Abstract. – We report a case series of three HBeAg positive and five HBeAg negative patients (7 males, mean age 50.6±14.6 years) with chronic HBV infection experiencing seroconversion after treatment with entecavir (0.5 mg/day or 1 mg/day), initiated in 2007. Overall, the mean time to HBsAg clearance was 9.4±4.5 months. Seroconversion occurred in all patients, after a mean time of 8.0±3.7 months. In HBeAg negative patients, mean time to HBsAg clearance and to seroconversion were 9.2±5.9 and 6.8±4.0 months, respectively. In HBeAg positive patients, mean time to HBsAg clearance and to seroconversion were 9.7±0.6 months and 10.0±2.6 months, respectively. In this case series, seroconversion was maintained and was observed both in HBeAg positive patients and in HBeAg negative patients. Therefore, it may be preliminarily suggested that treatment with entecavir could be associated to HBsAg seroconversion in a short period of time, in both HBeAg positive and HBeAg negative HBV patients.

Key Words: HBV infection, Entecavir, Seroconversion.

Introduction

Chronic hepatitis B virus (HBV) infection is a widespread condition affecting more than 350 million individuals worldwide1,2. These individuals have an increased risk of clinically important and potentially life-threatening hepatic sequelae, including cirrhosis and hepatocellular carcinoma1. The administration of an antiviral therapy plays a central role in the reduction of these complications3.

Short-term markers, such as alanine aminotransferase (ALT) levels and serum HBV DNA level, are currently used to monitor the efficacy in antiviral therapy3. In particular, hepatitis B e antigen (HBeAg) and s (surface) antigen (HBsAg) have a major importance in current clinical practice: the detection of these antigens in body fluids has emerged as a powerful predictive tool to evaluate the efficacy of antiviral therapy4. HBeAg can be detected after 6-12 weeks after exposure to viral particles4; the clearance of this antigen, generally associated with a reduction in viraemia and in ALT flares, is followed by the appearance of anti-HBe antibodies (HBeAb)4. HBsAg can be detected, with a high plasma concentration, early in during acute infection, on average 6-10 weeks after exposure to viral particles4; the clearance of this antigen, generally associated with a reduction in viraemia and in ALT flares, is followed by the appearance of anti-HBs antibodies (HBsAb)4. HBsAg can be detected, with a high plasma concentration, early in during acute infection, on average 6-10 weeks after exposure to viral particles4. The persistence of HBsAg for more than 6 months in body fluids, together with persistent or intermittent elevation of ALT levels and signs of chronic hepatitis B on liver biopsy, defines the evolution from acute hepatitis B to chronic HBV status4,5.

HBsAg clearance is now considered to be the primary goal of the antiviral treatment. This event may be followed by the seroconversion, i.e. the appearance of anti-HBs antibodies (HBsAb)4. Seroconversion indicates the recovery from the chronic HBV infection and is associated with a life-long immunity against HBV4. While the spontaneous seroconversion is sometimes report-
ed in HBeAg positive patients, this event is much less frequent in HBeAg negative subjects\(^6\). This finding may reflect the lower rates of sustained virological response in HBeAg negative subjects undergoing treatment with the anti-viral drugs and, possibly, some differences in immune features and duration of infection between the HBeAg negative and the HBeAg positive patients\(^6\).

Entecavir is a novel deoxyguanosine analogue, recently approved in U.S. and Europe for the treatment of HBV-infected patients\(^1\). We report here a case series of patients with chronic HBV infection experiencing the seroconversion after a treatment with entecavir. Of note, a full study may fail in identifying the occurrence of the seroconversion, since it is a relatively rare event especially in the HBeAg negative patients. Therefore, we combined single real-life experiences of different patients, collected among different clinical centers in Italy by experienced clinicians.

**Patients**

In total, eight patients affected by chronic HBV infection, defined as persistence of HBsAg for more than 6 months\(^5\) were included in this case series. They were treated in six different Italian hepatology centers. The observation started in September 2007 has been collected by one Author of this study (G. d’E.).

This prospective analysis complied with the Declaration of Helsinki and all patients gave written informed consent.

Baseline demographic and clinical characteristics for the patients included in this analysis are summarized in Table I. Most patients (n=7) were male; mean age of all patients was 50.6±14.6 years (range 19-70 years). Mean time from HBV diagnosis was 4.6±6.3 years (range 1-20 yrs). Detection of HBeAg in body fluids was performed via sandwich enzyme immunoassay (EIA). In total, HBeAg was identified in five patients.

Four patients had received lamivudine 100 mg/die (Zeffix, GlaxoSmithKline, Verona, Italy) prior to their inclusion in this case series analysis. Lamivudine was administered in combination with adefovir dipivoxil in one patient and with interferon in another patient. At the initiation of this case series, all patients started therapy with entecavir (Baraclude, Bristol-Myers Squibb, Rome, Italy) at a dose of 0.5 mg/day or 1.0 mg/day, either alone or in combination with pegylated-interferon alfa-2a: 180 μg/week (two subjects) [Pegasys, Roche, Monza, Italy]. The decision for the therapeutic switch from lamivudine was made, in most cases, because of the persistence of the symptoms of HBV infection.

The main evaluation parameters were: time to HBsAg clearance and time to seroconversion. HBs antigen and antibodies directed against the HBs antigen were detected via sandwich EIA. The results of both tests were analyzed with descriptive statistics, considering all patients and after stratification for the presence of HBeAg. Adverse events were also registered, and their potential correlation with the study drug was evaluated.

**Table I.** Baseline characteristics of the eight patients.

<table>
<thead>
<tr>
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<th>1</th>
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<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td>58</td>
<td>53</td>
<td>45</td>
<td>53</td>
<td>56</td>
<td>19</td>
<td>51</td>
<td>70</td>
</tr>
<tr>
<td><strong>Time from diagnosis (yrs)</strong></td>
<td>20</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td></td>
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<tr>
<td><strong>HBeAg status</strong></td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td><strong>HBV-DNA, cp/mL</strong></td>
<td>&gt;105</td>
<td>&gt;109</td>
<td>&lt;2000</td>
<td>&gt;107</td>
<td>&lt;2000</td>
<td>&gt;107</td>
<td>&gt;106</td>
<td>&gt;106</td>
</tr>
<tr>
<td><strong>ALT, IU/mL</strong></td>
<td>75</td>
<td>186</td>
<td>112</td>
<td>144</td>
<td>42</td>
<td>NA</td>
<td>54</td>
<td>144</td>
</tr>
<tr>
<td><strong>Previous therapy</strong></td>
<td>None</td>
<td>None</td>
<td>Lamivudine</td>
<td>Lamivudine*</td>
<td>Lamivudine**</td>
<td>None</td>
<td>None</td>
<td>Lamivudine</td>
</tr>
<tr>
<td><strong>Entecavir dose, mg/day</strong></td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
<td>1(^{1})</td>
<td>1(^{1})</td>
<td>1</td>
</tr>
</tbody>
</table>

*Plus adefovir dipivoxil; \(^+\): plus interferon; \(^{1}\): plus pegylated-interferon alfa-2a. ALT: alanine transaminase; cp: copies; HBeAg: hepatitis B e antigen; NA: not available; Neg: negative; Pos: positive.
HBsAg clearance and seroconversion were observed in all patients after the initiation of the entecavir treatment. Table II reports time to HBsAg clearance and to seroconversion for each patient. Overall, the mean time to HBsAg clearance was 9.4±4.5 months (range: 1-15 months). Seroconversion occurred after a mean time of 8.0±3.7 months from the initiation of entecavir therapy.

The stratification of the results according to the presence of HBeAg revealed that, in HBeAg negative patients, mean time to HBsAg clearance was 9.2±5.9 months and mean time to seroconversion was 6.8±4.0 months. In HBeAg positive patients these times were longer with a mean time to HBsAg clearance of 9.7±0.6 months and mean time to seroconversion of 10.0±2.6 months.

Seroconversion was sustained: at the time of the drafting of this report (January 2009), HBsAb were still detectable in all patients.

Treatment with entecavir was well tolerated by all patients during the entire observation period. Only two patients reported adverse events judged as potentially related with study is drug. All side effects reported were of mild intensity. In particular, asthenia, fatigue and musculoskeletal pain were observed in one patient (patient 2); of note, HBsAg clearance and seroconversion were observed after only 1 month in this patient. Another subject (patient 6) experienced moderate flu-like syndrome and weight loss. The resolution of these symptoms occurred spontaneously, and no interruption of the entecavir treatment was required.

Discussion

The clearance of HBsAg and detection of antibodies directed against HBsAg, i.e. seroconversion, are now considered to be the most desirable clinical endpoints in patients with chronic HBV infection, but are rarely observed. The case series described here documents eight cases of HBV-infected patients experiencing HBsAg clearance and seroconversion in association with therapy with entecavir, in different real-life scenarios. Although the number of patients included in this analysis is limited, both HBeAg positive and HBeAg negative subjects were observed. Of note, while the spontaneous HBsAg seroconversion sometimes occurs in HBeAg positive patients, HBeAg negative patients seldom experience a spontaneous seroconversion.

Noteworthy seroconversion was observed both in HBeAg positive patients and in HBeAg negative patients treated with entecavir, alone or in combination with pegylated interferon alfa-2a, therefore suggesting a potential effect of entecavir in determining seroconversion, especially in HBeAg negative patients, in which the spontaneous seroconversion is a very rare event. Seroconversion occurred, in all patients, within one year from the initiation of the entecavir treatment and was sustained up to 22 months. In most cases, this event was observed between 6 and 8 months, with no apparent differences related to HBeAg status.

Four out of eight patients received lamivudine, alone or in combination with other anti-viral agents (adefovir dipivoxil or interferon alfa) before switching to entecavir. No differences in time to seroconversion were observed between subjects previously assuming an anti-viral regimen and in those naive to such therapies. Entecavir treatment was well tolerated, with only minor adverse events potentially related to this drug.

These observations must be considered with particular caution. In fact, the observational nature of this analysis does not allow inference of

### Table II. Time to HBsAg clearance and detection of HBsAb in body fluids (seroconversion) in the eight patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
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<th>7</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Time to HBsAg loss, months*</td>
<td>7</td>
<td>1</td>
<td>8</td>
<td>15</td>
<td>15</td>
<td>10</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Time to seroconversion, months*</td>
<td>7</td>
<td>1</td>
<td>8</td>
<td>12</td>
<td>6</td>
<td>11</td>
<td>7</td>
<td>12</td>
</tr>
</tbody>
</table>

*From initiation of entecavir treatment.
any definite any direct cause/effect relationship. Moreover, this case-series presents several confounding factors, such as a time from diagnosis differing from one patient to another, a large majority of males (only one woman was observed) and different ages. However, these limitations may reflect, at least in part, the clinical-practice setting considered in this case series.

The observations reported here further strengthen the efficacy of entecavir in the treatment of chronic HBV infection in both HBeAg positive and negative patients. Entecavir is a potent deoxyguanine nucleoside analogue, selectively inhibiting HBV replication, and is characterized by a high genetic barrier. Entecavir is currently the most potent anti-HBV treatment, suppressing viral replication in almost 90% of treatment-naïve patients after 96 weeks of therapy. Two large studies have demonstrated significantly better rates of histologic, virologic, and biochemical improvement when compared with lamivudine in both HBeAg positive and negative patients.1,2 The efficacy of entecavir is sustained, thus reducing the rate of long-term liver complications, with a favourable cost/efficacy ratio.

In conclusion, despite the limitations discussed, this case series could provide preliminary evidence that a treatment with entecavir may be associated with the HBsAg seroconversion, thus indicating a clinical recovery and a life-long immunity from the HBV infection, in a short period of time. This event was observed in both HBeAg positive subjects and HBeAg negative patients, who seldom experience spontaneous seroconversion.

Further clinical trials are warranted to confirm these intriguing observations.

References

5) LOK AS, McMAHON BJ. Chronic hepatitis B. Hepatology 2001; 34: 1225-1241.

Acknowledgements

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