Inactivation of Rabies Diagnostic Reagents by Gamma Radiation

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Treatment of CVS-11 rabies adsorbing suspensions and street rabies infected mouse brains with gamma radiation resulted in inactivated reagents that are safer to distribute and use. These irradiated reagents were as sensitive and reactive as the nonirradiated control reagents.

The Center for Disease Control (CDC) produces and distributes diagnostic and reference reagents for a large number of viral diseases of public health importance. These reagents are supplied to state, county, and regional health laboratories, laboratories overseas, and commercial producers of diagnostic reagents.

The CDC reagents are intended for use as reference products and meet rigid specifications for production and evaluation. Qualified laboratories are supplied with working volumes of reagents when comparable commercial products are not available.

The Biological Products Division of CDC has a policy of shipping inactivated reagents when they demonstrate the same sensitivity and reactivity as their infectious counterparts. Investigators (4–8; L. A. White, H. E. Hall, W. A. Chappell, and A. P. Kendal, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, S193, p. 272) in other laboratories have shown the usefulness of gamma radiation for inactivation of a variety of serological reagents.

The performance of the rabies fluorescent antibody test is a potentially hazardous operation when infectious reagents are used. Street rabies mouse brain impression slides are potentially infectious, and rabies mouse brain adsorbing suspension has traditionally been inactivated by betapropiolactone, a carcinogen (9). Studies (10) have shown that antigenicity is greater in agents killed by radiation than in those killed by chemicals or heat, suggesting that irradiation is a preferred method of inactivation.

Experiments were conducted to see if exposure to gamma radiation would be an effective method of inactivating the rabies reagents used in the fluorescent antibody test.

MATERIALS AND METHODS

Street virus. A frozen bobcat brain was obtained from the Florida State Health Department. A 20% (wt/vol) suspension of the brain was made in phosphate-buffered saline, pH 7.2, containing 20% (vol/vol) normal rabbit serum. The suspension was mixed on an Omnimix apparatus for 2 min, leached for 30 min at 4°C, and centrifuged at 16,300 × g in a Sorvall RC2B centrifuge for 30 min. The supernatant fluid was dispens ed and used as the seed virus in the experiments.

The virus had a 50% lethal dose titer of 10^-4.8/0.02 ml when inoculated intracranially in 7-day-old mice.

CVS-11 Virus. Rabies virus (CVS-11) was prepared in the same way as the bobcat brain. The CVS-11 virus had a 50% lethal dose titer of 10^-6.8/0.02 ml when inoculated intracranially into 7-day-old mice.

Mice. All suckling, weanling, and adult mice, ICR strain, were supplied by the Veterinary Services Branch of the CDC.

Gamma radiation source. The gamma rays were emitted by 60Co contained in a Gammacell 220 apparatus purchased from Atomic Energy of Canada, Ltd. Exposure to radiation is expressed in megareads.

Preparation of mouse brain impression slides. The 20% bobcat brain suspension was made into 10-fold dilutions, 10^-1 to 10^-6, and 0.02 ml was inoculated intracranially into 7-day-old mice. The mice that received the 10^-3 dilution, showing a 50% death rate on day 13, were killed. Three mice were used to make brain impression slides (2), and their brains were pooled and made into a 10% suspension for preradiation titration. Eighteen mice were layered with crushed ice and placed in a 32-oz (960-ml) Nalgene bottle. The bottle was placed in the elevator of the Gammacell. At 15-min intervals of radiation, the bottle was retrieved from the Gammacell, three mice were removed, and slides and 10% suspensions of brain pools were made. Slides were examined by the fluorescent antibody test. The 10% suspensions were inoculated into 5-day-old mice for infectivity titrations. The procedure was repeated for up to 45 min of radiation, and the 10% suspensions from the various intervals were inoculated into 2-day-old mice.

Preparation of adsorbing suspension. The CVS-11 rabies mouse brain suspension was diluted 1:100 in 0.73% bovallalbumin fraction V in phosphate-buffered saline, and 0.02 ml was inoculated into 7-day-old mice. On day 4, the mice were killed, and their brains were removed by aspiration. The brains were made into a 20% suspension with phosphate-buffered saline, homogenized, leached, and centrifuged as described above. A sample of the supernatant fluid was removed for the adsorption test and preinactivation titration.
The remainder of the supernatant fluid was placed in a 1-liter Nalgene bottle and lowered into the Gam-macell. At 15-min intervals, samples were removed for adsorption tests and titration. The various CVS-11 adsorbing suspensions were mixed with fluorescein isothiocyanate-labeled rabies horse globulin. A normal mouse brain adsorbing suspension was mixed with the conjugate as a control. The fluorescein isothiocyanate-adsorbing suspension mixtures were then placed on microscope slides containing rabies street virus mouse brain impressions and normal mouse brain impressions, and the slides were incubated at 37°C for 30 min in a moist chamber. The slides were rinsed, air dried, and examined with a fluorescent microscope (1).

RESULTS

As the brains of the mice infected with street rabies virus were removed from the cranial cavity, the gross morphology of those exposed to 15, 30, and 60 min of radiation appeared to be similar to the brains that were not irradiated. At the 45-min interval, one brain appeared to have lost its integrity; this may have been due to the virus or the radiation. Brains exposed to 75- and 90-min intervals were bloody and not suitable for preparation of mouse brain impression slides. The ice that was layered between the mice was still evident at the 75-min interval. At the 90-min interval, the ice was reduced to a slush having a temperature of 8°C.

Slides made from every mouse were evaluated by the fluorescent antibody test. All of the slides demonstrated a variety of antigenic material varying in size from that of “dust particles” to Negri bodies. The brains from the 0-, 15-, 30-, 45-, and 60-min radiation intervals were sufficiently resilient to make thin impressions. The specific staining intensity and the background staining were satisfactory on all slides. The amount of antigenic material present was excessive in many cases, a variable that is difficult to control and that varies with the source of rabies street virus. The titration endpoints of the rabies street virus brain suspensions are shown in Table 1. No infectious virus was detected in the brain suspensions exposed to ≥1.26 megarads of radiation.

The titration endpoints of the CVS-11 adsorbing suspensions are shown in Table 2. All of the irradiated adsorbing suspensions completely inhibited the specific staining reaction between the antirabies conjugate and the street rabies mouse brain impression slides. No infectious virus was detected in the adsorbing suspension exposed to 1.64 megarads.

DISCUSSION

As early as 1960 (3), investigators were successfully inactivating rabies virus through cor-puscular radiation. A number of variables must be considered in determining the criteria for inactivating rabies street virus by gamma radiation. The temperature of the exposure chamber is critical in that frozen suspensions retain greater antigenicity than do fluid suspensions (10). Freezing the mice intact would not be satisfactory since a freeze-thaw cycle would possibly disrupt the morphology of the brains used to make impression slides. The ideal temperature of the exposure chamber should be between zero and 8°C.

Inactivation of street rabies-infected brains before removal from the cranial cavity of mice is a safer procedure than inactivation after removal of brains. The tolerance of brain tissue morphology to gamma radiation is limited. Gross morphological changes were observed in brain tissue after exposure to 2.1 to 2.5 megarads. An exposure of 1.26 megarads was needed to completely inactivate the street rabies-infected brains. Consequently, the optimal exposure to gamma radiation is between 1.2 and 1.6 megarads for street rabies-infected brains and ≥1.6 megarads for the CVS-11 rabies adsorbing suspensions.

The sensitivity of the host system for determining infectivity titers is critical in determining the dosage of gamma radiation required. Two-day-old mice were generally more sensitive than five-day-old mice in detecting infectious virus in

<table>
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<th>Exposure time (min)</th>
<th>Gamma radiation (megarads)</th>
<th>Infectivity titer* of:</th>
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<tr>
<td></td>
<td></td>
<td>5-day-old mice</td>
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<tr>
<td>0</td>
<td>0</td>
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<tr>
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<td>75</td>
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<td>90</td>
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* Infectivity titer = log_{10}/0.02 ml of intracranial inoculum.
irradiated mouse brains.

Currently, CDC has inactivated rabies mouse brain impression slides and adsorbing suspensions available at CDC for distribution to qualified diagnostic laboratories. Future lots of these reagents will be inactivated by gamma radiation, and infectivity tests will be made before reagents are released.

The use of inactivated rabies reagents in diagnostic laboratories does not alleviate the necessity for practicing reliable safety measures. Since laboratory personnel will continue to process infectious specimens, preventive measures should include effective rabies immunization. Postexposure treatment will depend upon the nature of the exposure and the immune status of the individual. The use of inactivated rabies reagents simply decreases the risk of exposure to rabies virus in a diagnostic laboratory.

LITERATURE CITED