ANTI-HEPATITIS B VIRUS ACTIVITIES OF PURINE DERIVATIVES OF OXETANOCIN A

Sir:

Hepatitis B virus (HBV) is a causative agent for hepatitis, but screening for anti-HBV agents has not been possible because no experimental animals or cell culture systems susceptible to its infection had been available. Recently, this problem was overcome by using transfection to establish a cell line, HB611, that continuously produces HBV-like particles. When this cell line was used to screen anti-HBV substances, β-interferon and adenine arabinoside (ara-A), which are clinically used for HBV infection, exhibited anti-HBV activity2).

Oxetanocin A (OXT-A), 9-(2-deoxy-2-hydroxymethyl-β-D-erythro-oxetanocyl)adenine, is a novel nucleoside isolated from a filtrate of the *Bacillus megaterium* culture medium3). This substance was shown to have antiviral activities against herpes simplex virus3) and human immunodeficiency virus4). OXT-A also inhibited HBV DNA replication in the HB611 cell system5). This paper reports on the anti-HBV activities of derivatives of oxetanocin such as 2-amino-OXT-A, OXT-G, OXT-H and OXT-X, which respectively have 2-amino-adenine, guanine, hypoxanthine, xanthine as the base moiety5).

HB611 cells were maintained in DMEM (Gibco) supplemented with 10% fetal bovine serum (General Scientific Laboratories), 100 µg/ml of streptomycin, 100 IU/ml of benzylpenicillin (Gibco) and 200 µg/ml of geneticin (Gibco) at 37°C in 5% CO₂ - 95% air.

The cells were seeded in 35-mm CORNING wells at a density of 1 × 10⁴ cells/well, using 1.2 ml of the medium. After 2 days of incubation, the medium was replaced with the same medium containing the test compound. The cells were incubated for a further 15 days, during which time the medium containing the drug was exchanged every 3 days. The cells were then harvested and cellular DNA was prepared5), and digested with restriction enzyme Hind III (Takara Shuzo Co., Ltd.). An aliquot (3 µg) was electrophoresed in 1.5% agarose gel, followed by blotting onto a nylon membrane GeneScreen Plus according to SOUTHERN6). The filter was hybridized to a random primed ³²P labeled HBV DNA probe, and washed twice with 2×standard saline citrate containing 1% SDS at 65°C for 30 minutes. It was then autoradiographed, and the results were analyzed using a densitometric analyzer (Shimadzu, Chromatoscana S930).

As can be seen from Fig. 1, because Hind III does not cleave the HBV DNA sequence7), the slow-migrating band (I) represents chromosomally integrated HBV DNA, and the fast-migrating bands (S, D1 and D2) are the extrachromosomal, replicative intermediates of HBV DNA. Among

Fig. 1. Southern blot analyses of Hind III digested cellular DNA of HB611 cells cultured in the presence of various drugs.

For drug and experimental protocol, see text.
these, S represents a single-stranded full-sized linear minus strand, D1 represents a partially double-stranded DNA consisting of the S and an incompletely synthesized plus strand, and D2 represents the D1 molecule in circular form, as is found in virions\(^1\). Our previous finding\(^2\) that OXT-A inhibits HBV DNA synthesis, as demonstrated by limited accumulation of the fast-migrating bands, was well supported. OXT-G and 2-amino-OXT-A seem to be much more potent inhibitors, as they are effective at much lower concentrations. These drugs affected the accumulation of viral replicative intermediates, leaving intact the cellular DNA synthesis that is represented by the component I. Judging from the effective concentrations, these two compounds may interfere with viral DNA replication without affecting cellular DNA replication in appropriate concentration ranges. Acyclovir and ara-A, used as controls, also inhibited viral DNA synthesis, but their effects were weaker than those of OXT-G and 2-amino-OXT-A.

To quantitatively evaluate the inhibitory activity of the compounds, we measured the band areas S, D1, D2 and I by densitometric analyzer, and calculated the inhibition percentage as follows:

\[
\text{Inhibition (\%)} = \left(1 - \frac{(S_{\text{drug}} + D1_{\text{drug}} + D2_{\text{drug}})/I_{\text{drug}}}{(S_{\text{control}} + D1_{\text{control}} + D2_{\text{control}})/I_{\text{control}}} \right) \times 100
\]

Anti-HBV activity ID\(_{50}\), expressed as the drug concentration required for 50\% inhibition of the viral DNA synthesis, was obtained by plotting the inhibition (\%) vs. drug concentration. The results are summarized in Table 1. Table 1 also includes minimum cytotoxic doses (MTD) of the drugs obtained by microscopic counting of viable cells just before extraction of cellular DNA. OXT-A and ara-A, which has been clinically used for viral hepatitis although its effect is incomplete\(^8\), behaved similarly with respect to the antiviral and cytotoxic activities. OXT-G and 2-amino-OXT-A showed antiviral effects 12 to 27 times as strong as that of ara-A and were less cytotoxic. Thus, these two compounds are better candidates for anti-HBV drugs. OXT-H and OXT-X showed no potent inhibitory effects on HBV DNA synthesis, as did acyclovir, a potent anti-herpes simplex virus drug\(^9\). Independent of this study, Shimada et al.\(^5\), examined the same set of oxetanocin derivatives, and found that OXT-A, OXT-G and 2-amino-OXT-A show anti-herpes simplex virus activity.

At present, the mechanism of inhibition of HBV DNA synthesis by OXT-G and 2-amino-OXT-A is not clear, although it is likely that they preferentially affect HBV-related reverse transcriptase\(^1\).

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Table 1. Anti-HBV activities of oxetanocin derivatives and some other compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MTD ((\mu)g/ml)</th>
<th>ID(_{50}) ((\mu)g/ml)</th>
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<tbody>
<tr>
<td>OXT-A</td>
<td>20.0</td>
<td>9.1</td>
</tr>
<tr>
<td>OXT-H</td>
<td>100.0</td>
<td>26.5</td>
</tr>
<tr>
<td>OXT-X</td>
<td>&gt;100.0</td>
<td>nd</td>
</tr>
<tr>
<td>OXT-G</td>
<td>100.0</td>
<td>0.72</td>
</tr>
<tr>
<td>2-Amino-OXT-A</td>
<td>50.0</td>
<td>0.32</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>&gt;50.0</td>
<td>47.0</td>
</tr>
<tr>
<td>ara-A</td>
<td>20.0</td>
<td>8.5</td>
</tr>
</tbody>
</table>

MTD represents the minimum cytotoxic dose. For details, see text. Antiviral activity is represented by ID\(_{50}\) on HBV DNA synthesis. These values were obtained by plotting the logarithm of the compound concentration vs. the inhibition percentage of the viral DNA synthesis in the treated cells (see text).

nd: Antiviral activity was not detected.

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References


