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Glycidol Degrades Scrapie Mouse Prion Protein

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ABSTRACT. Agents of transmissible spongiform encephalopathy (prion) are known to be extremely resistant to physicochemical inactivation procedures such as heat, radiation, chemical disinfectants such as detergents, alcohols, glutaraldehyde, formalin, and so on. Because of its remarkable resistance, it is difficult to inactivate prion. Chemical inactivation seems to be a practical method because it is applicable to large or fixed surfaces and complicated equipment. Here, three epoxides: β-propiolactone, propylene oxide, and glycidol (GLD) were examined of their inactivation ability against scrapie-mouse prion protein (PrP^{Sc}) under various conditions of chemical concentration, incubation time, and temperature. Among these chemicals, GLD worked most effectively and degraded PrP into small fragments. As a result of the bioassay, treatment with 3% GLD for 5 hr and 5% GLD for 2, 5 hr or 12 hr at room temperature prolonged the mean incubation time by 44, 30, 110 and 73 days, respectively. From dose-incubation time standard curve, the decrease in infectivity titers were estimated as 10^3 or more. Therefore, degradation of PrP^{Sc} by GLD decreased the scrapie infectivity. It is also suggested that pH and salt concentrations influence the effect of GLD. Although further study is necessary to determine the optimal condition, GLD may be a potential prion disinfectant.

KEY WORDS: decontamination, gycidol, prion, scrapie.

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Transmissible spongiform encephalopathies (TSEs) are neurodegenerative disorders of humans and animals such as Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-heinker syndrome (GSS), sheep scrapie, and bovine spongiform encephalopathy (BSE). The hallmark of TSEs are neuronal vacuolation, astrogliosis, and the accumulation of an abnormal isoform of prion protein (PrP^{Sc}) in the central nervous system [15]. PrP^{Sc} is generated from a host-encoded cellular form of prion protein by post-translational modification including conformational transformation. Many studies suggest that PrP^{Sc} is a major component of TSE agents (prion), however, it is still unknown whether prion consists solely of PrP^{Sc} [14, 15]. There are at present approximately 200 cases of iatrogenic CJD as a result of contaminated growth hormone and gonadotrophin, contaminated dura mater and corneal grafts [22]. Nosocomial transmission has also occurred via contaminated neurosurgical instruments and implantation of electroencephalographic electrodes from a patient with CJD to a new recipient [22].

Prion has a number of unusual characteristics including a remarkable resistance to physicochemical inactivation procedures such as heat, ionizing, UV, microwaves, radiation, and conventional chemical disinfectants such as detergents, alcohol, glutaraldehyde, and formalin [2, 4]. Consequently, prion decontamination requires very harsh treatment to eliminate all infectivity detectable through bioassay [1, 16]. Sodium hypochlorite is one of the effective chemical for prion inactivation, but on the other hand, has a practical disadvantage of being corrosive to skin, fabrics, and certain metals [3, 12]. Autoclaving at 134°C is a physical way to inactivate prion if medical instruments are heat-endurable [19].

It is known that the complete inactivation of prion is often difficult if the initial titter of infectivity is high [1, 20]. However, as long as the daily disinfection of medical equipment is concerned, the infectivity titer of the contaminated materials is relatively low [1]. Therefore, a mild decontamination procedure would be adequate so that contaminated materials are not damaged by severe chemical or physical treatments.

Ethylene oxide (EO) gas was reported to have failed prion inactivation [2, 12]. In contrast, we recently found that liquid ethylene oxide (LEO) was effective to inactivate prion (unpublished data). LEO is, however, flammable and volatile at temperature higher than 10°C, and the produced gas is highly toxic. Therefore, we screened three epoxides: β-propiolactone (β-PL), propylene oxide (PO), and glycidol (GLD), which resemble to EO in their structures but are easier in handling than LEO. β-PL is an alkylating substance known to be quite effective on virus inactivation [11]. PO is available as intermediary of propylene glycol and less toxic than EO. GLD is a slightly viscous liquid soluble in both water and organic solvents. It has been used as a stabilizer in the manufacture of vinyl polymers, an intermediate in the pharmaceutical products, an additive for synthetic hydraulic fluids and oils, and a diluent for epoxy resins.

Here we describe that among the three epoxides tested, GLD degraded scrapie-mouse PrP^{Sc} and inactivated scrapie

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infectivity. The results suggest that GLD may be a potential prion disinfectant.

MATERIALS AND METHODS

Preparation of scrapie-brain homogenates: The mouse-adapted scrapie strain Obihiro passaged by intracerebral inoculation [18] was used in this study. The brains of scrapie-affected ICR mice at the clinically final stage of disease were homogenized in 9 volumes of phosphate-buffered saline (PBS, pH 7.5). After sonication for 5 min, the homogenates were centrifuged at 1,000 rpm for 5 min. The supernatant was aliquoted and stored at –30°C as the scrapie-brain homogenates (10% homogenates) until use.

Preparation of recombinant mouse PrP: Recombinant mouse PrP (rMoPrP) corresponding to mouse PrP codon 23 to 230 was expressed in *E. coli* strain BL21 (DE3) pLysS as described previously [21]. Bacterial cells were collected by centrifugation at 7,000 rpm for 15 min. The pellets were suspended in 10 m*l* of 50 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl and 1 mM phenylmethylsulfonyl fluoride (PMSF), pH 8.0 per 1 g pellet and incubated with 0.1 mg of lysozyme for 10 min at 4°C. Then sodium deoxycholate was added at a final concentration of 0.1% and the bacterial suspensions were kept for 10 min at 4°C. In order to reduce the viscosity caused by bacterial chromosomal DNA, the suspensions were further treated with 0.5 mg of DNase I for 20 min at room temperature (r.t.). After the centrifugation at 9,000 rpm for 10 min, the pellets were washed with 0.5% Triton X-100, 10 mM EDTA, pH 8.0 for several times. Finally, the pellets of inclusion bodies were dissolved in 6 M guanidine hydrochloride (GdnHCl), 20 mM sodium phosphate buffer (pH 7.8) and adsorbed to a 3 m*l* column of nickel ion-charged Chelating Sepharose Fast Flow (Amersham Pharmacia, Uppsala, Sweden). The column was washed with 15 m*l* of 6 M GdnHCl and 20 mM sodium phosphate buffer (pH 7.8) then with 20 m*l* of 6 M GdnHCl and 20 mM sodium phosphate buffer (pH 4.9), and then the bound materials were eluted with 6 M GdnHCl and 20 mM sodium phosphate buffer (pH 4.3). The eluates were dialyzed against acetate buffer, pH 5.6 for 24 hr, then against acetate buffer, pH 4.4 for 24 hr, and against acetate buffer, pH 3.6 for 24 hr. The dialyzed eluates were centrifuged at 10,000 rpm for 10 min, and then the supernatant containing 1 to 3 mg of proteins was loaded to a TSK-GEL PA5PWRP reverse phase HPLC column (Tosoh, Tokyo, Japan). A monomeric rMoPrP with intramolecular disulfide bond was eluted with 30 to 50% acetonitrile linear gradient in the presence of 0.05% trifluoroacetic acid. Fractions containing the rMoPrP were lyophilized and dissolved with distilled deionized water (DDW).

Chemical treatment of the scrapie-brain homogenates, rMoPrP, and PrP-unrelated protein: Three epoxides, β-PL, PO, and GLD (2,3-epoxy-1-propanol), resembling to ethylene oxide in their structures were used. All of them were purchased as reagent-grade laboratory chemicals (Wako Pure Chemical; Tokyo, Japan).

The scrapie-brain homogenates were diluted to a final concentration of 1% with PBS and one of the epoxides used for the experiment. The samples were incubated with mild rotation. The reaction was stopped by incubating with 10 volumes of methanol at –20°C for 30 min and the remaining chemicals were removed by centrifugation at 15,000 rpm for 10 min at 4°C.

The rMoPrP was incubated with one of the epoxides under the conditions described in each experiment. The reaction was stopped by adding 50% trichloroacetic acid (TCA) to the samples at a final concentration of 5 to 10%. The samples were then held at –20°C for 10 min , and centrifuged at 15,000 rpm for 10 min. The pellets were washed with acetone and dissolved with SDS-PAGE sample buffer.

To measure the amount of protein in GLD-treated samples, 300–500 μg of rMoPrP was treated with GLD at r.t. and recovered by TCA precipitation. The pellets were resuspended with DDW and the protein concentration was measured using DC Protein Assay (Bio-Rad, Hercules, CA) according to the supplier's instruction. Triplicate samples were used for each reaction.

As a PrP-unrelated protein, 0.5 mg/m*l* human serum albumin (HSA) (Nihon Pharmaceutical; Tokyo, Japan) was used.

Soerensen's phosphate buffer [7] was made by mixing 1/15 M KH_2PO_4 and 1/15 M Na_2HPO_4 for adjusting pH, then NaCl was added to give a final concentration of 150 mM. Phosphate buffer was made by mixing 0.2 M NaH_2PO_4 and 0.2 M Na_2HPO_4 for adjusting pH. These buffers were used in some experiments instead of PBS to make 1% scrapie-brain homogenates. pH of the samples was estimated from the mixtures of phosphate buffer and 10% (w/v) brain homogenates of uninfected mice that were prepared by the same way as the scrapie-brain homogenates.

Bioassay: GLD-treated samples were methanol-precipitated and rinsed once with methanol to avoid the potential toxicity of residual GLD. The pellets suspended in original volume of PBS were inoculated intracerebrally (20 μl per mouse) into 4-week-old female ICR mice (n=6 to 8). The mice were kept until clinically final stage of disease and were put into euthanasia. One percent of chemical-untreated scrapie-brain homogenates, which were prepared by the same way as GLD-treated samples, were used as a chemical-untreated control. The infectivity titers of the samples were estimated by the dose-incubation period standard curve made by inoculating log dilutions of scrapie-brain homogenates with PBS intracerebrally (unpublished data). To confirm scrapie infection, brains and spleens of dead or sacrificed mice were examined for the presence of PrP^{Sc} by Western blot.

Sample preparation of brain and spleen: Twenty milligrams of brain tissues of mice were homogenized with 9 volumes of Triton-buffer (4% Triton X-100, 150 mM NaCl, 5 mM $MgCl_2$, 50 mM Tris-HCl pH 7.5) and digested with 20 μg of proteinase K at 37°C for 1 hr. The samples were centrifuged at 22,000 rpm for 10 min. Seventy milligrams of spleen tissues were homogenized with 9 volumes of Tri-

ton-buffer and digested with 30 μg of DNase I and 350 μg of collagenase at 37°C for overnight. After the digestion with 40 μg of proteinase K at 37°C for 2 hr, the reaction was stopped by adding 2 mM PMSF. The sample was then centrifuged at 40,000 rpm for 20 min and the pellet was suspended in 8 volumes of 6.25% Sarkosyl, 50 mM Tris. After incubation at 37°C for 30 min, the sample was centrifuged at 15,000 rpm for 5 min. The supernatants were salted out with 12% NaCl and centrifuged at 55,000 rpm for 40 min.

Detection of PrP^{Sc} and rMoPrP: Samples were suspended in SDS-PAGE sample buffer (50 mM Tris-HCl pH 6.8, 2% SDS, 6% 2-mercaptoethanol, 10% glycerol, and 0.02% bromophenol blue) and electrophoresed either with 12 or 15% polyacrylamide gels unless specifically mentioned. Kaleidoscope Polypeptide Standards (Bio-Rad, Hercules, CA) was used as a molecular mass marker. Western blot analysis was performed using B103 antibody by the method previously described [5, 8]. The image was visualized using ECL Western blotting detection reagents (Amersham Pharmacia; Buckinghamshire, England) and the following step either by Image Reader LAS-1000 (FUJI Photo Film; Tokyo, Japan) or by the exposure to FUJI Medical X-ray film, RX-U (FUJI Photo Film; Tokyo, Japan). The protein degradation was analyzed by silver staining with 2D-Silver Stain "Daiichi" (Daiichi Pure Chemicals, Tokyo, Japan) or with coomasie brilliant blue (CBB) staining.

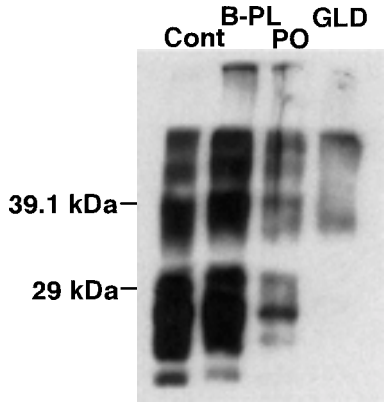

Fig 1. The effect of glycidol (GLD), propylene oxide (PO), and β-propiolactone (β-PL) on PrP^{Sc}. One percent scrapie-brain homogenates were treated with GLD, PO, or β-PL at 5% for 48 hr at r.t. The chemicals were removed by methanol precipitation and the pellets were dissolved with SDS-PAGE sample buffer. The antigenicity of PrP^{Sc} was examined by Western blot analysis using B-103 antibody. One hundred micrograms of brain equivalent was loaded in each lane. Cont: untreated one percent scrapie-brain homogenates. Molecular mass markers are indicated in kDa on the left.

RESULTS

Comparative analysis of the effect of three epoxides: The effect of β-PL, PO and GLD on mouse PrP^{Sc} was roughly estimated by monitoring the decrease in signals of PrP^{Sc} detected by Western blot analysis using B-103 antibody. When 1% (v/w) scrapie-brain homogenates were treated with 5% of respective chemicals at room temperature (r.t.) for 48 hr, the signals of PrP^{Sc} treated with β-PL did not change. GLD caused marked decrease of the signals of PrP^{Sc} and PO moderately decreased the signals (Fig. 1). These data revealed that GLD is the most effective in decreasing the immunoreactivity to B-103 antibody among the three epoxides tested. Thus, we focused on GLD in the following experiments.

Degradation of rMoPrP by GLD: To investigate whether the decrease in signals of PrP^{Sc} in Western blot analysis was caused by modulation of epitope recognized by B-103 antibody or by protein degradation, we treated rMoPrP with GLD. The molecular mass of rMoPrP was shifted from the original 29 kDa to slightly higher mass after the treatment with GLD (Fig. 2A). This implies that GLD covalently bound to rMoPrP molecules. Parallel to the molecular mass shifts, the bands of rMoPrP detected by CBB staining and Western blot analysis faded. These data suggest that the decrease in signals of PrP^{Sc} was not due to the modification of B-103 epitope, but due to the protein degradation. In Fig. 2A, the fragments of small molecular mass which would indicate the degraded product of rMoPrP were not observed, therefore, the samples were electrophoresed into a 20% gel in order to detect the degraded rMoPrP fragments generated by GLD treatment. The electrophoresis was stopped shortly after the line of bromophenol blue passed the stacking gel and the fragments were detected by silver staining. The faint bands smaller in molecular size than rMoPrP, which were not present in GLD-untreated rMoPrP were observed (Fig. 2B, indicated by arrow). These results support the idea that the loss of PrP^{Sc} by Western blot analysis was not due to the modification of B-103 epitope by GLD, but due to the degradation of rMoPrP by GLD. The degradation of rMoPrP into small fragment by GLD treatment was also confirmed that GLD treatment caused decrease of TCA precipitable protein (Fig. 2 C). Treatment with 5% GLD for 2, 6, and 24 hr reduced the amount of TCA-precipitable protein by 24 , 48 , and 56%, respectively (Fig. 2C). Therefore, we concluded the major cause of the loss of PrP^{Sc} bands (23–29 kDa in size) by GLD treatment was the degradation of PrP^{Sc}.

To examine whether the effect of GLD is PrP specific or not, we also treated HSA as PrP-unrelated protein with GLD at r.t. When incubated with 5% GLD for 6, 12, and 24 hr, the bands of HSA faded time-dependently and the slight increase in HSA molecular mass was observed (Fig. 2D). These results indicate that GLD bound to HSA and then cleaved into small peptides. Therefore, the effect of GLD was not PrP specific.

Influences of the concentration, duration and temperature on the effect of GLD: We further examined the condi-

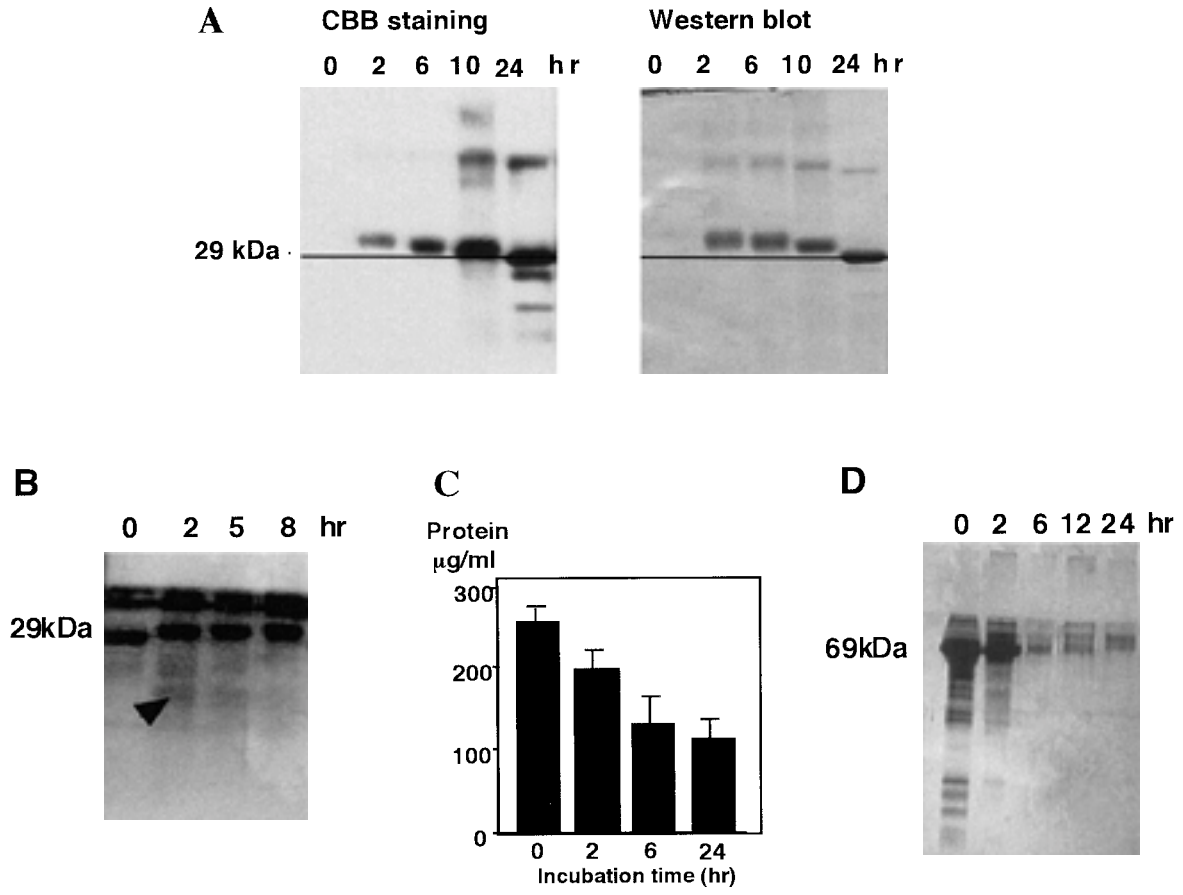

Fig. 2. Protein degradation by GLD treatment. A) Modulation of rMoPrP by GLD. rMoPrP was treated with 5% GLD for 2, 6, 12, and 24 hr at r.t. The rMoPrP was detected by CBB staining (left, 2 μg of rMoPrP was loaded in each lane) and by Western blot analysis (right, 0.1 μg of rMoPrP was loaded in each lane). B) Degradation of rMoPrP. rMoPrP was incubated with 5% GLD for 2, 5, and 8 hr at r.t. and detected by SDS-PAGE with a 20% acrylamide gel and the following silver staining. Five micrograms of rMoPrP was loaded in each lane. Arrow head indicates the degraded products. C) GLD treatment decreased the amount of rMoPrP. Three hundred micrograms of rMoPrP was incubated with 5% GLD at r.t. for 2, 6, and 24 hr. The reaction was stopped by TCA precipitation. The pellets were suspended in DDW for the protein assay. D) Degradation of HSA by GLD. HSA (0.5 mg/m*l*) was treated with 5% GLD for 2, 6, 12, and 24 hr at r.t. Four micrograms of HSA was loaded in each lane and detected by SDS-PAGE and silver staining. Molecular masses are indicated on the left.

tion under which GLD works more efficiently (Fig. 3). Although there was still much of PrP^{Sc} detected when the scrapie-brain homogenates were treated with 3 or 5% GLD at r.t. for 2 hr, treatment for 5 hr or more caused great reduction of PrP^{Sc} (Fig. 3A). Temperature also influenced the effect of GLD. Treatment at 35°C with 5% GLD for 2 hr diminished almost all the PrP^{Sc}, and the decrease of PrP^{Sc} was accelerated at 45°C or 55°C (Fig. 3B). These results suggest that the treatment with 3% GLD for 5 hr at r.t. is the minimum condition to eliminate all the detectable PrP^{Sc} by Western blot analysis and the treatment time can be shortened by the heating.

Effect of GLD on scrapie infectivity: Since GLD was able to degrade PrP^{Sc}, bioassay was carried out to examine whether GLD inactivates scrapie infectivity. Compared to the control mice inoculated with GLD-untreated scrapie-brain homogenates, the samples treated with 3% GLD for 2 hr, 5% GLD for 2 hr, 3% GLD for 5 hr, 5% GLD for 5 hr, and 5% GLD for 12 hr resulted in the prolongation of incubation time by 24, 30, 44, 110, and 73 days, respectively (Table 1). Among them, the differences of the incubation times of all the groups except for the group of mice inoculated the sample treated with 3% GLD for 2 hr were statistically significant. From the standard dose-incubation time curve, infectivity titer of GLD-untreated control was estimated as 1.6×10^5 LD_{50}/m*l*. Thus, the treatment with 3% GLD for 5 hr or 5% GLD for 2, 5, or 12 hr decreased the scrapie infectivity titer approximately by 10^2–10^3. This indicates that the degradation of PrP^{Sc} by GLD resulted in the inactivation of scrapie infectivity.

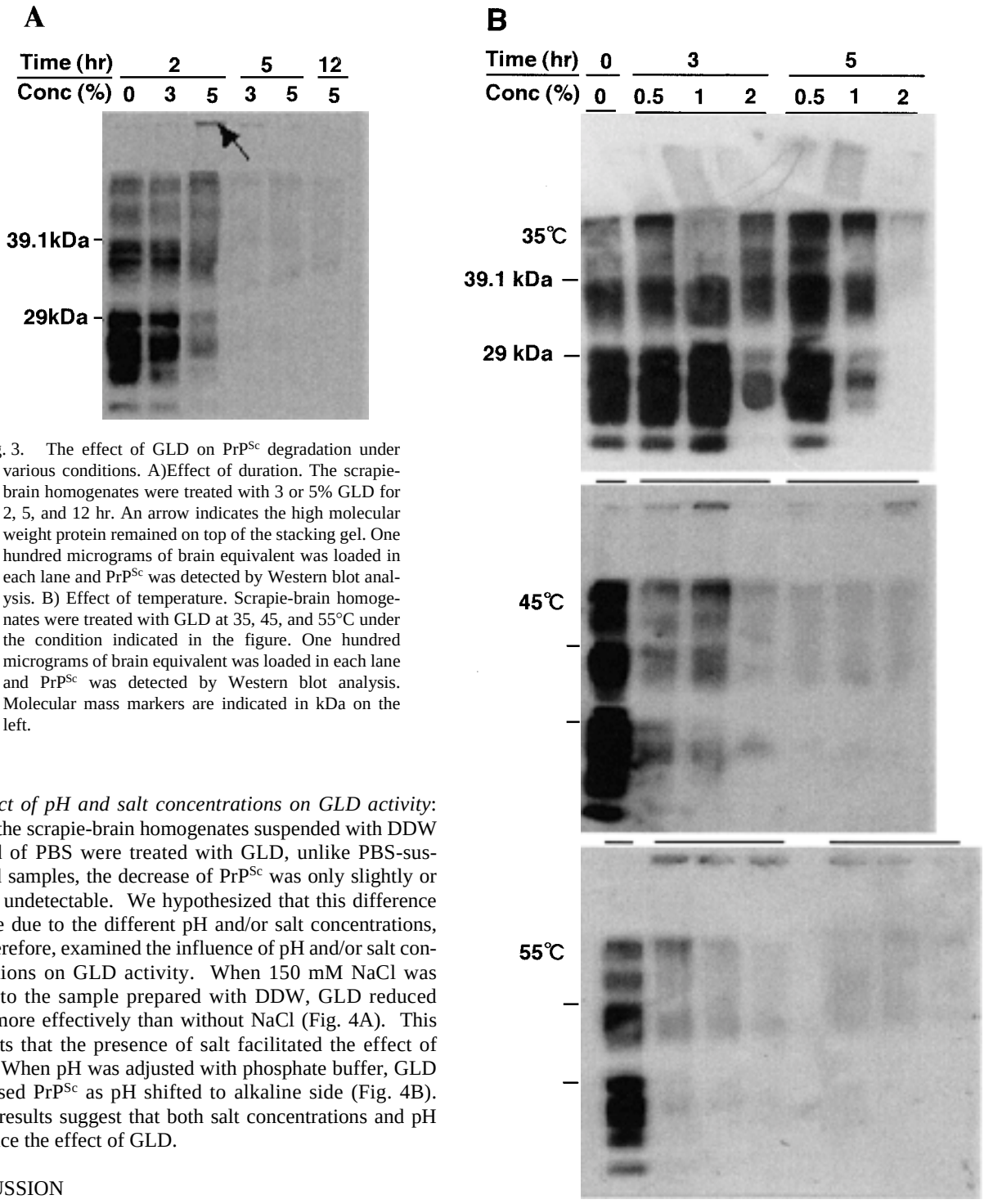

Fig. 3. The effect of GLD on PrP^{Sc} degradation under various conditions. A)Effect of duration. The scrapie-brain homogenates were treated with 3 or 5% GLD for 2, 5, and 12 hr. An arrow indicates the high molecular weight protein remained on top of the stacking gel. One hundred micrograms of brain equivalent was loaded in each lane and PrP^{Sc} was detected by Western blot analysis. B) Effect of temperature. Scrapie-brain homogenates were treated with GLD at 35, 45, and 55°C under the condition indicated in the figure. One hundred micrograms of brain equivalent was loaded in each lane and PrP^{Sc} was detected by Western blot analysis. Molecular mass markers are indicated in kDa on the left.

Effect of pH and salt concentrations on GLD activity: When the scrapie-brain homogenates suspended with DDW instead of PBS were treated with GLD, unlike PBS-suspended samples, the decrease of PrP^{Sc} was only slightly or almost undetectable. We hypothesized that this difference may be due to the different pH and/or salt concentrations, and therefore, examined the influence of pH and/or salt concentrations on GLD activity. When 150 mM NaCl was added to the sample prepared with DDW, GLD reduced PrP^{Sc} more effectively than without NaCl (Fig. 4A). This suggests that the presence of salt facilitated the effect of GLD. When pH was adjusted with phosphate buffer, GLD decreased PrP^{Sc} as pH shifted to alkaline side (Fig. 4B). These results suggest that both salt concentrations and pH influence the effect of GLD.

DISCUSSION

It is desirable that iatrogenic prion diseases such as iatrogenic CJD caused by contaminated dura mater transplantation must be prevented by simple decontamination procedure. Although there are some effective ways to inactivate prion, the more effective the procedure is, the decontamination procedures can become more hazardous [16, 20]. Therefore, we examined three epoxides: βPL, PO, and GLD, which resemble to EO in their structures but are easier in handling than LEO.

The result of Fig. 1 is consistent with the previous reports that β-PL is ineffective against prion inactivation [6]. Treat-

Table 1. Decrease in scrapie infectivity by GLD treatment

Treatment	Scrapie mice/ Inoculated mice	Incubation time (mean days ± SD)[a)]	Infectivity titre (LD_{50}/m*l*)	Decrease in infectivity titre
Untreated	6/6	184 ± 12	1.6×10^5	0
3%GLD 2 hr	6/6	208 ± 27	1.6×10^4	10
3%GLD 5 hr	6/6	228 ± 10*	$<1.6 \times 10^3$	$>10^2$
5%GLD 2 hr	6/6	214 ± 7*[b)]	$<1.6 \times 10^3$	$>10^2$
5%GLD 5 hr	6/7[c)]	294 ± 44*	$<1.6 \times 10^3$	$>10^2$
5%GLD 12 hr	7/8[d)]	264 ± 51*	$<1.6 \times 10^3$	$>10^2$

a) Mean incubation time was calculated from the number of days until death or cull.
b) Mice died at 207, 208, 215, 219, 222, and 328 days post inoculation (dpi). Although the mouse died at 328 dpi was positive for PrP^{Sc}, the considerable delay suggests certain failure in intracerebral incubation, and thus this mouse was excluded from a statistical analysis.
c) One mouse died of tumor at 328 dpi, but was negative for PrP^{Sc} both in brain and spleen.
d) One mouse died not show any clinical signs of scrapie during the observation period (up to 560 dpi). This mouse was negative for PrP^{Sc} both in brain and spleen.
e) *P<0.01 (student's T test).

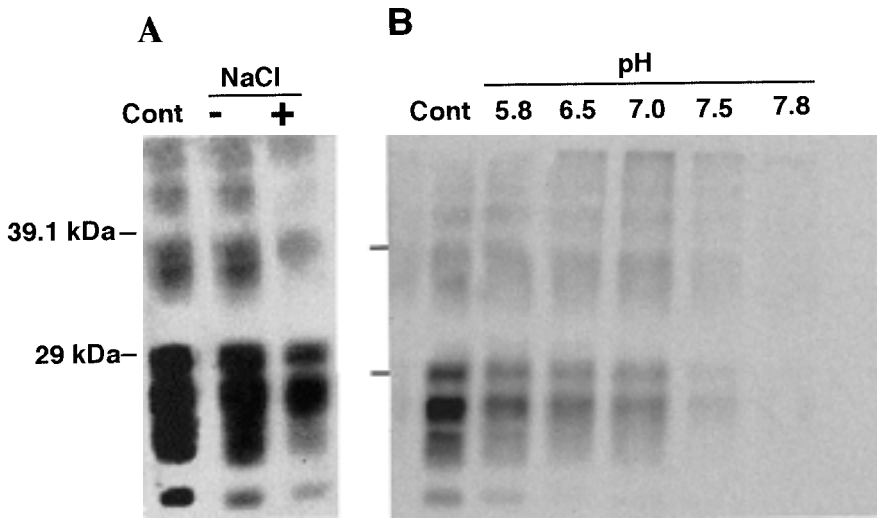

Fig. 4. Salt concentrations and pH influence the effect of GLD. A) Scrapie-brain homogenates diluted with DDW were treated with 5% GLD at r.t. for 2 hr in the presence or absence of 150 mM NaCl. One hundred micrograms of brain equivalent was loaded in each lane. Cont: GLD-untreated control sample in PBS (pH 7.5). B) Scrapie-brain homogenates were suspended with phosphate buffer and treated with 5% GLD at 55°C for 1 hr at indicated pH. Eighty micrograms of brain equivalent was loaded in each lane. Cont: GLD-untreated scrapie-brain homogenates in PBS. PrP^{Sc} was detected by Western blot analysis. Molecular mass markers are indicated in kDa on the left.

ment with 5% PO slightly reduced PrP^{Sc} antigenicity after 48 hr of incubation, which seems to be unpractical for disinfection. In contrast, treatment with 3 or 5% GLD for 12 hr dramatically reduced the detectable antigenicity of PrP^{Sc}. In addition, GLD is far more easier to handle than LEO and is stable in aqueous solution because its hydrolysis occurs rather slowly (t1/2 at 37°C is 100 hr) [17]. Therefore, GLD is the most potential on prion inactivation among the three epoxides.

Figure 2 shows that the loss of PrP^{Sc} by Western blot analysis was not due to the modification of B-103 epitope by GLD, but due to the protein degradation. Although it is not clear whether GLD cleaves amino acid-specific or not, GLD covalently binds and degrades protein into small peptide fragments that was not detectable in molecular size by SDS-PAGE using a 12% acrylamide gel. We used purified rMoPrP for this analysis of modification and degradation of the protein, because the signals of rMoPrP and PrP^{Sc} in Western blot decreased similarly by GLD treatment (Figs. 2 and 3) and large amounts of miscellaneous proteins in the homoge-

nates hampered the analysis of PrP^{Sc} by protein staining.

The result of the bioassay suggests that GLD reduced the scrapie infectivity. Consistent with the result of Western blot analysis, treatment with 3% GLD for 2 hr had a small effect on prion infectivity and the mean incubation period was only slightly prolonged compared to the control mice inoculated the GLD-untreated scrapie-brain homogenates. On the other hand, treatment with 3% GLD for 5 hr, 5% GLD for 2, 5, and 12 hr decreased the prion infectivity level by 10^2–10^3. Mean incubation time of the mice inoculated with the sample treated with 5% GLD for 5 hr was longer than the mice received the sample treated with 5% GLD for 12 hr because of the disparity in the incubation time of each mouse (Table 1). This suggests that a few portions of infectivity are considerably resistant to inactivation with GLD. It seems to support this explanation that in Fig. 3A antigenicity of most PrP^{Sc} was destroyed until 5 hr, but smear like signals of PrP^{Sc} oligomers with larger than 29 kDa were observed faintly in the sample even after 12 hr of incubation. Infectivity found in the samples treated with 5% GLD for 5 or 12 hr may associate with such GLD resistant oligomers.

Although 5% GLD could not prevent the onset of scrapie, it did reduce the infectivity titer by 10^2–10^3. Infectivity levels in human tissues are substantially lower than those of the mouse-passaged lines of scrapie prions [1]. Therefore, as long as the daily disinfection of medical equipments are concerned, the infectivity titer of the contaminated materials are relatively low. Application of pretreatment such as wash and wipe can also reduce the initial titer of the contaminated materials. In this case, GLD treatment may reduce enough of the agent to establish an infection.

We found that the effect of GLD increases dose-dependently and the treatment with 5% GLD for 5 hr at room temperature reduced most of the detectable PrP^{Sc} (Fig. 3A). When incubated at 35°C, the reduction efficiency was improved and the treatment with 5% GLD for 2 hr resulted to the same effect as 5% GLD treatment for 5 hr at room temperature (Fig. 3B). When incubated at 45°C or 55°C, GLD degraded PrP^{Sc} more efficiently as the incubation temperature increased. Therefore, either the increase in GLD concentration or the incubation at higher temperature can facilitate the GLD activity and consequently, can shorten the incubation time. The bioassay revealed that 2 hr treatment with 3% GLD at r.t. did not reduce much of the infectivity titer. However, as long as the results of in Fig. 4B are concerned, 3% GLD also degrades much of the PrP^{Sc} at 45°C and 55°C. It is likely that the treatment with 3% GLD for 2 hr at 55°C is as effective as other GLD treatment conditions.

Figure 4 suggested that pH and salt concentrations influence the effect of GLD. The results suggest that the influence of salt concentrations differed from pH and the contents of the buffers used but GLD degraded PrP^{Sc} more efficiently at alkaline environment if salt concentrations are unchanged. GLD is known to be reactive with sodium and degrades in acidic pH [17]. These factors need to be considered to optimize the GLD treatment condition. GLD is also known to be mutagenic and carcinogenic, however, there are great differences in susceptibility among species [9, 10]. For safety, it may be desirable to wash GLD off from the treated materials with weak acid because GLD degrades to glycerol in acidic environment [17].

However, it needs to be emphasized that GLD inactivation should be regarded as a treatment to lower titers of prion infectivity, thus, it is suboptimal such as for laboratories where in frequent use of high infectivity materials. This study implies that GLD may be a potential prion disinfectant such as for daily hospital use where drastic inactivation procedures cannot be applied and requires the treatment which is effective relatively in short time and lower chemical concentration.

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