

Letters to the Editor

Changes in the Antigenicity of a Hepatitis B Virus Mutant Stemming from Lamivudine Therapy

Lamivudine, the negative enantiomer of 2'-dideoxy-3'-thiacytidine, is a reverse transcriptase inhibitor for both human immunodeficiency virus (HIV) and human hepatitis B virus (HBV) (4). Its application has so far resulted in a reduction of serum HBV DNA in chronic carriers and also improved liver functions (6).

Emergence of mutant strains with decreased sensitivity to lamivudine has been reported after prolonged treatment (5, 7, 9). Most of these mutations have been identified in the catalytic region of the HBV DNA polymerase, in particular Met552Ile or Met552Val in the conserved Tyr-Met-Asp-Asp (YMDD) motif (1). Although the entire coding region of the viral surface antigen (HBsAg) is contained in that of the HBV DNA polymerase, the YMDD motif (located in domain C) does not overlap the antigenic "a" determinant of HBsAg (positioned in the variable linker region between domains A and B of the polymerase) (3). While altered antigenicities have been shown for mutations in the "a" determinant (amino acid residues 124 to 147) (2), it is conceivable that concurrent amino acid substitutions in HBsAg from lamivudine-related mutations in the YMDD motif should not lead to changes in the antigenicities of the mutants (8).

We have recently reported an HBV strain related to lamivudine treatment, which carried an unusual amino acid substitution in the conserved "a" determinant of HBsAg (Gly130Asp) independently of the YIDD mutation detected in the same virus (9). This virus also carried a pretreatment Gly145Arg HBsAg mutation. To investigate the changes in the antigenicity of this HBV mutant, three mutant genomes (Gly130Asp, Gly145Arg, and Gly130Asp/Gly145Arg) were generated by site-directed mutagenesis (QuickChange kit; Stratagene). The wild-type HBV genome, used as the template for the mutagenesis, was cloned into the mammalian expression vector pcDNA 3.1 (Invitrogen) in a replication-competent form (10). The mutant constructs and the wild type were then transiently transfected separately into mammalian HepG2 cells (Lipofectin reagent; GibcoBRL), and their antigenicities were measured by commercial antibody-based kits (Auszyme and Ausria; Abbott Laboratories). In addition, the transfection efficiency was measured by the luciferase activity (Turner Designs Instrument) expressed from the cotransfected plasmid pRL-CMV (Promega) carrying the firefly luciferase reporter gene. Cultures of cells transfected with similar efficiencies were selected for the measurement of antigenicity. The liquid hybridization assays (Genostics; Ab-

bott Laboratories) were chosen to quantitate the amount of HBV DNA in the culture of transfected HepG2 cells. Culture supernatants with comparable amounts of HBV DNA (picograms per milliliter) were assayed for the reactivities of a particular HBsAg to Auszyme and Ausria kits. Changes in antigenicity were represented by percent reactivity of a particular HBsAg mutant compared with the wild type. Our results demonstrated similar magnitudes of antigenic changes in single and double mutants compared with the wild type (Table 1).

Our findings therefore reveal antigenic changes in HBV mutants that emerged from lamivudine treatment. The significance of the observed altered antigenicity of the Gly130Asp mutant is further supported by its recent detection in a vaccinated Singapore infant and in several other apparently healthy individuals who were HBsAg negative but anti-HBc immunoglobulin G positive (unpublished observation). Closer monitoring would therefore be required for this new class of lamivudine-associated mutants, as they are less sensitive to lamivudine and also may escape vaccination or detection due to changes in their viral antigenicities.

REFERENCES

1. Bartholomew, M. M., R. W. Jansen, L. J. Jeffers, K. R. Reddy, L. C. Johnson, H. Bunzendahl, L. D. Condreay, A. G. Tzakis, E. R. Schiff, and N. A. Brown. 1997. Hepatitis-B-virus resistance to lamivudine given for recurrent infection after orthotopic liver transplantation. *Lancet* **349**:20-22.
2. Carman, W. F., A. R. Zanetti, P. Karayiannis, J. Waters, G. Manziolo, E. Tanzi, A. J. Zuckerman, and H. C. Thomas. 1990. Vaccine-induced escape mutant of hepatitis B virus. *Lancet* **336**:325-329.
3. Chen, W. N., and C. J. Oon. 1999. Human hepatitis B virus mutants: significance of molecular changes. *FEBS Lett.* **453**:237-242.
4. Doong, S.-L., C.-H. Tsai, R. F. Schinazi, D. C. Liotta, and Y.-C. Cheng. 1991. Inhibition of the replication of hepatitis B virus in vitro by 2',3'-dideoxy-3'-thiacytidine and related analogues. *Proc. Natl. Acad. Sci. USA* **88**:8495-8499.
5. Gauthier, J., E. Bourne, M. W. Lutz, L. M. Crowther, J. L. Dienstag, N. A. Brown, and L. D. Condreay. 1999. Quantitation of hepatitis B viraemia and emergence of YMDD variants in patients with chronic hepatitis B treated with lamivudine. *J. Infect. Dis.* **180**:1757-1762.
6. Lai, C.-L., R. N. Chien, N. W. Leung, T. T. Ching, R. Guan, D. I. Tai, K. Y. Ng, P. C. Wu, J. C. Dent, J. Barber, S. L. Stephenson, and D. F. Gray. 1998. A one-year trial of lamivudine for chronic hepatitis B. *N. Engl. J. Med.* **339**:61-68.
7. Ling, R., D. Mutimer, M. Ahmed, E. H. Boxall, E. Elias, G. M. Ducheiko, and T. J. Harrison. 1996. Selection of mutations in the hepatitis B virus polymerase during therapy of transplant recipients with lamivudine. *Hepatology* **24**:711-713.
8. Locarnini, S. 1998. Hepatitis B virus surface antigen and polymerase gene variants: potential virological and clinical significance. *Hepatology* **27**:294-297.
9. Oon, C. J., W. N. Chen, N. Lim, S. Koh, G. K. Lim, A. L. Leong, S. W. Teng, and G. S. Tan. 1999. Hepatitis B virus variants with lamivudine-related mutations in the DNA polymerase and the "a" epitope of the surface antigen are sensitive to ganciclovir. *Antivir. Res.* **41**:113-118.
10. Weiss, L., A. S. Kekule, U. Jakubowski, E. Burgelt, and P. H. Hofschneider. 1996. The HBV-producing cell line HepG2-4A5: a new in vitro system for studying the regulation of HBV replication and for screening anti-hepatitis B virus drugs. *Virology* **216**:214-218.

TABLE 1. Altered antigenicity in lamivudine-related HBV mutants

Mutation	Auszyme (monoclonal) (A_{492}) ^a	Ausria (polyclonal) (cpm) ^b	HBV DNA (pg/ml)	Antigenicity (%) ^c
None: wild type	1.760	1,650	3.00	100.00
Gly130Asp	0.008	130	2.30	1.90
Gly145Arg	0.005	195	2.30	0.40
Gly130Asp/Gly145Arg	0.037	120	2.80	1.80
None: nontransfected cell culture	0.000	130	0.00	0.00

^a Average for two independent transfections; cutoff was calculated to be 0.050.

^b Average for two independent transfections; cutoff was calculated to be 155.

^c Remaining reactivity to Auszyme or Ausria compared with the wild type, after normalization for HBV DNA amount and luciferase activity.

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