PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

WJG, 2000 February; 6(1):138-139
World Journal of Gastroenterology
 ISSN 1007-9327

Expression of IGF-II, p53, p21 and HBxAg in precancerous events of hepatocarcinogenesis induced by AFB1 and/or HBV in tree shrews

Qin LL, Su JJ, Li Y, Yang C, Ban KC and **Yian RQ**

Subject heading liver neoplasms; carcinoma, hepatocellular; hepatitis B virus; IGF-II; p53 gene; p21 gene; HBxAg; aflatoxin B1

INTRODUCTION

In order to study the relationship between oncogene expression and HCC generation, we observed the precancerous hepatic GGT foci, IGF-II, p53 and p21 expression during hepatocarcinogenesis of tree shrew induced by hepatitis B virus (HBV)and/or aflatoxin B1 (AFB1).

MATERIALS AND METHODS

Materials

One hundred and twenty tree shrews (Tupaia Belangeri Chinese) were purchased from Yunnan Province of China. Their body weight was 100g-159g.

HBV infected serum was got from the patients with hepatitis B, positive for HBsAg and HBeAg. Monoclonal antibodies (MoAb) to HBsAg, *p*21 (MAB-0143), *p*53 D0.7 (MAB-0142) and S-P Kit were purchased from Maxim Technic Co. Anti-HBxAg MoAb was presented by Professor Wu GH in Liver Disease Institute, General Hosp ital of PLA Beijing Military Area Command. IGF-II (Lot 12850) RbAb was from Ups tate Co. HBV DNA-Bio probe and *in situ* hybridization kit were provided by Liver Disease Institute of Beijing Medical University.

Methods

HBV infection marks were negative in serum of the animals before inoculation. Liver biopsy (LB) was taken from each animal which was negative for HBV-infected marks in serum before inoculation. It was used as self-control. Each of the 80 tree shrews was inoculated with HBV-infected serum, injected through the femoral vein. Blood was taken from the animals were from wk2 to wk8 after inoculation. The animals positive for HBsAg, HBeAg and anti-HBcAg were divided randomly into group A and group B. The remaining 40 animals that did not receive inoculation of HBV were divided into group C and group D. Group A was HBV positive and fed with AFB1; group B was HBV infected; group C was fed with AFB1 and group D was as control. AFB1 mixed in the milk was freely lapped by animals, 6 d/w at a dose of 150 $\mu g \cdot kg^{1} \cdot d^{1}$. LB was taken from all animals anaesthetized by ketamini. The size of the tissue s was about 0.8 cm×0.4 cm×0.4 cm, and they were fixed immediately. LB was taken repeatedly in every other 15wk after fed with AFB1.

HBsAg, HBxAg, IGF-II, *p*53 and *p*21 were detected by immunohistochemistry. Microwave citrate buffer strengthened HBxAg and *p*53. After testing suitable concentration of antibodies, slides were stained according to the S-P kitins truction. Positive and negative controls were set in each test to differentiate false reaction. GGT was stained by the method of Rutenberg. *In situ* hybridization of HBV- DNA was made according to the kit information.

The data was statistically evaluated by using *t* and χ^2 tests.

RESULTS

HCC was not found after 75 wk of observation. The average amount of AFB1 taken by each animal in groups A and C was 9.9 mg and 10.4 mg respectively ($P>0.05$).

The number of GGT positive foci was larger in group A than in groups B and C ($P<0.05$). GGT positive foci in groups A, B and C were more and bigger than in group D ($P<0.01$) (Table 1).

Table 1 GGT positive foci at wk75

Group	HBV	AFB1	Animal No	GGT positive foci ($\bar{x}\pm s$) No/cm^2	mm^2/cm^2	mm^2/focus
A	+	+	15	143.3±96.4	7.12±7.50	0.04±0.03
B	+	-	14	71.1±72.0	1.90±2.09	0.03±0.01
C	-	+	16	72.9±70.4	2.22±2.42	0.23±0.01
D	-	-	10	19.4±19.9	0.35±0.36	0.01±0.01

Liu Liang Qin[1], Jian Jia Su[1], Yuan Li[1], Chun Yang[1], Ke Chen Ban[1] and Rue Qi Yian[2]
[1]Department of Pathology, Guangxi Cancer Institute, Nanning 530021 , China
[2]Department of Pathology, Cancer Institute, Sun Yat Sen University of Medical Sciences, Guangzhou, 510060, China
Dr. Liu Liang Qin, female, born on 1963-10-21 in Liuzhou, Guangxi, gr aduated from Guangxi Medical University with a master degree in 1996, research fellow in pathology, having 6 papers published.
Supported by the National Natural Science Foundation of China, No. 3926 0033.
Correspondence to: Liu Liang Qin, Department of Pathology, Guangxi Cancer Institute, Nanning 530021, P.R. China
Tel. +86-771-5313022 Ext.3021
Received 1999-01-03 **Accepted** 1999-03-25

The HBsAg positive rates were 86.7% and 95.7%, and HBxAg rates were 92.9% and 82.6% respectively in groups A and B (Table 2).

Table 2 HBsAg, HBxAg and HBV DNA in group A and B

Group	HbsAg		HBxAg		HBV DNA	
	Animal *n*	Positive *n* (%)	Animal *n*	Positive *n* (%)	Animal *n*	Positive *n* (%)
A	15	13(86.7)	14	13(92.9)	10	7(70.0)
B	23	22(95.7)	23	19(82.6)	16	12(75.0)
Total	38	35(92.1)	37	32(86.5)	26	19(73.1)

At wk 15, IGF-II positive liver cells in brown color were dispersely distributed in plasma. At wk 75, however, the positive cells were found to be near the margin of the proliferation foci of liver cells. The positive rate was quite different between wk 45 and wk 75($P<0.05$, Table 3). Nuclear p53 was not found at wk75. p21 positive rate was about 5% in groups A, B and C, with small brown grains in the plasma of liver cells.

Table 3 IGF-II at wk 45 and wk 75

Group	wk 45		wk 75	
	Animal *n*	Positive *n*(%)	Animal *n*	Positive *n*(%)
A	15	13(86.7)	15	5(33.3)
B	23	17(73.9)	20	13(65.0)
C	18	18(100.0)	17	11(64.7)
D	11	6(54.5)	11	4(36.4)

DISCUSSION

Hepatocarcinogenesis was found only in the precancerous phase of the tree shrews. The reason for the unequal numbers among the tests was that the LB tissue was too small to detect the marks.

Both HBsAg and HBxAg were found in the liver of tree shrew by immunohistochemistry. HBV-DNA was detected by *in situ* hybridization. These results showed that the tree shrew model for HBV infection was reliable.

GGT positive foci did not form in the early stage of the experiment. Small and irregular GGT positive foci were found at wk 75. There were more GGT foci in group A (treated by HBV and AFB1) than in groups B and C (treated by HBV or AFB 1), indicating the synergistic effect of HBV and AFB1 in hepatocarcinogenesis, which was also reported by other researchers. Group B (infected with HBV) had more GGT positive foci than group D (control), indicating that the effect of HBxAg trans-activation could induce precancerous lesions in the liver of tree shrew.

IGF-II was over expressed in human liver cancer and its surrounding tissues. Us ually the expression of cancer-surrounding tissues is higher and is almost the same as that in fetal liver. In normal liver tissue, however, IGF-II was negative[1,2]. IGF-II has high expression rate at wk 45, but significantly low at wk 75. In the HCC tissue of tree shrew, IGF-II was 100% positive[3]. This revealed that IGF-II expression did not persist in a high level during hepatocarcinogenesis of tree shrew. IGF-II in group D probably caused by LB might repeatedly activate the expression.

Over expression of oncogenes and anti-oncogenes was found in AFB1 and HBV induced liver cancer. Many studies showed p21 in the early stage of carcinogenesis, and it was related with liver cell transformation. High rate of p21 was not observed. The expression of p21 in the later stage of the exper iment is to be studied. p53 was not found in the precancerous tissues, which is in agreement with that reported in the literature[4].

REFERENCES

1 Yang DH, Liu WW, Gu JR, Wan DF, Liu SL. Localization and expression of IGF-II, IGF-II receptor and CSF-1 receptor/c-fms oncogene products in hepatocellular carcinoma and noncancerous liver tissues. *Zhonghua Xiaohua Zazhi*,1993;13:189-192

2 Yang D, Rogler CE. Analysis of insulin-like growth factor II (IGF-II) expression in neoplastic nodules and hepatocellular carcinomas of woodchucks utilizing in situ hybridization and immunocytochemistry.*Carcinogenesis*,1991;12:1893-1901

3 Su JJ, Qin GZ, Yang C, Yan RQ, Lotlikar PD. Expressions of IGF-II, HBxAg and ras gene in the experimental hepatocellular carcinomas in tree shrews.*Guangxi Kexue*,1997;4:309-311

4 Tsuda H, Sakamoto M, Hirohashi S. Different mutation of p53 gene in nodule in nodule hepatocellular carcinoma as an evidence for multistage progression.*Cancer Lett*,1994;83:197-200

Edited by Wang XL