 ng/ml at day 6 on an average, respectively) as the GOT levels reduced. These regenerating liver cells had already disappeared from the specimen of liver on day 6, while AFP levels maintained relatively high values.

From the above results, a close relationship between the synthesis of AFP and regenerating liver cells in CCl_4 induced hepatitis was suggested. However, the present authors do not know whether the so-called regenerating liver cells in CCl_4 induced hepatitis arise from the division of the parent cells, or have their origin in cells in the liver tissue other than liver cells.

REFERENCES

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