Inhibition of Influenza Virus Replication in Mice by GG167 (4-Guanidino-2,4-Dideoxy-2,3-Dehydro-N-Acetylenuraminic Acid) Is Consistent with Extracellular Activity of Viral Neuraminidase (Sialidase)

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We demonstrate the potent antiviral activity of a novel viral neuraminidase (sialidase) inhibitor, 4-guanidino-2,4-dideoxy-2,3-dehydro-N-acetylenuraminic acid (GG167), administered by the intranasal route in comparison with those of amantadine and ribavirin in experimental respiratory tract infections induced with influenza A and B viruses. In an extended study in which mice were infected (day 0) with influenza A/Singapore/1/57 virus, with treatments given prophylactically plus twice daily over days 0 to 3 and with mice observed to day 10, we show that intranasally administered GG167 at 0.4 and 0.01 mg/kg of body weight per dose reduced mortality, lung consolidation, and virus titers in the lungs, with no virus growing back following the cessation of treatment. In other studies with influenza B/Victoria/102/85 virus in which infected mice were culled after the cessation of treatment, the calculated intranasal dose required to reduce virus titers in the lungs of treated animals to 10% of that seen in untreated controls (ED_{AUC} [where AUC is area under the virus titer days curve]) was 0.085 mg/kg per dose. GG167 was inactive against influenza viruses A and B when given by the intraperitoneal or oral route (ED_{AUC} > 100 mg/kg per dose). GG167 was metabolically stable, with an elimination half-life of 10 min following intravenous administration. While readily bioavailable by systemic routes, it was poorly bioavailable by the oral route. Its potent efficacy by the intranasal route but lack of efficacy by other routes, relative to those of amantadine and ribavirin, was explicable in terms of its vitro activity, bioavailability, and pharmacokinetic properties and with the extracellular activity of viral sialidase.

Influenza viruses cause respiratory tract infections in a wide range of species, some of which, notably mice (9) and ferrets (11), have been widely used as models for influenza virus infection in humans.

We have used both of these species for the evaluation of the antiviral activities of novel inhibitors of influenza virus sialidase and have reported that one of these inhibitors, 4-guanidino-2,4-dideoxy-2,3-dehydro-N-acetylenuraminic acid (GG167), is an effective inhibitor of influenza A/Singapore/1/57 virus in mice and ferrets when the compound is administered intranasally but not when it is administered intraperitoneally to mice (14). Although influenza virus replicates primarily in the surface epithelial cells of the respiratory tract and effective treatment by direct (respiratory) application is to be expected, the lack of activity seen after intraperitoneal administration was disappointing since both ribavirin and amantadine are reported to effectively inhibit influenza virus replication when they are administered systemically (3, 13).

We therefore examined further the activity of GG167 against both influenza A and B viruses in influenza virus-infected mice and its pharmacokinetic properties when administered intranasally, orally, or parenterally.

**MATERIALS AND METHODS**

*Reagents.* Unless stated otherwise all reagents, medium components, Madin-Darby canine kidney (MDCK) cells, non-mouse-adapted virus strains influenza A/Singapore/1/57 and influenza B/Victoria/102/85, and the antiviral agents amantadine (1-amino-adamantine), ribavirin (Virazole; 1β-d-ribofuranosyl-1,2,4-triazole-3-carboxamide), and GG167 (5-acetylamino-2,6-anhydro-4-guanidino-3,4,5-trideoxy-D-galacto-non-2-enoic acid) were obtained from the sources given previously (15).

**Influenza virus infection in mice.** The protocol for infecting mice with influenza virus was essentially that described previously (14). Briefly, mice were anesthetized by the inhalation of ether and were inoculated intranasally with 50 μl of virus suspension (approximately 25 μl into each nostril). When subsequent treatments were to be given by the intraperitoneal or oral route, each mouse received 1.5 × 10³ 50% tissue culture infective doses (TCID₅₀ₐₘ) of influenza A/Singapore/1/57 or 1.0 × 10⁴ PFU of influenza B/Victoria/102/85, which was a nonlethal challenge. However, when treatments were to be given by the intranasal route, each mouse received a 10-fold reduction in the challenge dose to compensate for the exacerbation of infection associated with repeated inoculations of fluid into the respiratory tract (12). Under these conditions the challenge contained 10 to 100 50% lethal doses. Two types of efficacy tests were used. In one, the period of observation was over the 10 days postinfection, and we assessed efficacy in terms of the death in the virus titer in lung homogenates (percent area under the virus titer days curve [AUC] relative to that for the controls), reduction in lung consolidation (superficial macroscopic discoloration), and reduction in mortality. In the other type of test, the period of observation was limited to
TABLE 1. Efficacy of GG167 by intranasal dosing against influenza virus A/Singapore/1/57

<table>
<thead>
<tr>
<th>Treatment (mg/kg/dose)*</th>
<th>No. of mice</th>
<th>Group median (minimum, maximum) virus titers in lung homogenate (log_{10} TCID_{50}/ml) on days**</th>
<th>No. of mice dead/total no. of mice</th>
<th>% AUCd</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>39</td>
<td>&lt;2.3 3.0 (&lt;2.3, 4.6) 4.2 (3.5, 5.1) 5.6 (&lt;2.3, 5.8) 4.8 (&lt;2.3, 5.6) &lt;2.3 (&lt;2.3, 5.6) 3.7 (2.6, 3.8) 100</td>
<td>0/39</td>
<td>0.49</td>
</tr>
<tr>
<td>0.01</td>
<td>37</td>
<td>3.8 (3.3, 3.8) 5.9 (5.0, 6.8) 6.9 (6.1, 7.8) 7.1 (6.6, 7.3) 5.1 (4.1, 6.6) 4.9 (&lt;2.3, 5.6) 4.2 (&lt;2.3, 4.6) 23/37</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>Control#</td>
<td>64</td>
<td>4.9 (3.3, 3.8) 6.2 (3.3, 7.6) 7.1 (7.1, 7.8) 6.3 (5.8, 6.8) 5.9 (5.1, 6.8) 5.8 (5.6, 6.6) No survivors</td>
<td>40/40</td>
<td>100</td>
</tr>
</tbody>
</table>

* Mice were dosed intranasally with 50 μl at −18, −3, and 3 h and then twice daily on days 1 to 3.
# Four mice were culled from each group indicated otherwise.
Unscheduled deaths.
Unscheduled AUC was calculated from mean virus titers (log_{10} TCID_{50} per milliliter) in lung homogenates over days 0 to 6.
No control animals survived beyond day 6, and all virus titers on days 8 to 10 for the two treatment groups were <2.3.
There were only three mice per group.
P < 0.01.
Mice were dosed with vehicle.

3 to 6 days postinfection, and assessments of efficacy were confined to measuring the reduction in virus titers in lung homogenates on days 2, 3, 4, and 6, as appropriate. Except where indicated otherwise, drug administration was essentially performed as described previously (14) and involved prophylactic and postinfection treatments.

Assay of virus in lung homogenate samples. At designated intervals, four animals from each group were culled, the lungs were removed aseptically, and the extent of consolidation was expressed as a percentage of the total lung surface (10). The titer of influenza A/Singapore/1/57 infectious virus in lung homogenate was determined by enzyme-linked immunosorbent assay by a modification of a method described previously (2), while the titers of influenza B/Victoria/102/85 were determined by a plaque assay essentially as described previously (7).

The methods for deriving the AUC for virus titers in lung homogenates relative to those in lung homogenates of placebo-treated mice (AUC, in percent) for a specific dose of compound and the dose (milligrams per kilogram per dose) required to reduce the AUC in a treated group to 10% of that seen in a corresponding placebo-dosed control group (ED_{50}AUC_d) were as described previously (14). Statistical analysis for comparing treatment groups was conducted by logrank tests (mortality data), the Kruskal-Wallis rank sum test (lung consolidation scores), and analysis of variance on ranked data and then a t test with Sidak adjustment (lung homogenate virus titers).

Pharmacokinetics. GG167, amantadine, and ribavirin were administered to mice by the oral, intraperitoneal, intravenous (0.2 ml), and intranasal (50 μl) routes to compare their relative bioavailabilities. At predetermined time intervals, three to five mice were culled and samples of blood were removed and pooled. Serum was recovered from the blood by centrifugation. Urine samples were collected and pooled from similar groups of mice kept in metabolism chambers over the 24-h period after compound administration. Blood level profile studies were not conducted with ribavirin. Serum and urine samples were then assayed for their content of parent compound, which was expressed in terms of the concentration in serum or the percentage of the original material recovered from urine.

Assay of amantadine, GG167, and ribavirin in serum and urine samples. (i) Amantadine. The concentrations of amantadine in serum and urine samples were measured by previously described methods by means of a gas-liquid chromatography system (4).

(ii) Ribavirin. The concentrations of ribavirin in urine samples were measured by a high-pressure liquid chromatography method. Urine samples were diluted 1 in 10 in distilled water and were then filtered through a 0.2-μm-pore-size Millipore filter, and the filtrate was transferred to an autosampler vial. A total of 50 μl of the filtered urine was directly chromatographed onto a Lichrosorb RP18 (Hichrome) column (250 by 46 mm) by using a distilled water mobile phase. The eluate was monitored with a UV detector at 220 nm and 0.02 absorbance units, full scale, range, with a flow rate of 1 ml/min. Quantitation was achieved by peak area ratio determinations. The limit of quantitation in serum and urine was 5.0 μg/ml.

(iii) GG167. The concentrations of GG167 in serum and urine samples were measured by a high-pressure liquid chromatography method. Serum samples were acidified with equal volumes of 4% perchloric acid and were centrifuged at 7,000 × g for 3 min, and the supernatant was transferred to an autosampler vial. Urine samples were diluted 1 in 10 in distilled water and were then filtered through a 0.2-μm-pore-size Millipore filter, and the filtrate was transferred to an autosampler vial. A total of 50 μl of the serum supernatant or filtered urine was directly chromatographed onto a Hypercarb C18 (Shandon) column (100 by 46 mm) by using a mobile phase consisting of 18% methanol and 0.5% trifluoroacetic acid in distilled water. The eluate was monitored with a UV detector at 230 nm and 0.01 absorbance units full scale range, with a flow rate of 2 ml/min. Quantitation was achieved by peak area ratio determinations. The limits of quantitation in serum and urine were 0.1 and 1.0 μg/ml, respectively.

RESULTS

Assessment of efficacy of GG167 by intranasal dosing against influenza A/Singapore/1/57. We assessed the efficacy of GG167 in treated mice over a 10-day period (in groups of three to four mice each) in terms of reductions in mortality, virus titers in lung homogenates, and lung consolidation scores relative to those in placebo-treated control mice (Table 1).

The mean time to death in the placebo group was 4.4 days, and all animals died (or were culled for analysis) by day 7. In the group treated with 0.4 mg/kg per dose there was a significant reduction in mortality, with no unscheduled deaths occurring over the 10-day period (P < 0.001). Similarly, there were only two unscheduled deaths (5.4%) in the group treated with 0.01 mg/kg per dose (P < 0.001), one on day 5 and the other on day 8. There were no significant differences in the reductions in mortality between groups treated with 0.4 and 0.01 mg/kg per dose (P > 0.05). Twenty-four of the placebo-treated control mice were culled (in groups of four mice each) over days 1 to 6 to estimate virus titers in lung homogenates and lung consolidation scores. However, because of 40 unscheduled deaths no mice were available for culling over days 7 to 10 postinfection. Over the period from days 0 to 6, the
TABLE 2. Efficacy of GG167 when treatments are delayed

<table>
<thead>
<tr>
<th>GG167 treatment regimen</th>
<th>% AUC (days 0–3) at doses (mg/kg/dose) of a,b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylactic</td>
<td></td>
</tr>
<tr>
<td>-18 h</td>
<td>12.5</td>
</tr>
<tr>
<td>-3 h</td>
<td>0.4</td>
</tr>
<tr>
<td>Therapeutic</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>GG167</td>
</tr>
<tr>
<td>Vehicle</td>
<td>GG167</td>
</tr>
<tr>
<td>Vehicle</td>
<td>GG167</td>
</tr>
</tbody>
</table>
| a Mouse were dosed intranasally with 50 μl at -18, -3, and 3 h and then twice daily on days 1 and 2 and were given a single dose (a.m.) on day 3.
| b Four mice were culled from each group unless indicated otherwise. Percent AUC was calculated from the mean virus titers (log10 TCID50s/ml per milliliter) in lung homogenates over days 0 to 3.
| c Virus titers were significantly reduced relative to those in controls days 2 and 3 (P < 0.05).
| d Virus titers were significantly reduced relative to those in controls on day 3 (P < 0.05).

was again a significant reduction in virus titers on day 3 (P ≤ 0.05) compared with those in a vehicle-dosed control group and an AUC (days 0 to 3) of 0.68%. However, when the two standard prophylactic treatments were replaced with treatment with the vehicle, thus effectively delaying treatment until 3 h postinfection, GG167 was less active, with no significant reduction in virus titers on days 2 and 3 (P > 0.05) compared with those in a placebo-dosed control group and an AUC (days 0 to 3) of 5.7%. At the higher dose of 12.5 mg/kg per dose, GG167 was active even when treatments were delayed until 3 h postinfection, with an AUC (days 0 to 3) of <0.005%.

Assessment of relative efficacy of GG167 by oral dosing against influenza A/Singapore/1/57. GG167 and ribavirin were inactive by oral dosing, with no significant reductions in virus titers in lung homogenates on days 2 and 3 (P > 0.05) compared with those in lung homogenates of vehicle-dosed controls. This is reflected in ED50 values of >200 mg/kg per dose extrapolated from AUCs (days 0 to 3) of 10 and 16%, respectively, obtained at 200 mg/kg per dose and 97 and 23%, respectively, obtained at 100 mg/kg per dose. Amantadine was more active and at doses of 200 and 100 mg/kg per dose but not 25 mg/kg per dose, there were significant reductions in virus titers in lung homogenates on days 2 and 3 (P < 0.05) compared with those in lung homogenates of vehicle-dosed controls, which are reflected in AUCs (days 0 to 3) of 0.2, 0.8, and 12.5, respectively. An ED50 value of 28 mg/kg was calculated from these AUCs.

Assessment of efficacy of GG167 by multiple intraperitoneal dosing against influenza A/Singapore/1/57. Studies were conducted to investigate if GG167 could be shown to be active by the intraperitoneal route when, in addition to the standard once-daily prophylactic doses given at 18 and 3 h preinfection, the total amount of compound (milligrams per kilogram per day) given daily postinfection on days 0 to 3 or 0 to 4 was administered as multiple doses. GG167 was inactive when given prophylactically at 100 mg/kg per dose and then postinfection doses of 100 mg/kg per day were given as one, two, or six equally spaced daily doses on days 0 to 3, with AUCs (days 0 to 3) of 98, 52, and 106%, respectively. Ribavirin was also inactive when it was given prophylactically at 200 mg/kg per dose and then after postinfection doses of 200 mg/kg per day as one or two equally spaced daily doses on days 0 to 4, with AUCs (days 0 to 6) of 98 and 75%, respectively. While there was some indication of activity when the postinfection doses of 200 mg/kg/day on days 0 to 4 were given as three equally spaced daily doses, with an AUC (days 0 to 6) of 14%, there was a significant reduction in virus titers in lung homogenates on days 2 and 3 (P < 0.05) in mice given 200 mg/kg/day in four equally spaced daily doses compared with those in lung homogenates of vehicle-dosed controls, which was reflected in an AUC (days 0 to 6) of 5%. Amantadine showed some indication of activity when given prophylactically at 50 mg/kg per dose and then as once-daily postinfection doses on days 0 to 4, with an AUC (days 0 to 6) of 23%. Its activity improved when the postinfection doses of 50 mg/kg/day on days 0 to 4 were given as two, three, or four equally spaced daily doses, with significant reductions (P < 0.05) virus titers in lung homogenates on days 2, 3, and 6, corresponding to AUCs (days 0 to 6) of 1, 1, and 8%, respectively.

Assessment of efficacy of GG167 against influenza B/Victoria/102/85. Intranasal GG167 at 6.25, 1.6, and 0.4 mg/kg per dose produced significant reductions (P < 0.05) in virus titers in lung homogenates on days 2 and 3, corresponding to AUCs (days 0 to 3) of 0.12, 0.5, and 2.6%, respectively (Table 3). Ribavirin was active at 100 mg/kg per dose, with significant reductions (P < 0.05) in virus titers in lung homogenates on
days 2 and 3, corresponding to an AUC (days 0 to 3) of 1.0%. There was a good dose-response for GG167 and ribavirin in terms of AUCs at the doses used, and the ED\textsubscript{AUC0.05} values of 0.085 and 64 mg/kg/dose, respectively, calculated from fitted lines, showed that GG167 was 753 times more active than ribavirin following intranasal dosing.

Following intraperitoneal administration, GG167 and amantadine were inactive at 400 and 100 mg/kg per dose, respectively. Although a dose-response in terms of AUCs (days 0 to 3) was seen with ribavirin over the dose range tested, the ED\textsubscript{AUC0.05} of 269 mg/kg per dose calculated from a fitted line shows that it, too, was inactive and there were no significant reductions ($P > 0.05$) in virus titers in lung homogenates on days 2 and 3.

**Pharmacokinetic studies.** The availabilities of the test compounds to the systemic circulation were monitored by measuring levels in serum or recovery in urine following oral, parenteral, or intranasal administration.

GG167 was readily bioavailable following intravenous, intraperitoneal, and intranasal but not oral administration (Fig. 1). After intravenous administration of a single 50-mg/kg dose, a level of 106 µg/ml was seen in serum at 5 min, but this decreased rapidly to 1.5 µg/ml at 1.0 h, with a calculated elimination half-life of 10 min. After intraperitoneal administration of a single 50-mg/kg dose, a peak level of 92 µg/ml was seen within 10 min of dosing, and this, too, decreased rapidly to 6 µg/ml at 1.0 h. After intranasal administration of a single 25-mg/kg dose, a peak level of 12 µg/ml was seen at 10 min; this also decreased rapidly to a level of 3.7 µg/ml at 1.0 h. After oral administration of a single 100-mg/kg dose, a peak level of 2.0

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**FIG. 1.** Comparative concentrations in serum of GG167, amantadine, and ribavirin in mice following administration by several routes. ○, ribavirin administered intraperitoneally at 45 mg/kg (1); ○, amantadine administered intraperitoneally at 100 mg/kg; □, amantadine administered orally at 100 mg/kg; ■, GG167 administered intraperitoneally at 50 mg/kg; □, GG167 administered orally at 50 mg/kg; ▲, GG167 administered intranasally at 25 mg/kg; Δ, GG167 administered intravenously at 50 mg/kg.
μg/ml was seen at 10 min after dosing; it was possible to detect the compound (1.2 μg/ml) in only one of three mice at 20 min after dosing, and it was not detected (<0.1 μg/ml) at 40 min after dosing.

Amantadine was readily bioavailable following intraperitoneal and oral administration. After intraperitoneal administration a peak level of 33 μg/ml was seen within 15 min of dosing; this decreased slowly, with an elimination half-life of several hours, to 1.8 μg/ml at 4.0 h and a trace amount at 8 h. After oral dosing, a peak level of 19.4 μg/ml was seen within 15 min; again, the levels were still relatively high at 6.8 μg/ml at 4.0 h.

As shown by recovery in urine (0 to 24 h), GG167 was not readily bioavailable following oral administration (Table 4), but it was readily bioavailable following intraperitoneal and intranasal administration, with recoveries in urine after administration of a 50-mg/kg dose of 3, 68, and 43%, respectively. Both amantadine and ribavirin were readily bioavailable following oral, intraperitoneal, and intranasal administration (Table 4).

**DISCUSSION**

We have previously reported (14, 15) that GG167 is a highly effective inhibitor of influenza A/Singapore/1/57 and influenza A/Stockholm/24/90 viruses in mice when given intranasally, with an ED_{AUC_{0-5}} of 0.027 mg/kg. In these experiments the period of observation was confined to the 3 days following infection, and assessments of efficacy were restricted to measuring the reduction in mean virus titers in lung homogenates.

We reported here on further work in which mice were treated with an identical regimen at 0.4 and 0.01 mg/kg per dose, but the period of observation was extended to 10 days postinfection. Even at the 0.01-mg/kg per dose level, which is below the reported ED_{AUC_{0-5}} for A/Singapore in this model (14, 15), while there was no reduction in virus titers in lung homogenates, there were substantial reductions in mortality and lung consolidation scores over the 10-day period. Thus, we confirmed and extended the data given in our previous reports, which demonstrated that GG167 is a highly effective inhibitor of influenza A virus in mice following intranasal administration (14, 15). We further showed that the 3-day test data reported previously and that were based solely on reduction in virus titers in lung homogenates substantially underestimate the accompanying clinical effect of this compound in this experimental system.

In all of the experiments conducted to date, GG167 was shown to be very active when administered by the intranasal route when treatments were given prophylactically and postinfection. Subsequent experiments reported here show that at the treatment level of 12.5-mg/kg per dose, GG167 significantly reduced virus titers in lung homogenates on days 2 and 3, even when the first dose was delayed until 3 h postinfection.

However, when treatments at 0.4 mg/kg per dose were delayed until 3 h postinfection, there was no significant reduction in virus titers in lung homogenates on days 2 and 3, even though the AUC of virus titers was reduced to 5.7%. Therefore, we can be confident that in mice GG167 will be active when given in a therapeutic situation but that the ED_{AUC_{0-5}} will be higher than that seen following a prophylactic treatment regimen.

In other experiments in the present study, GG167 was compared directly with amantadine and ribavirin against influenza A and B viruses in mice by using the intranasal, intraperitoneal, and oral routes of administration. While various in vivo activities have been reported for amantadine (6) and ribavirin (for a review, see reference 5) for each of these selected routes, there are no in vivo data for ribavirin administered by the intranasal route. Since the different degrees of efficacy reported for the different model systems were probably attributable to variations in virus susceptibilities to the drugs in vitro, in vivo challenge levels, treatment routes, and the therapeutic end points used, further comments on comparative efficacy will be restricted to the data obtained with our model system.

In mice infected with influenza B/Victoria/102/85 virus, intranasally administered GG167 was 753 times more active than ribavirin, which is in agreement with its similarly superior in vitro activity and the fact that it was previously shown to be more active than ribavirin in vivo and in vitro tests against influenza A/Singapore/1/57 (14). GG167 was more than 5,000 times less active in vivo by the intraperitoneal route than by the intranasal route; this agrees with the 4,000 times reduction in activity that we have already reported against influenza A/Singapore/1/57 when GG167 was given by the intraperitoneal route (14). A totally different picture was seen with amantadine and ribavirin, at most they were only 4.2 to 6.6 times more active against influenza B/Victoria/102/85 and influenza A/Singapore/1/57 viruses by the intranasal route (Table 3) (14). Thus, we are led to the conclusion that the intranasal route of administration allows GG167 to maximize its therapeutic potential as an inhibitor of viral sialidase; the same cannot be claimed for amantadine or ribavirin because of their different modes of action.

Pharmacokinetic experiments following the administration of a single intraperitoneal dose showed that the levels of amantadine in plasma were prolonged, a feature that has also been described for ribavirin (1), and as such might be expected to give prolonged antiviral concentrations intracellularly and extracellularly at the site of virus replication, unlike GG167, which is rapidly eliminated from the plasma and additionally does not penetrate cells (5a). Also, other efficacy studies with GG167 showed that the efficacies of amantadine and ribavirin, but not that of GG167, improved in proportion to the frequency of dosing, probably as a consequence of slow elimination and the maintenance of effective antiviral concentrations of these agents at their sites of action.

Thus, it is likely that the relatively poor in vivo activity seen with GG167 in mice following intraperitoneal administration, despite good bioavailability, is due to its rapid clearance from the plasma, permitting poor penetration into respiratory secretions, coupled with its inability to penetrate and persist inside cells. In this respect GG167 probably behaves like another neuraminidase inhibitor, 2-deoxy-2,3-dehydro-N-acetylmuramic acid, which was also rapidly eliminated and was also inactive following parenteral administration (8). Similarly, the poor efficacy following oral dosing is probably a consequence of poor oral bioavailability in addition to these other factors. Better oral bioavailabilities in terms of levels in plasma and recovery in urine were seen with amantadine and ribavirin.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>% Recovery in urine (0–24 h)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG167</td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td>Amantadine</td>
<td>100</td>
<td>36</td>
</tr>
<tr>
<td>Ribavirin</td>
<td>100</td>
<td>45</td>
</tr>
</tbody>
</table>

* Mice were confined to a metabolism chamber.

The dose was administered in 0.5 ml of phosphate-buffered saline.

The dose was administered in 50 μl of phosphate-buffered saline.
(Table 4 and Fig. 1), which result in effective inhibitory antiviral concentrations at the site of virus replication at the doses used.

In addition to its superior activity to amantadine or ribavirin in reducing the virus titers of influenza A/Singapore/1/57 in the lungs of mice (14), we have now shown in subsequent tests with influenza A/Singapore/1/57 virus that after the cessation of therapy virus does not grow back, as determined by the mean virus titers in lungs, and at a treatment level (0.01 mg/kg per dose) at which there was no demonstrable reduction in virus titers in lungs, GG167 protected mice from lung consolidation and death. We have also shown here that intranasally administered GG167 has activity superior to ribavirin in reducing influenza B/Victoria/102/85 virus titers in the lungs of mice, but as with influenza A/Singapore/1/57 virus infections, it has no significant activity when given by the oral or intraperitoneal routes. We conclude that because of its specific activity against viral sialidase, the superior activity of intranasally administered GG167, relative to its activity when administered by other routes, in inhibiting virus replication in the respiratory tracts of mice is consistent with the extracellular activity of the viral sialidase.

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REFERENCES