

Inactivation of Hepatitis B Virus by Intermediate-to-High-Level Disinfectant Chemicals

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In five separate tests, hepatitis B virus in dried human plasma was exposed for 10 min at 20°C to disinfectant chemicals having activity levels ranging from intermediate (e.g., 70% isopropyl alcohol) to high (e.g., 2% aqueous glutaraldehyde at pH 8.6). Five chimpanzees (one animal per disinfectant chemical) received treated material intravenously, and none showed signs of infection after post-inoculation periods of 9 months. Two animals were rechallenged with inoculum treated in the same manner, except that saline was used instead of a disinfectant chemical; both were infected within 4 weeks. Our results showed that hepatitis B virus was not as resistant to disinfectant chemicals as once thought and suggested that chemicals with similar activity levels (intermediate to high) might possibly be used on hepatitis B virus contamination with a margin of safety.

Epidemiological studies done after the development of specific serodiagnostic tests have shown that hepatitis B virus (HBV) may be transmitted by routes other than classic percutaneous exposures such as blood product transfusion or needlestick inoculation with contaminated material (11, 12, 18). Hepatitis B surface antigen (HBsAg) has been detected on a wide variety of inanimate environmental surfaces (3, 4, 6, 7, 9, 10, 17, 19, 21, 22, 31), and investigators have hypothesized that HBV contamination of these surfaces explains instances of disease transmission in the absence of overt percutaneous or mucous membrane exposures (4, 11, 12, 18). This hypothesis is strengthened by the fact that HBV in human plasma survived and caused infection in a chimpanzee after being dried and then stored at 25°C and 42% relative humidity for at least 1 week (2).

Since HBV cannot be propagated in tissue culture, comparative virucidal testing has not been performed as it has been for other types of viruses that can be conveniently cultured and tested in the laboratory. Consequently, little is known about the precise inactivation kinetics of HBV by physical or chemical agents, and this situation has led many experts to recommend nothing less than a sterilizing treatment when dealing with contamination by this virus. This has fostered the concept that HBV is some sort

of "super virus" in terms of its resistance to germicidal treatments. In 1977, we suggested that although HBV may be comparatively more resistant to a variety of physical or chemical agents than most viruses, it was unreasonable to assume that its level of resistance is equivalent to that of bacterial endospores (4). At that time, using the scheme of Spaulding et al. for classifying germicidal chemicals (25), we proposed that the resistance of HBV be considered greater than that of the tubercle bacillus but less than that of bacterial spores and probably much closer to the former. Accepting this consideration, it may be assumed that a normal physical or chemical sterilizing treatment capable of killing large numbers of bacterial spores would also kill large numbers of HBV. With disinfection processes, however, one must rely on empirical observations. HBV is not as resistant as bacterial spores, nor does its resistance even approach that of spores; this is known because HBV in human serum is inactivated by boiling for 1 min (12), a treatment that bacterial spores can withstand for hours. Therefore, a physical or chemical treatment known to exhibit sporicidal activity should also be virucidal for HBV.

We also used an alternate approach to determine the action of a germicidal agent on HBV by studying its effects on the immunological reactivity of the HBV-associated and comparatively stable antigen, HBsAg (4). Others have also used this approach (15, 23). We assumed that if a physical or chemical agent can destroy the reactivity of HBsAg, it can also destroy the infectivity

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ity of HBV. Similar strategies have been used by determining the effect of germicidal agents on the morphology of HBV (29) or effects on other associated markers such as endogenous DNA-dependent polymerase activity (20). Although studies such as these have produced useful information to increase confidence levels and lessen the severity of physical or chemical germicide recommendations, the results of the studies are often difficult to interpret. For example, there are certain germicides, such as formaldehyde, whose actions are fixative in nature, and HBsAg immunological reactivity is not destroyed at concentrations high enough to kill bacterial spores. In addition, the concentrations of germicides necessary to inactivate HBsAg within a reasonable period of time are often comparatively high, resulting in very conservative protocols of decontamination to be used in high-risk environmental settings.

We report here the first in a continuing series of direct chimpanzee infectivity studies to determine whether HBV will survive certain common physical or chemical treatments.

MATERIALS AND METHODS

Source of HBV. The single-source human plasma (designated HLD-1) was the same as that used in recent reports on transmission of HBV infection via eye inoculation of a chimpanzee (5) and survival of HBV after drying and storage at 25°C and 42% relative humidity for 1 week (2). The HLD-1 inoculum is known to be positive for HBsAg, with a radioimmunoassay (RIA; Ausria II, Abbott Laboratories, North Chicago, Ill.) endpoint titer (4) of approximately $1:10^6$. This inoculum is also positive for hepatitis B *e* antigen HBeAg by RIA (Abbott Laboratories) and positive for DNA polymerase, and numerous intact HBV particles (Dane particles) have been visualized by immune electron microscopy. The \log_{10} titer of HBV in HLD-1 is known to be 10^8 chimpanzee infectious doses per ml by direct chimpanzee titration of 10-fold plasma dilutions in homologous serum. The chimpanzees receiving the 10^{-7} and 10^{-8} dilutions were infected within 60 days, and the animal receiving the 10^{-9} dilution remained negative for a post-inoculation period of 9 months.

Exposure of inoculum to disinfectants. The disinfectant chemicals tested are listed below. For each test, 0.1-ml portions of a 1:10 normal saline dilution of HLD-1 plasma were placed in each of three sterile, siliconized (Prosil-28, PCR Research Chemicals, Gainesville, Fla.), chlorine demand-free, screw-capped tubes (16 by 125 mm) to effect an HBV concentration of 10^6 chimpanzee infectious doses per tube. The tubes were then loosely capped and dried for 16 h under vacuum at 25°C over anhydrous calcium carbonate. It is known that the inoculum will survive this treatment (2), and dried microorganisms are considered to be generally more resistant to physical or chemical agents than those in the wet state. A 1-ml volume of disinfectant (or aqueous dilution of disinfectant prepared with chlorine demand-free commercial

distilled water) tempered at 20°C was added to each tube, and the tubes were then placed in a stationary 20°C water bath incubator for 10 min. After this time, the disinfectants were neutralized by adding 10 ml of homologous chimpanzee plasma at 4°C to each tube and mixing immediately on a vortex mixer. For halogen disinfectants, one drop of sterile 5% (wt/vol) sodium thiosulfate was added to each tube before the homologous plasma was added. The contents of each tube were pooled and chilled in an ice-water bath until a chimpanzee was inoculated. The elapsed time between elution and chimpanzee inoculation did not exceed 15 min. To determine whether complete removal of the inoculum from the tubes had occurred, we gently rinsed each tube twice with 10 ml of distilled water to remove residual plasma eluate and then sampled the inside surfaces of the tubes for HBsAg, using the swab-rinse technique (4). All swab eluates were negative for HBsAg by RIA, and the only distilled water rinse fluids positive for HBsAg were the first rinses from the tubes in the iodophor tests (see below). This finding was probably due to small amounts of residual plasma eluate in the tubes and the fact that the iodophor did not completely inactivate the immunological reactivity of HBsAg. Also, comparative HBsAg endpoint titrations were done by using both wet and dried HLD-1 inocula. The dried material was eluted from the tube by adding 1 ml of 1% bovine serum albumin in saline and mixing for 15 s on a vortex mixer. The RIA endpoint titers were as follows: wet inoculum, $1:8 \times 10^4$; dried inoculum, $1:5 \times 10^4$.

The first disinfectant tested was an aqueous dilution (pH 9.2) of reagent-grade sodium hypochlorite (Fisher Scientific Co., Fair Lawn, N.J.) having 500 mg of free available chlorine per liter, as determined by the DPD colorimetric test method (LaMotte Chemical Products Co., Chestertown, Md.). After reaction with the HLD-1 inoculum for 10 min at 20°C, there was 300 mg of free available chlorine remaining per ml. No HBsAg was detected by RIA in the homologous plasma pool. The second disinfectant tested was 2% aqueous glutaraldehyde adjusted to pH 8.4 ("activated") with a phosphate-bicarbonate buffer (Cidex CX-250, Arbrook, Inc., Arlington, Tex.). This solution was chosen for testing because it represents the oldest and perhaps the most widely distributed of the currently available commercial glutaraldehyde disinfectant-sterilants registered with the U.S. Environmental Protection Agency (EPA). No HBsAg was detected by RIA in the homologous plasma pool after treatment with glutaraldehyde. The third compound tested was a 1:16 aqueous dilution (pH 7.9) of a commercial product initially containing a mixture of 2% glutaraldehyde and 7% phenol (Sporicidin, Sporicidin Co., Washington, D.C.). This product was chosen for testing because it has recently been marketed as an effective disinfectant for, among other things, dental instruments. The plasma pool from this test used for chimpanzee inoculation was found to be positive for HBsAg by RIA (four sample ratio units; counts per minute of the test divided by the mean negative control value; sample ratio unit values ≥ 2.1 are considered positive). The fourth chemical tested was 70% (vol/vol) aqueous isopropyl alcohol (pH 8.0; Fisher), since alcohols are generally thought to have limited virucidal capabilities and also because this is the most common alcohol formulation used in the medical professions. No

TABLE 1. Chimpanzees used in testing disinfectant chemical activity against HBV

No.	Sex	Age (yr)	Weight (kg)
1028	M	3.0	14
980	M	4.0	15
1089	F	2.0	8
854	M	6.5	29
1085	M	2.0	8

HBsAg was detected in the homologous plasma pool in this test. The fifth germicide tested was a 1:213 aqueous dilution (pH 2.0) of an iodophor detergent-disinfectant (Wescodyne, West Chemical Co., Lynbrook, N.Y.) having 80 mg of available iodine per liter as determined by a colorimetric test method (La-Motte). This compound was chosen for testing because, after hypochlorite solutions, the iodophors are the most widely used halogen disinfectants. After reacting with the HLD-1 inoculum for 10 min at 20°C, there was 32 mg of available iodine per liter remaining in the solution. Also, the homologous plasma pool in this test was shown to be positive for HBsAg by RIA (91 sample ratio units).

Inoculation and monitoring of chimpanzees. Characteristics of colony-born chimpanzees (*Pan troglodytes*) used in this series of tests are shown in Table 1, and the chimpanzees are matched, in order, to the disinfectant chemicals listed above. None of the chimpanzees had ever been inoculated with HBV material, and each received intravenously 11 ml (one-third of a triplicate pool) of inoculum treated with a particular disinfectant chemical. The clinical monitoring of the animals consisted of pre-inoculation liver biopsies and base-line serological tests for liver enzymes (aspartate and alanine aminotransferases), HBsAg, and antibody to HBsAg (anti-HBs; RIA, Ausab, Abbott Laboratories). After inoculation, serum specimens were collected weekly for 9 months and were tested for liver enzymes, HBsAg, and anti-HBs. Also, the final serum specimens collected were tested for antibody to hepatitis B core antigen (Corab, Abbott Laboratories). Liver biopsy was scheduled in the event of any abnormal serological finding.

Rechallenge of chimpanzees. At the end of the 9-month post-inoculation period, two of the chimpanzees (no. 1089 and 854) received intravenous inoculation with HLD-1 plasma treated exactly as described above (11 ml of a homologous plasma eluate pool), except that 1-ml portions of sterile normal saline were used instead of a disinfectant chemical.

RESULTS AND DISCUSSION

None of the chimpanzees showed abnormal serological test results during the 9-month observation periods after inoculation, indicating that all of the disinfectant chemicals were effective in killing the HBV in dried plasma. After rechallenge of the two chimpanzees with dried but otherwise untreated HLD-1 plasma, both were infected within 4 weeks; results of post-inoculation serological monitoring are shown in Table 2.

TABLE 2. Post-inoculation serological monitoring of rechallenged chimpanzees

Chimpanzee no.	Time (wk) of first positive result post-inoculation					
	ALT, AST ^a	HBsAg	Anti-HBs	HBeAg ^b	Anti-HBe ^b	Anti-HBc ^c
1,089	6	2	6	5	9	6
854	12	4	30	9	21	10

^a Alanine and aspartate aminotransferases.

^b As measured by RIA (Abbott Laboratories).

^c Antibody to hepatitis B core antigen as measured by RIA, Corab, Abbott Laboratories.

This result indicated that our test method was capable of delivering potentially infectious material to the animals in the disinfectant tests and confirmed our earlier observation that all of the chemicals tested were capable of killing 10⁶ chimpanzee infectious doses of HBV in dried human plasma. Obviously, the data in this study are not applicable to a great deal of statistical analysis (one disinfectant chemical, one chimpanzee), and certainly they do not show the superiority of one of the tested chemicals over another. In effect, what was shown was that HBV does not appear to be as resistant to chemical germicides as once thought. These results may be helpful in designing disinfection strategies in high-risk areas, but they should not be construed as meeting the EPA criteria for specific effectiveness claims on labels of commercially available germicides containing the chemicals tested. The study examined disinfectant chemicals ranging in activity levels from intermediate (e.g., isopropyl alcohol) to high (e.g., 2% aqueous glutaraldehyde), and all were shown to be effective against HBV under a "worst-case" situation (dried plasma, 20°C, 10 min). This suggests that any chemical germicide having an activity level in this range might also be used on HBV contamination with a margin of safety, provided that the chemical is properly used. Proper use of disinfectant chemicals is discussed in detail elsewhere (1, 8, 25), but the most important step in any disinfecting protocol is adequate precleaning of surfaces before conducting the actual disinfecting step.

There are many questions remaining regarding the resistance or sensitivity of HBV to other physical or chemical agents (e.g., temperatures between 60 and 100°C, phenolics, and quaternary ammonium compounds), but the scarcity and expense of chimpanzees hamper the gathering of any statistically valid data. Until a laboratory culture method for HBV is developed, the interruption of the environmentally mediated transmission of HBV infection will have to depend primarily on judicious use of the small amount of existing direct data on infectivity (2, 14, 16, 24,

26–28, 30; H. Kobayashi, personal communication), as well as vaccination of employees working in high-risk areas (13, 14) and application of the time-honored principles of asepsis.

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