Character of HBV (hepatitis B virus) polymerase gene rtM204V/I and rtL180M mutation in patients with lamivudine resistance

LI Min-wei (李敏伟)[†], HOU Wei (侯伟), WO Jian-er (沃健儿)[‡], LIU Ke-zhou (刘克洲)

(Institute of Infectious Diseases, First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China)

[†]E-mail: limw@zju.edu.cn

Received Nov. 12, 2004; revision accepted Mar. 11, 2005

Abstract: Objectives: To investigate the relationship between HBV (hepatitis B virus) polymerase gene 180 and 204 sites mutation and lamivudine resistance. Methods: One hundred forty-one patients with lamivudine resistance after lamivudine treatment and 60 chronic hepatitis B patients without lamivudine treatment were enrolled in this study. The serum HBV DNA mutation was analyzed by sequence detection via polymerase chain reaction (PCR). The sequences of the same patient were analyzed before and after lamivudine treatment. Results: One hundred and nine lamivudine resistance patients had HBV YMDD (tyrosine-methionine-aspartate-aspartate) mutation. Among them, 45 patients had rtL180M/M204V mutation (41.28%), 28 patients had rtL180M/M204I mutation (25.70%) and 36 patients had rtM204I mutation (33.02%). There were 6 patients with rtL180M mutation in 32 lamivudine resistance patients. Sixty chronic hepatitis patients without lamivudine treatment had no mutations. Conclusions: HBV mutations, which play an important role in lamivudine resistance usually locate at polymerase gene 204 site; 180 site mutation was also observed in these patients. Evaluation of the anti-virus therapy by surveillance of the two sites mutations is of importance.

Key words: Hepatitis B virus, Lamivudine, YMDD mutant, Sequence analysis

doi:10.1631/jzus.2005.B0664

Document code: A

CLC number: R512.6²

INTRODUCTION

Hepatitis B virus (HBV) infection is a major health problem leading to around one million deaths annually worldwide (Lee, 1997). A wide range of clinical manifestations has been established for chronic hepatitis B virus infection, from asymptomatic carriers to severe chronic liver disease, including those with cirrhosis and hepatocellular carcinoma (Chen, 1993; Chu and Liaw, 1997). Lamivudine, an oral nucleoside analogue, inhibits HBV replication (Lai *et al.*, 1997; 1998) and can markedly reduce serum HBV DNA levels and normalise alanine aminotransferase (ALT) levels associated with improvement in liver necroinflammatory activity (Lai *et al.*, 1998), but the greatest drawback with lamivudine treatment is the emergence of drug-resistant HBV mutants, the mutation of the ty-

rosine-methionine-aspartate-aspartate (YMDD) motif in the C domain of the HBV DNA polymerase gene (Lai *et al.*, 1998; Dienstag *et al.*, 1999). The serum HBV DNA mutation of 141 patients with lamivudine resistance and 60 chronic hepatitis B patients without lamivudine treatment was analyzed by sequence detection via polymerase chain reaction (PCR) in order to investigate the relationship between HBV polymerase gene 180 and 204 sites mutation and lamivudine resistance.

MATERIALS AND METHODS

Patients

Two-hundred and one subjects were inpatients and outpatients in our hospital from June 2002 to June 2003. They were 135 men and 66 women, aged 18–53 years. Among them were 141 patients with lamivudine resistance after lamivudine treatment (100

[†]Corresponding author

mg/d) with mean treatment period of one year and a half; and 60 chronic hepatitis B patients without lamivudine treatment. None of the patients was ever treated with interferons and other anti-viral drugs.

Design and synthesis of primers

Upstream primer: 5' CTCCAATCACTCACCA AC 3'; downstream primer: 5' GGGTTTAAATGT ATACCCA 3'; sequencing primer: 5' GTAATTCCC ATCCC 3'. All the primers above were synthesized by Sangon Company, Shanghai (China).

PCR

The amplification reaction contained 1 µl each of 25 µmol/L specific primers, 1 µl 10 mmol/L dNTP mixture (dATP, dGTP, dCTP, dTTP), 4 µl 25 mmol MgCl₂, 2.5 U *Taq* DNA polymerase (Promega) and 5 µl 10×PCR buffer solution. The total volume was brought to 50 µl using ddH₂O. The PCR amplifications were performed in a PTC-200 peltier thermal cycler (MJ Research, USA) under the following conditions: After an initial denaturation for 5 min at 94 °C, samples were subjected to 35 cycles of amplification (94 °C 45 s, 55 °C 45 s, 72 °C 1 min), followed by a final extension of 5 min at 72 °C. The purification of PCR products was performed by QIAquick PCR purification kit according to manufacture's instructions (QIAGEN, USA).

Sequence analysis

Sequence analysis of the PCR products was performed by DYEnamicTM ET dye terminator cycle sequencing Kit (AmershamBioscience) in a MegaBACETM 500 according to manufacture's instructions; sequence analysis software was used to analyze the results.

RESULTS

Types of HBV polymerase gene 180 and 204 sites mutation

There were 109 lamivudine resistance patients who had HBV YMDD mutation and 141 patients with lamivudine resistance. Among them, 45 patients (45/109; 41.28%) had rtL180M/M204V mutation that played the most important role in YMDD mutation. In addition, 28 patients (28/109; 25.70%) had rtL180M/

M204I mutation and 36 patients (36/109; 33.02%) had rtM204I mutation. There were 6 patients with rtL180M mutation in the other 32 lamivudine resistance patients; 60 chronic hepatitis patients without lamivudine treatment had no mutations (Table 1).

Table 1 Types of HBV polymerase gene 180 and 204 sites mutation

Group	Type	<i>n</i>
Patients with lamivudine treatment	rtL180M/M204V	45
	rtL180M/M204I	28
	rtM204I	36
	rtL180M	6
	No classification	26
Patients without lamivudine treatment	No mutation	60

Combinatorial mutation rate of M204V/I

All 45 patients with rtM204V mutation also had rtL180M mutation; the rate of this kind of combinatorial mutation was 100%. Among 64 patients with rtM204I mutation, only 28 patients also had rtL180M mutation; the occurrence rate of this kind of combinatorial mutation was 43.75%.

Combinatorial mutation rate of rtL180M

Among 79 patients with rtL180M mutation were 45 patients who had rtM204V mutation, 28 patients who had rtM204I mutation, the combinatorial mutation rate was 56.96% and 35.44%, respectively. In addition, there were only 6 patients who had rtL180M mutation without rtM204V mutation or rtM204I.

Relationship between HBV polymerase gene mutation and lamivudine treatment

Among 141 patients with lamivudine resistance, 115 patients had polymerase gene mutation, either at 204 site or at 108 site. The mutation rate was 81.56%. While in the other group, 60 chronic hepatitis patients without lamivudine treatment did not have any mutations.

DISCUSSION AND CONCLUSION

Chronic hepatitis B virus (HBV) can be treated with a nucleoside analogue, lamivudine (2',3'-dideoxy-

3'-thiacytidine). In the short term, substantial inhibition of HBV replication can be achieved. However, resistance to lamivudine emerges in approximately 14% of patients after 1 year, rising to 43% after 3 years. Resistance is associated with mutations in the highly conserved tyrosine-methionine-aspartate-aspartate (YMDD) motif (codons 203–206 of the reverse transcriptase (rt)), which is part of the catalytic site of the HBV polymerase (Allen *et al.*, 1998; Chayama *et al.*, 1998; Gutfreund *et al.*, 2000; Kirishima *et al.*, 2002; Kobayashi *et al.*, 2001; Leung, 2002; Yeh *et al.*, 2000). Three types of mutations are observed in the polymerase gene. The consensus rt domain numbering system described in Stuyver *et al.* (2001) has been used to describe the variants. There is the M204V associated with L180M (Group I), the M204I alone (Group II) or the M204I with L180M (Group III) (Pillay *et al.*, 1998). The L180M and M204V mutations act synergistically to increase resistance to lamivudine (Allen *et al.*, 1998).

One hundred and forty-one patients with lamivudine resistance and 60 chronic hepatitis B patients without lamivudine treatment were studied in this work in the Hangzhou area. The serum HBV DNA mutation was analyzed by sequence detection via polymerase chain reaction (PCR). Our results indicated 109 lamivudine resistance patients had HBV YMDD mutation. There were three main types of mutation: rtL180M/M204V, rtM204I and rtL180M/M204I. Among them, 45 patients had rtL180M/M204V mutation (41.3%), which played the most important role in YMDD mutation. In addition, 28 patients had rtL180M/M204I mutation (25.7%) and 36 patients had rtM204I mutation (33.0%).

Our results indicated that rtM204V mutation is always combined with rtL180M; the occurrence rate of this kind of combinatorial mutation was 100%; while rtM204I mutation could exist alone. These results accorded with those of other research groups' (Pillay *et al.*, 1998; Allen *et al.*, 1998).

Our study revealed that there were 6 patients with rtL180M mutation among the 32 lamivudine resistance patients. This kind of mutation was novel, and requires further study to explore its clinical significance.

Though in this study, 60 chronic hepatitis patients without lamivudine treatment had no mutations, it had been reported that patients without lamivudine

treatment could also have mutations; we considered that these contradictory findings had something to do with the fewer samples.

In conclusion, HBV mutations, which play an important role in lamivudine resistance, usually locate at polymerase gene 204 site; 180 site mutation was also observed in the patients. It is of importance to evaluate anti-virus therapy by surveillance of the two sites mutations.

References

- Allen, M.I., Deslauriers, M., Andrews, C.W., Tipples, G.A., Walters, K.A., Tyrell, D.L., Brown, N., Condeay, L.D., 1998. Identification and characterization of mutations in hepatitis B virus resistant to lamivudine. *Hepatology*, **27**(6):1670-1677.
- Chayama, K., Suzuki, Y., Kobayashi, M., Tsubota, A., Hashimoto, M., Miyano, Y., Koike, H., 1998. Emergence and takeover of YMDD motif mutant hepatitis B virus during long-term lamivudine therapy and re-takeover by wild-type after cessation of therapy. *Hepatology*, **27**(6):1711-1716.
- Chen, D.S., 1993. From hepatitis to hepatoma: Lessons from type B viral hepatitis. *Science*, **262**(5132):369-370.
- Chu, C.M., Liaw, Y.F., 1997. Natural history of chronic hepatitis B virus infection: An immunopathological study. *J Gastroenterol Hepatol*, **12**(9-10):S218-S222.
- Dienstag, J.L., Schiff, E.R., Wright, T.L., Perrillo, R.P., Hann, H.W., Goodman, Z., 1999. Lamivudine as initial treatment for chronic hepatitis B in the United States. *N Engl J Med*, **341**(17):1256-1263.
- Gutfreund, K.S., Williams, M., George, R., Bain, V.G., Ma, M.M., Yoshida, E.M., 2000. Genotypic succession of mutations of the hepatitis B virus polymerase associated with lamivudine resistance. *J Hepatol*, **33**(3):469-475.
- Kirishima, T., Okanoue, T., Daimon, Y., Itoh, Y., Nakamura, H., Morita, A., 2002. Detection of YMDD mutant using a novel sensitive method in chronic liver disease type B patients before and during lamivudine treatment. *J Hepatol*, **37**(2):259-265.
- Kobayashi, S., Ide, T., Sata, M., 2001. Detection of YMDD motif mutations in some lamivudine-untreated asymptomatic hepatitis B virus carriers. *J Hepatol*, **34**(4):584-586.
- Lai, C.L., Ching, C.K., Tung, A.K.M., Li, E., Young, J., Hill, A., 1997. Lamivudine is effective in suppressing hepatitis B virus DNA in Chinese hepatitis B surface antigen carriers: A placebo-controlled trial. *Hepatology*, **25**(1):241-244.
- Lai, C.L., Chien, R.N., Leung, N.W., Chang, T.T., Guan, R., Tai, D.I., 1998. A one-year trial of lamivudine for chronic hepatitis B. *N Engl J Med*, **339**(2):61-68.
- Lee, W.M., 1997. Hepatitis B virus infection. *N Engl J Med*, **337**(24):1733-1745.

- Leung, N., 2002. Treatment of chronic hepatitis B: Case selection and duration of therapy. *J Gastroenterol Hepatol*, **17**(4):409-414.
- Pillay, D., Bartholomeusz, A., Cane, P.A., Mutimer, D., Schinazi, R.F., Locarnini, S.A., 1998. Mutations in the hepatitis B virus DNA polymerase associated with antiviral resistance. *Int Antiviral News*, **6**:167-169.
- Stuyver, L.J., Locarnini, S.A., Lok, A., Richmann, D.D., Carman, W.F., Dienstag, J.L., 2001. Nomenclature for antiviral-resistant human hepatitis B virus mutations in the polymerase region. *Hepatology*, **33**(3):751-757.
- Yeh, C.T., Chien, R.N., Chu, C.M., Liaw, Y.F., 2000. Clearance of the original hepatitis B virus YMDD-motif mutants with emergence of distinct lamivudine-resistant mutants during prolonged lamivudine therapy. *Hepatology*, **31**(6):1318-1326.

Welcome Contributions to JZUS-B

1. Introducing a New One---Journal of Zhejiang University SCIENCE B (Biomedicine and Biotechnology), ISSN 1673-1581, Monthly

- JZUS-B is indexed & abstracted by the famous database MEDLINE, and will also be accepted by PubMed Central (PMC) which is a new core full text-online database of MEDLINE in Life Science Periodicals and today, is among 189 Biology and Science journals accepted by PMC. JZUS-B will be the only one from China.
- JZUS-B has linked its website (<http://www.zju.edu.cn/jzus>) to:
MEDLINE: <http://www.ncbi.nlm.nih.gov/PubMed>;
CrossRef: <http://www.crossref.org>; (doi:10.1631/jzus.xxxx.Bxxxx), etc.
 Therefore, your latest research would be rapidly spread on-line and read by researchers in biology and medicine area in the world.
- JZUS-B has an International Editorial Board, International Peer Review System, International Author-Pool and International network, all of which are highly appraised by MEDLINE, Ei and SCI, etc.

2. Welcome Your Contributions to JZUS-B

JZUS-B warmly and sincerely welcome scientists all over the world to contribute to JZUS-B in the form of Review, Article and Science Letters focused on biomedicine and biotechnology areas. Especially, Science Letters (3–4 pages) would be published as soon as about 30 days (Note: detailed research articles can still be published in the professional journals in the future after Science Letters are published by JZUS-B).

3. Contributions requests

- (1) Electronic manuscript should be sent to **jzus@zju.edu.cn** only. If you have any question, please feel free to visit our website: <http://www.zju.edu.cn/jzus>, and hit "For Authors".
- (2) English abstract should include Objective, Method, Result and Conclusion.
- (3) Tables and figures could be used to prove your research result.
- (4) Full text of the Science Letters should be in 3–4 pages. The length of articles and reviews are not limited.
- (5) Please visit our website (<http://www.zju.edu.cn/jzus/pformat.htm>) to see paper format.