Cloning and *in vitro* antiviral susceptibility of clevudine-resistant mutants of hepatitis B virus isolated from chronic hepatitis B patients

Kyun-Hwan Kim\(^1\), Yong Kwang Park\(^1\), Won Hyeok Choe\(^2\), Chang Hong Lee\(^2\), Byung Kook Kim\(^2\), Soon Young Ko\(^2\), Eun Sook Cho\(^1\), Hyo Sun Choi\(^1\), Sung Hyun Ahn\(^1\), So Young Kwon\(^2\)*

\(^1\)Department of Pharmacology, Konkuk University School of Medicine, Seoul, Korea.  
\(^2\)Department of Internal Medicine, Konkuk University School of Medicine, Seoul, Korea.

**Backgrounds/Aims:** Clevudine (CLV) is a nucleoside analog with potent antiviral activity against chronic hepatitis B virus (HBV) infection. Viral resistance to CLV in patients receiving CLV therapy has not been fully identified. The aim of this study was to characterize CLV-resistant HBV in patients with viral breakthrough (BT) during long-term CLV therapy. **Methods:** The gene encoding HBV reverse transcriptase (RT) was analyzed from chronic hepatitis B patients with viral BT during CLV therapy. Sera collected from the patients at baseline and at the time of viral BT were studied. To characterize the mutations isolated from the patients, we subjected the mutant replicons to *in vitro* drug susceptibility assays. **Results:** Several conserved mutations were identified in RT domain during viral BT, with M204I being the most common. *In vitro* phenotypic analysis showed that mutation M204I was predominantly associated with CLV resistance, whereas L229V was a compensatory mutation for the impaired replication of the M204I mutant. A quadruple mutant (L129M+V173L+M204I+H337N) was identified that conferred greater replicative ability and strong resistance to both CLV and lamivudine. All of the CLV-resistant clones were lamivudine resistant. They were susceptible to adefovir, entecavir, and tenofovir except for one mutant clone. **Conclusions:** Mutation M204I in HBV RT plays a major role in CLV resistance and leads to viral BT during long-term CLV treatment. Several conserved mutations may have a compensatory role in replication. Drug susceptibility assays reveal that adefovir and tenofovir are the most effective compounds against CLV-resistant mutants. These data may provide additional therapeutic options for CLV-resistant patients.