

Susceptibility of Hepatitis B Virus to Disinfectants or Heat

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Using direct chimpanzee inoculation as an assay method, we tested the abilities of the following chemical or physical treatments to inactivate hepatitis B virus in human plasma: 1% aqueous glutaraldehyde at 24°C for 5 min, 0.1% aqueous glutaraldehyde at 24°C for 5 min, 80% ethyl alcohol at 11°C for 2 min, and heat at 98°C for 2 min. All treatments were shown to be effective, indicating that the resistance level of the hepatitis B virus is not extreme.

Disinfection or sterilization procedures for viral hepatitis B (HB) contamination have been suggested by a number of workers (2-6, 11-14), yet the majority of these procedures are based either on empirical data or on retrospective observations obtained during or after an outbreak investigation. Since HB virus cannot be propagated in the laboratory, few direct infectivity studies have been reported (1, 7-9, 16, 18). The only acceptable animal model for such studies is the chimpanzee, and the scarcity and expense of these animals make the gathering of statistically valid data extremely difficult. In this report, the virucidal activities of glutaraldehyde, ethanol, and heat on HB virus in human plasma were studied by direct chimpanzee inoculation.

MATERIALS AND METHODS

HB virus. Pooled human plasma (JHB001) was employed as the inoculum. The plasma pool is positive for HB surface antigen (HBsAg), hepatitis B e antigen (HBeAg), and DNA polymerase, and the log₁₀ titer of HB virus is known to be 10⁸ chimpanzee infectious doses per ml (17).

Treatment of inoculum. The disinfectant chemicals tested were aqueous glutaraldehyde (Sterihyde; Maruishi Pharmaceutical Co., Osaka, Japan) at two concentrations (1 and 0.1%) and 80% (vol/vol) aqueous ethanol. Exposures of HB virus to the chemicals were conducted as described below.

(i) **1% Glutaraldehyde.** Duplicate 1-ml portions of a 1:1,000 JHB001 plasma dilution in phosphate buffer (pH 7.2) were mixed with 1-ml portions of 2% (wt/vol) glutaraldehyde to effect a final glutaraldehyde concentration of 1%. The tubes were held at 24°C for 5 min and then the glutaraldehyde was neutralized by adding 1 ml of 4% (wt/vol) aqueous sodium bisulfite to each tube.

(ii) **0.1% Glutaraldehyde.** Duplicate 1-ml portions of a 1:500 JHB001 plasma dilution in phosphate buffer (pH 7.2) were mixed with 1-ml portions of 0.2% (wt/vol) glutaraldehyde to effect a final glutaraldehyde concentration of 0.1%. The tubes were held at 24°C for 5 min and then the glutaraldehyde was neutralized by adding 1 ml of 0.4% (wt/vol) aqueous sodium bisulfite to each tube.

(iii) **80% Ethanol.** Duplicate 0.2-ml portions of a 1:300 JHB001 plasma dilution in phosphate buffer (pH 7.2) were mixed with 0.8 ml of 99.5% (vol/vol) ethanol to effect a final ethanol concentration of 79.6%. Tubes containing 1 ml were

held at 11°C for 2 min and then neutralized by dilution in 200 ml of saline solution.

To determine the effects of heating at 98°C for 2 min on the infectivity of HB virus, duplicate 1-ml ampoules containing 1 ml of a 1:1,000 dilution of JHB001 plasma in phosphate buffer (pH 7.2) were heated in a bath of liquid paraffin at 98°C. In preliminary studies with thermocouples (EM12006; Chino Works, Tokyo, Japan) to determine the temperature profile of the ampoules, it was shown that it took 4 min to reach 98°C. In the actual test, the ampoules were placed in the paraffin bath for 6 min (4 min to reach 98°C and 2 min at 98°C). After heating, the ampoules were cooled immediately in an ice-water bath.

Inoculation and monitoring of chimpanzees. Nine wild-caught chimpanzees (*Pan troglodytes*) from West Africa between the ages of 2 and 4 years were used in these tests. Before importation, they were shown in two separate samplings to be negative for HBsAg, antibody to HBsAg (anti-HBs), and antibody to HB core antigen (anti-HBc) by radioimmunoassay (Ausria II, Ausab, and Corab, respectively; Abbott Laboratories, North Chicago, Ill.). Before inoculation, there was no evidence of hepatitis infection as determined by liver biopsy and serological tests for liver functions.

Each chimpanzee was inoculated intravenously with a JHB001 plasma dilution treated as shown in Table 1. The total amount of each inoculum was 3 ml treated with glutaraldehyde and neutralized, 201 ml treated with ethanol and diluted in saline, or 1 ml heated. After inoculation, serum specimens were collected weekly for 14 to 17 weeks and then every 2 weeks until 30 weeks after inoculation and tested for HBsAg, anti-HBs, anti-HBc, and liver functions (total protein, bilirubin, alkaline phosphatase, aspartate and alanine aminotransferases, lactic dehydrogenase, γ -glutamine transpeptidase, cholinesterase, leucine aminopeptidase, triglycerides). Liver biopsies were performed every month.

Challenge of chimpanzees. To prove that the untreated dilution of plasma was potentially infectious to the animals, a single chimpanzee received intravenously 1 ml of a 1:1,000 dilution of JHB001 plasma in phosphate buffer (pH 7.2).

RESULTS

None of the chimpanzees inoculated with treated plasma dilutions showed either abnormal liver functions or positive

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TABLE 1. Chimpanzees used in the test and plasma dilution at treatment

No.	Sex	Wt. (kg)	Treatment of inoculum	Plasma dilution at treatment
1	M	9.8	1% Glutaraldehyde, 5 min	1 : 2,000
2	M	7.8		
3	F	16.3	0.1% Glutaraldehyde, 5 min	1 : 1,000
4	M	15.4		
5	M	14.9	80% (vol/vol) ethanol, 2 min	1 : 1,500
6	F	20.4		
7	M	10.3	98°C, 2 min	1 : 1,000
8	F	8.8		
9	M	30.0	Untreated	1 : 1,000

results for HBsAg, anti-HBs, and anti-HBc for 30 weeks after inoculation. Liver biopsies demonstrated no histologically abnormal findings.

The chimpanzee inoculated with the untreated plasma dilution was infected within 6 weeks after inoculation. In the serological monitoring, the first positive results after inoculation were obtained at week 6 for HBsAg, week 9 for anti-HBc, week 32 for anti-HBs, and week 11 for alanine and aspartate aminotransferases, and the length of time after inoculation before HBsAg was detected was consistent with past observations (17).

DISCUSSION

Although the number of chimpanzees used in this study was not sufficient for statistical evaluation of the results, the human plasma inoculum used was of relatively high HB virus titer, and the physical or chemical treatments studied were comparatively mild. Our results are in agreement with the currently small amount of direct infectivity data, which indicate that HB virus is not unusually resistant to germicidal treatments (1, 17).

Glutaraldehyde (2% aqueous) has been recommended for inactivation of HB virus infectivity (2, 11, 13, 14), but only one direct transmission study has been reported (1). In this study, we showed glutaraldehyde in low concentration (1 or 0.1%) to be effective for HB virus inactivation within 5 min at 24°C.

Horst et al. (4) showed that immunological reactivity of HBsAg was not inactivated after treatment with 80% ethanol for 3 h, and ethanol has been suggested as ineffective for killing HB virus (3, 10, 12, 15). These recommendations were, however, not based on prospective, direct transmission studies, but rather on retrospective observations. In our chimpanzee study, 80% ethanol treatment for 2 min at a rather low temperature (11°C) was shown to be effective in killing HB virus. Bond et al. have reported similar results with isopropanol (1), although dried inoculum was employed in their study.

Krugman and Giles (8) reported that boiling at 98°C for 1 min inactivated infectivity of HB virus in diluted serum without affecting antigenicity. Upon reexamination of results at a later date (9), however, it was shown that administration of this heat-inactivated HB virus induced inapparent infections. Soulier et al. (18) and Shikata et al. (16) reported that treatment at 60°C for 10 h may not, depending on titer, inactivate HB virus, and these reports are the major reasons

for the HB virus having a reputation for being highly resistant to heat. In the past, we have also recommended relatively severe treatments for routine processing of HB virus-contaminated materials (6). In the study reported here, however, we showed by direct infectivity testing that HB virus in plasma was inactivated by heating at 98°C for 2 min. This result indicated that the heat resistance of HB virus is not extreme.

Although the current amount of direct infectivity data on HB virucidal treatments is small (1, 7), our results add to the evidence that HB virus is not exceptionally resistant to chemical or physical germicidal treatments. Thus far, reported data suggest that existing rigorous protocols for HB contamination should be carefully reevaluated for maximum effectiveness with a minimum of time and expense, especially since ethanol is one of the most widely used disinfectants in hospitals, and was shown to be effective in killing HB virus in our study. Careful attention should be paid to proper contact time, however, since ethanol evaporates rapidly. Also cleaning of surfaces before actual disinfection is important for effective disinfection.

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LITERATURE CITED

- Bond, W. W., M. S. Favero, N. J. Petersen, and J. W. Ebert. 1983. Inactivation of hepatitis B virus by intermediate-to-high-level disinfectant chemicals. *J. Clin. Microbiol.* **18**:535-538.
- Bond, W. W., N. J. Petersen, and M. S. Favero. 1977. Viral hepatitis B: aspects of environmental control. *Health Lab. Sci.* **14**:235-252.
- Council on Dental Therapeutics. 1976. Type B (serum) hepatitis and dental practice. *J. Am. Dent. Assoc.* **92**:153-159.
- Horst, H., I. Tripatzis, and I. Konstantinidis. 1972. Australia antigen: effect of chemical and physical measures upon antigenicity. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A* **219**:1-6.
- Kobayashi, H. 1979. The hepatitis controversy: what should be "practical" standards of treatment? *Med. Instrum.* **13**:56-57.
- Kobayashi, H., Y. Takahashi, M. Tsuzuki, Y. Yoshihara, and H. Toyama. 1978. Sterilization of hepatitis B surface antigen-contaminated materials. *Med. Instrum.* **12**:171-173.
- Kobayashi, H., M. Tsuzuki, T. Oda, H. Toyama, K. Koshimizu, N. Yoshihara, T. Shikata, T. Karasawa, and K. Abe. 1980. Inactivation of hepatitis B virus. *Jpn. J. Med. Instrum.* **50**:524-525.
- Krugman, S., and J. P. Giles. 1970. Hepatitis virus: effect of heat on the infectivity and antigenicity of the MS-1 and MS-2 strains. *J. Infect. Dis.* **122**:432-436.
- Krugman, S., L. R. Overby, I. K. Mushahwar, C. M. Ling, G. G. Frösner, and F. Deinhardt. 1979. Viral hepatitis, type B: Studies on natural history and prevention re-examined. *N. Engl. J. Med.* **300**:101-106.
- Levin M. L., W. C. Maddrey, J. R. Wands, and A. I. Mendeloff. 1974. Hepatitis B transmission by dentists. *J. Am. Med. Assoc.* **228**:1139-1140.
- Marmion, B. P., and R. W. Tonkin. 1972. Control of hepatitis in dialysis units. *Br. Med. Bull.* **28**:169-179.
- Murray, R. 1955. Viral hepatitis. *Bull. N.Y. Acad. Med.* **31**:341-358.
- Percy-Robb, I. W., J. Proffitt, and L. G. Whitby. 1970. Precautions adopted in a clinical chemistry laboratory as a result of an outbreak of serum hepatitis affecting hospital staff. *J. Clin. Pathol.* **23**:751-756.
- Report of a WHO Scientific Group. 1973. Viral hepatitis. WHO Tech. Rep. Ser. 512.

15. Schmidt, N. J., and E. H. Lennette. 1972. Safety precaution for performing test for hepatitis-associated "Australia" antigen and antibodies. *Am. J. Clin. Pathol.* **57**:526-530.
16. Shikata, T., T. Karasawa, K. Abe, T. Takahashi, M. Mayumi, and T. Oda. 1978. Incomplete inactivation of hepatitis B after heat treatment at 60°C for 10 hours. *J. Infect. Dis.* **138**:242-244.
17. Shikata, T., T. Karasawa, K. Abe, T. Uzawa, T. Mayumi, M. Imai, M. Kako, M. Torii, K. Mitamura, H. Suzuki, T. Oda, Y. Moritsugu, N. Yoshihara, and K. Koshimizu. 1977. Study on infectivity of carrier-sera positive or negative for HBe Ag. *Acta Hepatol. Jpn.* **18**:388-393.
18. Soulier, J. P., C. Blatix, A. M. Courouce, D. Benamon, P. Amouch, and J. Drouet. 1972. Prevention of virus B hepatitis (SH hepatitis). *Am. J. Dis. Child.* **123**:429-434.