JOURNAL OF VIROLOGY, Feb. 2004, p. 2164–2165 Vol. 78, No. 4
0022-538X/04/$08.00+0 DOI: 10.1128/JVI.78.4.2164–2165.2004

Inactivation of Transmissible Spongiform Encephalopathy (Prion) Agents by Environ LpH

Richard E. Race* and Gregory J. Raymond

Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana

Received 28 July 2003/Accepted 16 October 2003

Agents causing transmissible spongiform encephalopathy (TSE) diseases are resistant to inactivation by several conventional decontamination methods. Using an animal bioassay, we compared the TSE agent disinfectant efficacy of a commercially available product referred to alternatively as LpH-SE, LpH-AG, or LpH-st to that of a similarly named but differently formulated product, Environ LpH, which was found to be an effective TSE agent disinfectant in a previous study. Here, we found LpH-SE to be at least 10^4-fold to 10^5-fold less effective than Environ LpH.

Transmissible spongiform encephalopathy (TSE) agents are notoriously difficult to destroy by treatments that are effective against other infectious agents. Nevertheless, several compounds with at least some anti-TSE agent activity have been reported (1–6). Of these, Environ LpH (Steris Corp., St. Louis, Mo.) was found to be particularly effective (4). Additional advantages of Environ LpH are that it is more stable and less hazardous than other chemicals commonly used for TSE agent decontamination, such as sodium hydroxide or sodium hypochlorite (6). As a result, research and diagnostic laboratories, hospitals, and other medical facilities began using Environ LpH to inactivate items contaminated by TSE agents. At the time of the previous study (4), the Environ LpH formulation was designated LpH. After the previous study, it was also referred to as Canadian LpH. Causing even more confusion, the manufacturer introduced a different formulation of LpH, variously marketed as LpH-SE, LpH-AG, or LpH-st. Though the chemical formulations of these three products are identical to each other, they differ considerably from that of Environ LpH. Some users of these products assumed that the newer LpH formulations have the same anti-TSE activity as the Environ LpH originally tested. However, since the chemical composition of the newer formulation is different from that of the originally tested formulation, it is possible that these differences might alter TSE inactivation properties (Table 1).

To determine if the alternative LpH compounds have anti-TSE activity equal to that of Environ LpH, we tested the effect of 1% (vol/vol) LpH-SE on a 9% (wt/vol) brain suspension derived from clinically sick, 263K scrapie agent-infected Syrian golden hamsters for a 16-h contact period as described previously (4). Groups of normal hamsters were inoculated with three successive 10-fold dilutions of the LpH-SE-treated brain suspensions. All hamsters inoculated with the LpH-SE-treated brain developed scrapie, indicating at least some resistance of the 263K agent to LpH-SE. This result differs from earlier data, where all hamsters inoculated with Environ LpH-treated brain suspensions survived when inoculated with the identical dilution series (4). LpH-SE did have some inactivating effect, however, because the incubation periods of hamsters inoculated with LpH-SE-treated brain were longer than the incubation periods of hamsters receiving similar dilutions of untreated brain (Table 2). Comparison of the incubation periods for LpH-SE-treated brain to a standard curve relating incubation period to infectivity titer for untreated brain indicated a 100- to 1,000-fold inactivation of the 263K hamster agent by LpH-SE (data not shown). In an earlier study, Environ LpH inactivated $>10^7$ $\log_{10}$ of 263K hamster infectivity (4). Thus, Environ LpH is several orders of magnitude ($\geq 10^4$ to 10^5) more effective than LpH-SE as a TSE disinfectant.

We also tested the effect of autoclaving the LpH-SE-treated brain. A 0.9% suspension of 263K-infected hamster brain was autoclaved at 121°C for 60 min in a 1% LpH-SE solution. This combination treatment removed all detectable infectivity, similar to earlier results using Environ LpH (4).

These studies show that LpH-SE has limited anti-TSE efficacy unless combined with autoclaving. Without autoclaving, Environ LpH is a far superior disinfectant for the hamster

TABLE 1. Chemical composition of LpH compounds

Chemical component	% by wt[a]	
	Environ LpH[b]	LPH-SE[b], LPH-AG[b], LPH-st[b]
o-Benzyl-*p*-chlorophenol	6.4	
p-Tertiary-amylphenol	3.0	7.6
o-Phenyl phenol	0.5	7.7
Hexylene glycol	4.0	
Glycolic acid (hydroxyacetic)	12.6	
Isopropanol	8.0	7.5
Phosphoric acid		15.0
Dodecyl benzene sulfonic acid		<5

[a] Percent by weight from material safety data sheet.
[b] Manufactured by Steris Corporation, St. Louis, Mo.

* Corresponding author. Mailing address: Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 903 S. Fourth St., Hamilton, MT. Phone: (406) 363-9358. Fax: (406) 363-9286. E-mail: rrace@niaid.nih.gov.

TABLE 2. Effect of LpH-SE and Environ LpH on 263K scrapie infectivity in Syrian golden hamsters[a]

Treatment	% Brain inoculated	No. of Sc+ hamsters/no. inoculated	Interval to sacrifice (days) (mean ± SD)
LpH-SE	0.1	4/4	89 ± 0
	0.01	4/4	100 ± 4.5
	0.001	4/4	133 ± 38
None	0.1	4/4	82 ± 0
	0.01	4/4	89 ± 0
	0.001	4/4	92 ± 3
LpH-SE + autoclave	0.1	0/4	*[b]
Environ LpH	0.1	0/3	*[b]
Environ LpH + autoclave	0.1	0/4	*[b]

[a] Data relative to Environ LpH is taken from an earlier study (4) and is shown for comparison to LpH-SE. The Environ LpH result for 1% LpH and 0.1% brain homogenate is shown. Hamsters were inoculated intracerebrally with 50 μl of the indicated suspension of LpH-treated or untreated brain. The interval to sacrifice is the time from inoculation to definite clinical scrapie. Sc+, scrapie positive.

[b] *, no clinical scrapie disease during a 15-month postinoculation observation period.

263K agent, one of the highest-titered TSE agents known (1). We did not determine which of the Environ LpH ingredients provides the anti-TSE effect. Three Environ LpH components—glycolic acid, hexylene glycol, and *o*-benzyl-*p*-chlorophenol—are not present in LpH-SE. Presumably, one of these or a combination of one or more of them provides the effect.

Based on our studies, we recommend Environ LpH, but not the other LpH products, for use as a TSE disinfectant.

Animal research complied with all relevant federal guidelines and NIH/NIAID/Rocky Mountain Laboratories' institutional policies.

REFERENCES

1. **Brown P., E. H. Rau, B. K. Johnson, et al.** 2000. New studies on the heat resistance of hamster-adapted scrapie agent: threshold survival after ashing at 600 degrees C suggests an inorganic template of replication. Proc. Natl. Acad. Sci. USA **97:**3418–3421.
2. **Chatigny, M. A., and S. B. Prusiner.** 1970. Biohazard and risk assessment of laboratory studies on the agents causing the spongiform encephalopathies, p. 491–514. *In* S. B. Prusiner and W. J. Hadlow (ed.), Slow transmissible diseases of the nervous system, vol. 2. Academic Press, New York, N.Y.
3. **Chesebro, B., and B. N. Fields.** 1996. Transmissible spongiform encephalopathies: a brief introduction, p. 2845–2849. *In* B. N. Fields, D. M. Knipe, P. M. Howley, et al. (ed.), Fields virology. Raven Publishers, Philadelphia, Pa.
4. **Ernst, D. R., and R. E. Race.** 1993. Comparative analysis of scrapie agent inactivation methods. J. Virol. Methods **41:**193–202.
5. **Taylor, D. M.** 2000. Inactivation of transmissible degenerative encephalopathy agents: a review. Vet. J. **159:**10–17.
6. **World Health Organization.** 1999. WHO infection control guidelines for transmissible spongiform encephalopathies. Report of a WHO consultation, Geneva, Switzerland, 23–26 March 1999, p. 1–35. World Health Organization, Geneva, Switzerland.