Experimental Transmission of Hepatitis B Virus by Semen and Saliva

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The ability of human semen and saliva to transmit hepatitis B virus (HBV) by parenteral and nonparenteral routes was studied. Semen, donated by a hepatitis B surface antigen (HBsAg)- and hepatitis B e antigen (HBeAg)-positive carrier, was administered to one gibbon by subcutaneous inoculation and to another by intravaginal instillation. Both developed HBsAg, followed by the development of antibody to HBsAg (anti-HBs) and antibody to hepatitis B core antigen (anti-HBc). Saliva from two donors who were HBsAg- and HBeAg-positive was pooled and administered subcutaneously to two gibbons and orally to five others. The animals inoculated subcutaneously developed HBsAg followed by anti-HBs, but none of the gibbons exposed orally developed evidence of HBV infection. Thus, semen and saliva of HBsAg carriers can be infectious, and venereal transmission of HBV by semen can occur. Transmission of HBV in saliva can also occur through breaks in the skin, but experimental transmission of HBV by saliva administered orally has not been accomplished.

Epidemiologic and serologic evidence has been accumulating that implicates secretions as vehicles of hepatitis B virus (HBV) transmission. Hepatitis B surface antigen (HBsAg) has been identified in the saliva [1-7], vaginal secretions [8], and semen [4, 9] of infected persons. A number of possible routes has been suggested through which transmission by secretions might occur. Venereal contact by vaginal or rectal intercourse and oral exchange of saliva by kissing or the common use of contaminated implements have been implicated [10-13], and HBV has been transmitted by a human bite [14]. In experimental studies using gibbons, transmissions occurred following parenteral, but not oral, administration of human saliva [5]. An attempt to infect a chimpanzee by the inoculation of HBsAg-positive human semen was inconclusive [9]. In this case, the animal died suddenly four weeks after the inoculation of the semen. Although HBsAg was first detected in the blood just prior to death, extensive autopsy showed no evidence of HBV infection, and death prevented the definitive proof of infection by later antibody development. There have been no reports of experimental transmission of HBV by the vaginal administration of semen. In this study we have employed both parenteral and transmucosal routes in an attempt to infect gibbons with HBsAg-positive semen and saliva.

Materials and Methods

Semen collection. Semen was donated by a 35-year-old American, a former dentist, who had been diagnosed two years previously as having...
Table 1. Analysis of samples of serum and saliva from carriers of hepatitis B e antigen (HBeAg) and hepatitis B surface antigen (HBsAg), subtype adw, used in the saliva pool.

<table>
<thead>
<tr>
<th>Donor</th>
<th>Serum</th>
<th>Saliva</th>
<th>Contribution to pool</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBeAg</td>
<td>HBsAg titer*</td>
<td>Occult blood reaction†</td>
</tr>
<tr>
<td>Female (FM317)</td>
<td>+</td>
<td>1:181</td>
<td>+</td>
</tr>
<tr>
<td>Male (SH)</td>
<td>+</td>
<td>1:128</td>
<td>+</td>
</tr>
</tbody>
</table>

* HBsAg titers were determined by CF. The titer for FM317 is the geometric mean CF titer over four samples.
† Occult blood reaction was determined by orthotolidine method.

acute hepatitis B [9]. The diagnosis was made following the development of HBsAg-positive hepatitis in two of his dental patients. Subsequently, two of his sexual partners developed acute icteric HBsAg-positive hepatitis. On initial diagnosis his serum alanine aminotransferase (ALT) level was 1,000 international units [IU]/liter; it later dropped to between 100 and 400 IU/liter. Liver biopsy specimens revealed chronic active hepatitis, positive for both HBsAg and hepatitis B core antigen (HBcAg) by fluorescence microscopy. Serologic studies were positive for HBsAg, subtype adw (HBsAg/adw), hepatitis B e antigen (HBeAg), and antibody to HBcAg (anti-HBc). At the time of our semen collection, about two years after diagnosis, the serum was still positive for HBsAg, HBeAg, and anti-HBc, and the serum ALT level was 119 IU/liter. Semen was collected and pooled in a sterile container and frozen at −20°C for storage.

Saliva collection. Whole mouth saliva, collected and prepared as described previously [5], was obtained from two Thai volunteers. The donors were selected to maximize the probability of infectious secretions. Although neither donor had an evaluation in the serum transaminases, each of them carried HBeAg and HBsAg/adw with a radioimmunoassay (RIA) titer of >1:1,000 in their serum, and both had detectable HBsAg in their saliva (table 1). Further, one had transmitted HBV to her offspring during the neonatal period. Saliva was stored at −70°C and pooled prior to use.

Gibbon management and serology. Gibbons (Hylobates lar) were housed in mosquito-proof rooms in individual galvanized iron cages designed to prevent contact between animals. The animals had individual supplies of food and water and were fed on commercial primate diet supplemented with fruits and vegetables. The animals were handled and examined by personnel who were negative for HBsAg and antibody to HBsAg (anti-HBs).

The day of exposure was designated as day 0. Feeding habits and rectal temperatures were recorded daily. Weekly examinations, following sedation with ketamine hydrochloride, were performed for evidence of jaundice, weight loss, hepatomegaly, or other abnormalities. Blood was obtained weekly for analysis of complete blood count, serum ALT, and HBV serology. Transaminase assays were performed using a colorimetric assay (Sigma Laboratories, St. Louis, Mo.), and HBsAg was detected by a commercially available RIA, Ausria II (Abbott Laboratories, North Chicago, Ill.). Results were expressed as the ratio of sample counts to the mean counts of six negative controls (P/N ratio), and a sample was considered positive when this ratio exceeded 2.1. All positive results were confirmed by duplicate tests, and specificity was established by neutralization with high-titered human anti-HBs [15]. HBsAg was titrated by RIA or CF [16], and HBsAg subtypes were identified using an immunodiffusion method [17]. For anti-HBs testing, we employed an RIA of commercial origin (Ausab®, Abbott Laboratories). HBeAg assays were performed by a modified double immunodiffusion technique in agarose gel [18]. Detection of anti-HBc was done at the Walter Reed Army Institute of Research with an RIA developed by Dr. Stanley Lemon. Saliva and semen were tested for occult blood using an orthotolidine-impregnated plastic strip (N-multistix®, Ames Co., Elkhart, Ind.) and/or by a quaiac slide test (Hemoccult®, Smith, Kline, and French, Philadelphia, Pa.).

Gibbon exposure. Saliva inoculations were carried out first. Gibbons that were not infected by saliva were reused for the semen experiment one and a half years later. All gibbons were
Transmission of HBV by Semen and Saliva

Table 2. Routes of exposure and outcome in gibbons exposed to a human saliva pool from hepatitis B surface antigen- and hepatitis B e antigen-positive donors.

<table>
<thead>
<tr>
<th>Exposure category</th>
<th>Gibbon no.</th>
<th>Age (year)</th>
<th>Total dose (ml)</th>
<th>Hepatitis B virus infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculation sc</td>
<td>PC-26</td>
<td>1.5</td>
<td>5.0</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>B-40</td>
<td>10</td>
<td>5.0</td>
<td>Yes</td>
</tr>
<tr>
<td>Oral aerosol</td>
<td>PC-21</td>
<td>2</td>
<td>5.0</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>PC-20</td>
<td>3</td>
<td>5.0</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>S-81</td>
<td>12</td>
<td>5.0</td>
<td>No</td>
</tr>
<tr>
<td>Oral aerosol and toothbrush</td>
<td>P-16</td>
<td>10</td>
<td>5.0</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>PC-16</td>
<td>2.5</td>
<td>5.0</td>
<td>No</td>
</tr>
</tbody>
</table>

Two gibbons (PC-26 and B-40) received a total of 5 ml of the saliva pool sc in two 2.5-ml doses on two consecutive days. Five other gibbons were divided into two groups. One group of three (PC-21, PC-20, S-81) received 5 ml of the pooled saliva in two 2.5-ml doses administered by aerosol to the nose and mouth. The remaining two animals (P-16, PC-16) received the same doses of the pooled saliva by an oral aerosol followed by toothbrushing. The toothbrushing invariably lead to gingival injury and bleeding (table 2).

Two gibbons received semen (table 3). One gibbon, PC-16, received a total of 1.8 ml sc divided into three daily doses. The other, PC-21, received a total of 3.6 ml intravaginally, divided into three 1.2-ml daily doses. The semen was instilled deeply into the vagina through a plastic catheter, following which a gloved finger was repeatedly inserted into the vagina to simulate copulation. Following the last administration of semen, the vaginal secretions were found to be slightly tinged with blood.

Results

Characterization of the inocula. The saliva pool was colorless but was minimally positive for occult blood using the orthotolidine method.\(^1\)

\(^1\) The orthotolidine methods often give false-positive results with secretions due to the presence of nonheme peroxidases [9].

HBsAg was detected only by RIA with a P/N ratio of 3.2 and an RIA titer of 1:4. The semen sample was also minimally positive for occult blood by the orthotolidine method but negative by the quaiac method. It was positive for HBsAg only by RIA, with a P/N ratio of 8.4 and an RIA titer of 1:20.

Results of saliva administration. Gibbons PC-26 and B-40 developed specific evidence of HBV infection by week 10 and week 16, respectively, after the sc inoculation of 5 ml of the saliva pool. Antigenemia with HBsAg/adw lasted from two to four weeks and was accompanied in each case by a modest rise in the serum ALT. The disappearance of antigenemia was followed by the development of anti-HBs in PC-26. In B-40, despite the disappearance of HBsAg by week 19, anti-HBs was not detected until 50 weeks after inoculation.

None of the gibbons that received saliva via the oral route, either with or without the trauma of toothbrushing, showed any evidence of HBV infection at any time during the year following exposure.

Results of semen administration. In the gibbons exposed to semen by the sc and intravaginal routes, HBV infection occurred as evidenced by the development of HBsAg followed by anti-HBs and anti-HBc. Gibbon PC-16 became antigenemic, with a P/N ratio of 9.5, eight weeks after the sc inoculation of 1.8 ml of semen (figure 1). The subtype of HBsAg was adw, the same as that

Table 3. Routes of exposure and outcome in gibbons exposed to human semen from a hepatitis B surface antigen- and hepatitis B e antigen-positive donor.

<table>
<thead>
<tr>
<th>Exposure category</th>
<th>Gibbon no.</th>
<th>Age (year)</th>
<th>Total dose (ml)</th>
<th>Hepatitis B virus infection</th>
<th>Onset (weeks after exposure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculation sc</td>
<td>PC-16</td>
<td>3</td>
<td>1.8</td>
<td>Yes</td>
<td>8</td>
</tr>
<tr>
<td>Intravaginal instillation</td>
<td>PC-21</td>
<td>3.5</td>
<td>3.6</td>
<td>Yes</td>
<td>7</td>
</tr>
</tbody>
</table>
carried by the donor. At the time of seroconversion, there were no abnormal physical findings; however, a modest rise in the serum ALT concentration (peak ALT, 32 IU/liter) was noted. The animal was antigenemic from weeks 8–11 after exposure with a peak P/N ratio of 70.3. Anti-HBs was detected in week 12 following exposure. Although insufficient serum was available for anti-HBc assays during the period of antigenemia, anti-HBc was present by week 12 following exposure. The antibodies were detected through week 18 after inoculation. At that time, the animal stopped eating, lost 1.1 kg, developed a leukocytosis with a shift to the left and an increase in serum transaminase levels, and died. Necropsy revealed no cause of death, and there was no evidence of hepatitis in microscopic sections of the liver.

Gibbon PC-21, which received 3.6 ml of semen intravaginally, developed HBsAg/adw, with a P/N ratio of 4.9, seven weeks after exposure (figure 1). The subtype of HBsAg again matched that of the donor. The antigenemia lasted three weeks, with a peak P/N ratio of 37.4, and was followed by the development of anti-HBs. Here again there was insufficient serum during the antigenemic period to determine anti-HBc; however, anti-HBc was present in the 12-week serum sample at the time anti-HBs was first detected. The serum ALT rose to 36 IU/liter from a base-line value of 19 IU/liter three weeks after the appearance of HBsAg and remained elevated intermittently from the ninth through the eighteenth week after inoculation. There were no physical signs of illness through 22 weeks of observation.

Discussion

This study clearly demonstrates that semen from an HBsAg-positive donor can contain viable HBV and that this material can be infectious when administered intravaginally. This finding supports the epidemiologic evidence that HBV can be spread by the venereal route [13, 14]. Whether this transmission can occur in the absence of mucosal injury remains a question. Vaginal manipulation of gibbon PC-21 following deposition of the semen produced blood-tinged secretions indicating that some trauma had occurred.

Szmuness et al. [12] reported a high prevalence of hepatitis B among homosexuals practicing rectal intercourse. As rectal tears are often associated with this activity, it may be that mucosal damage is necessary for sexual transmission of infectious virus. This supposition may explain why individuals who engage in oral or vaginal intercourse, where injury is less likely to occur, tend to have a lower frequency of HBV infection [13]. Sexual
promiscuity with transient relationships and large numbers of partners would increase the probability of mucosal injury and of exposure to HBV carriers and, therefore, enhance the probability of virus transmission.

Saliva was able to transmit HBV only when it was inoculated parenterally. Transmission did not occur with the five gibbons to which the virus was administered orally, despite the relatively large amounts of saliva given and the careful selection of the saliva donors, which should have optimized the probability of infectious secretions. Injury to the oral mucosa by toothbrushing also appeared to have no enhancing effect. The inability in this experiment to transmit hepatitis B by oral administration of saliva in conjunction with the previously reported [5] negative experience in eight animals would suggest that oral transmission by saliva or saliva-contaminated objects occurs infrequently in nature. This infrequent transmission might be due to low concentrations of infectious virus in oral secretions and/or to inactivation of virus by enzymic action in the mouth or gastrointestinal tract.

The death of gibbon PC-16 following inoculation of semen is reminiscent of a previous experiment in which a pool of semen containing a sample from the same donor was inoculated iv into a chimpanzee [9]. In that case, death followed administration of the semen by four weeks. As in our case, no specific cause of death could be determined, and microscopic examination of liver sections revealed no evidence of hepatitis. It may be that there was an unidentified toxin, allergen, or infectious agent in the semen of this donor that was lethal when inoculated parenterally into primates.

References