参考資料1

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Practical Methods for Chemical Inactivation of Creutzfeldt-Jakob Disease Pathogen

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Abstract Chemical inactivation of pathogen of Creutzfeldt-Jakob disease (CJD) was examined using the mouse-adapted CJD strain. A high concentration of formic acid, guanidine compounds, trichloroacetate and phenol prevented CJD transmission. NaOH between 0.25 and 2 N lengthened the incubation periods. Sodium dodecyl sulfate (SDS) in a concentration between 1 and 3% did not alter incubation at room temperature but did completely block the transmission after boiling for 3 min in 3% SDS. This method is recommended for practical disinfection.

Infectious pathogens of Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler syndrome (GSS) and kuru in New Guinea share the same or similar unconventional properties with those of scrapie in sheep and in other species to which infection had spread from sheep. Due to the strong resistance of the pathogen against physicochemical inactivation procedures, disinfection is difficult. Using the mouse-adapted CJD Fukuoka-1 strain, we noted the strong resistance of the pathogen for heat (8, 9), ultraviolet irradiation (8), long-term fixation in 10% formalin (8, 9) and other chemicals (8, 10). Chemical treatments suitable for practical disinfection were examined in the present experiment.

NZW mouse brain infected with the mouse-adapted CJD strain was homogenized in normal saline 10% (W/V). After centrifugation at $4,000\times g$ for 30 min, the supernatant was obtained. The residue was burnt out, as usually done for contaminated tissues. Then 0.2 ml of the supernatant was mixed with saline and chemicals in each final concentration listed in Table 1 and kept for 2 hr at room temperature, except for 3% sodium dodecyl sulfate (SDS), which was additionally kept for 2 hr at 60 C or boiled for 3 min at 100 C. Then the mixed materials were dialyzed against distilled water for 48 hr through membranes (Spectrapor membrane tubing: MW cutoff 3,500), hence exposure of the homogenate supernatant to chemicals of decreasing concentrations continued for more than 2 hr until chemicals disappeared. Chemical reagents used in this experiment are listed in Table 1. The non-treated supernatant was diluted with saline and dialyzed in the same manner as for treated materials, and inoculated as control. After these treatments, the final volume of each inoculum ranged between 2.4 and 7.3 ml, corresponding to

Table 1. Effect of chemical treatments on CJD transmission

Treatment	Final dilution of homogenate (%)	Nos. of mice affected/ examined	Incubation period (Days, M±S.D.)	Infectivity $\log ID_{50}$
Control (dialysis only)	0.80	7/7	148±14	7.0
Alkali and acid				
NaOH 0.25 N	0.74	9/9	191±20*	5.6
1 N	0.71	5/9	347±128**	4.1
2 N	0.74	7/9	209±19*	5.2
HCl 1 N	0.80	9/9	137±5	7.4
Formic acid				
20%	0.71	8/8	155±12	6.9
60%	0.52	0/4		—
80%	0.49	0/6		—
Chaotropic ions				
Gdn-HCl 1 M	0.83	7/7	145±8	7.1
7 M	0.57	0/6		—
Gdn-SCN 1 M	0.83	7/9	140±5	7.3
3 M	0.77	0/7		—
TCA 0.2 M	0.77	7/7	148±14	7.0
3 M	0.32	0/7		—
Denaturants				
SDS 1%	0.57	7/7	131±3	7.6
3%	0.36	5/5	156±20	7.0
60 C, 3%	0.29	7/7	155±21	7.1
100 C, 3%	0.27	0/6		—
Organic solvents				
Phenol 50%	0.42	0/9		—
80%	0.43	0/6		—

—: Infectivity was not detected.

Significant delay of incubation period from that of control by Mann-Whitney's u-test for unpaired samples with $P<0.01$* or $P<0.05$**.

Chemicals used: formic acid (Katayama Chemicals); Gdn-HCl, guanidine-HCl (Nakarai Chemicals); Gdn-SCN, guanidine-thiocyanate (Fluka AG); K-SCN, potassium thiocyanate (Katayama Chemicals); phenol (Katayama Chemicals); SDS, sodium dodecyl sulfate (Wako Pure Chemical).

the dilution of brain homogenate as shown in Table 1. The range of this different dilution caused no significant delay in the incubation periods (8, 9).

Twenty microliters of the inoculum was injected into brains of 5-week-old female NZW mice and these mice were kept until distinct clinical signs of CJD appeared or for their entire life span. All mice except a few of early or accidental death were examined pathologically, including immunostain with anti-mouse prion protein (6). Infectivity ($\log ID_{50}$/g mouse brain) was calculated from incubation periods and final dilution of the homogenates (9).

No mouse showed serious side effects after intracerebral inoculation. According to the length of the incubation period, the experimental animals were grouped into 3: short-incubation groups without significant delay from control; long-incu-

bation groups with incubation periods longer than 2 standard deviations (SD) of that of control; survivor groups.

In the short-incubation groups, mice treated with 1 N HCl and 1% SDS showed earlier onset than did the control. The long-incubation groups included 3 groups treated with NaOH. The 1 N NaOH treatment was done as an additional experiment and exceptionally long incubation period was observed in a few mice. The effect of NaOH did not seem to depend on the concentration, between 0.25 and 2 N.

In 8 groups, no mouse developed the disease during its life span of over 2 years. Disinfection with formic acid, guanidine-HCl (Gdn-HCl), guanidine-thiocyanate (Gdn-SCN) and trichloroacetate (TCA) was concentration dependent, and that with 3% SDS was temperature dependent. Three percent SDS at room temperature or 60 C did not alter the incubation periods, but did completely block the transmission after boiling at 100 C for 3 min.

Concentrations of chemicals needed for total disinfection were high, except for SDS, and were most corrosive to skin, fabrics and metals, as is sodium hypochlorite in a high concentration (>0.5%) used for CJD inactivation (1–5, 8). NaOH was less corrosive and the incubation period was lengthened but was not totally effective, contrary to the findings of other workers (1–3).

SDS was reported to be effective on brain supernatant of scrapie-infected animals when the SDS: protein ratio exceeded 1.8 (7) or to reduce infectivity by $10^{3.3}$, when the brain homogenate with 5% SDS was treated for 1 hr at 70 C (5). In our experiment, SDS in 1 and 3% concentration at below 60 C did not lengthen the time of incubation, yet boiling in 3% SDS did block the transmission. It is not known whether SDS has a direct biocidal effect on the pathogen or reduces heat stability of the pathogen (5). SDS is not corrosive, not expensive and has cleaning effects. Therefore, more than 3 min boiling in 3% SDS can be recommended, alone or in combination with other methods in hospital and laboratory practice.

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