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GG167 (4-Guanidino-2,4-Dideoxy-2,3-Dehydro-*N*-Acetylneuraminic Acid) Is a Potent Inhibitor of Influenza Virus in Ferrets

D. MICHAEL RYAN,* JULIA TICEHURST, AND MARIA H. DEMPSEY

Glaxo Wellcome Research and Development Limited, Stevenage, United Kingdom

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GG167 (4-guanidino-2,4-dideoxy-2,3-dehydro-*N*-acetylneuraminic acid) is a novel viral neuraminidase (sialidase) inhibitor which, following intranasal administration in ferrets, is at least 100 to 1,000 times more effective than ribavirin and amantadine against influenza A and B viruses. It retains its activity even when treatments are delayed until 24 h postinfection and has no effect on the serum antibody response to infection.

Neuraminidase is primarily responsible for promoting the release of progeny virus from infected cells, and the selective neuraminidase inhibitor GG167 has been shown to inhibit the growth of a wide range of influenza A and B viruses in vitro (4, 5). When given intranasally to mice infected with influenza A and B viruses, GG167 was 100 to 1,000 times more active than amantadine or ribavirin and retained efficacy when treatments were delayed until the postinfection period (2). We now report on the efficacy of GG167 by the intranasal route against strains of influenza A and B viruses in a ferret model and the effects on efficacy of delaying treatments until the postinfection period.

All reagents, medium components, MDCK cells, non-ferret-adapted virus strains, and antiviral agents were obtained from sources given previously (2, 5). Ferrets (female, 0.6 to 1.2 kg) were obtained from Glaxo Wellcome Research and Development, Bury Green, Hertfordshire, United Kingdom, and were used in groups of three to six animals. Methods for inducing infection, for assessing and quantifying infection in terms of the area under the curve values (AUC) over days 1 to 9 of the nasal wash virus titers ($\log_{10}$ 50% tissue culture infective doses or PFU per milliliter), and for the telemetric monitoring of body temperature were as described previously (2, 4). Significant pyrexia is defined here as the elevation of body temperature to 1°C (or more) above the preinfection (baseline) temperature for at least 12 h within the 30- to 96-h-postinfection period. Unless stated otherwise, the standard treatment regimen used in efficacy tests involved 13 individual doses, starting with a single dose at −26 h (day −1) and continuing with doses at −2 and 5 h on the day of infection (day 0), at 22 and 30 h (day 1), and twice daily 8 h apart on days 2 to 5 inclusive. Where possible, ribavirin and amantadine were included at relevant dose levels and tested in parallel with at least one dose level of GG167 for each of the three viruses. Statistical analyses on nasal wash virus titer AUCs to compare treatment groups within experiments were conducted with analysis of variance; where only two groups were involved, comparisons were made with Duncan's multiple range test and the *t* test. Reduction in the incidence of significant pyrexia was analyzed with Fisher's exact test.

* Corresponding author. Mailing address: Department of Chemotherapy, Biology Division, Glaxo Wellcome Limited, Gunnels Wood Rd., Stevenage, Hertfordshire SG1 2NY, United Kingdom. Phone: 01438 76 3870. Fax: 01438 76 4229. Electronic mail address: DMR3766@GGR.CO.UK

The profiles of nasal wash virus titers and temperature responses over days 1 to 9 in 31 vehicle-dosed infected ferrets used in these studies were as described previously (4) and were similar for the three viruses at the challenge levels used. A total of 29 of the 31 ferrets developed a significant pyrexia. Results of efficacy studies are given in Table 1. In influenza A/Singapore/1/57 virus-infected animals, GG167 significantly reduced nasal wash virus titer AUCs and the incidence of significant pyrexia ($P < 0.05$) with an $ED_{AUC_{10}}$ (effective dose required to reduce the AUC in a treated group to 10% of that seen in a vehicle-dosed control group) of <0.05 mg/kg of body weight per dose. Ribavirin, at 12.5 mg/kg per dose and tested in parallel with GG167, also significantly reduced nasal wash virus titers (AUC % [ratio of the AUCs for treated and vehicle-dosed controls] = 25, range of 12.3 to 43%) and the incidence (1/4) of significant pyrexia ($P < 0.05$). Amantadine was ineffective in reducing nasal wash virus titers (AUC % of 68%) and the incidence of significant pyrexia at 6.25 mg/kg per dose, the highest dose tolerated by this route. In influenza A/Mississippi/1/85 virus-infected ferrets, GG167 significantly reduced nasal wash virus titer AUCs and the incidence of pyrexia with an $ED_{AUC_{10}}$ of 0.06 mg/kg per dose. Ribavirin at 12.5 mg/kg per dose significantly reduced nasal wash virus titers (AUC % = 13.2, range of 6.6 to 22%) and the incidence (1/4) of significant pyrexia ($P < 0.05$). In ferrets infected with influenza B/Victoria/102/85 virus, GG167 significantly reduced nasal wash virus titer AUCs and the incidence of pyrexia ($P < 0.05$) with an $ED_{AUC_{10}}$ of <0.75 mg/kg per dose. Ribavirin at 50 mg/kg per dose and tested in parallel with GG167 also significantly reduced nasal wash virus titers (AUC % = 15, range of 5.8 to 69%) and abrogated (0/4) significant pyrexia ($P < 0.05$). The effect of delaying dosing on the efficacy of GG167 was studied in influenza A/Mississippi/1/85 virus-infected ferrets over the period of 1 to 9 days postinfection by directly comparing the standard treatment protocol involving prophylactic and postinfection doses and protocols in which the early doses were delayed until 5 or 22 h postinfection. In these experiments, GG167 at 0.3 and 0.05 mg/kg per dose reduced nasal wash virus titer AUCs and abrogated pyrexia (0/5), giving AUC % of 1.4 (range, 0.48 to 6.2%), 1.5 (range, 0.68 to 5.3%), and 2.9 (range, 1.7 to 5.4%) for GG167 at 0.3 mg/kg and 11.5 (range, 1.4 to 71%), 14 (range, 4.2 to 21%), and 32 (range, 10.2 to 82%) for GG167 at 0.05 mg/kg. With the exception of the AUC % value of 32%, all these reductions in nasal wash virus titers and pyrexia were significant ($P > 0.05$). Where treatments were omitted from the early part of the standard pro-

TABLE 1. Efficacy of GG167 by intranasal dosing against influenza virus infections in ferrets

Challenge virus	AUC % (range) and incidence of significant pyrexia (in brackets) at mg/kg per dose:									$ED_{AUC_{10}}$ (mg/kg per dose)
	6.25	1.5	0.75	0.3	0.2	0.1	0.05	0.0125	0.003	
A/Singapore 1/57 H2N2			0.06[a] (<0.05–0.15) [0/4][a]		0.16[a] (0.05–0.44) [0/4][a]		0.58[a] (0.23–2.6) [0/4][a]			<0.05
A/Mississippi 1/85 H3N2				1.4[a] (0.48–6.2) [0/4][a]		3.7[a] (1.5–6.5) [0/4][a]	19[a] (7.6–46) [1/5][a]	68 (33–195) [1/5][a]	106 (30–224) [4/5]	0.06
B/Victoria 102/85	0.5[a] (0.16–1.3) [0/4][a]	1.6[a] (1.2–2) [0/4][a]	2.4[a] (1.3–3.6) [0/4][a]							<0.75

[a] Response significantly reduced relative to vehicle-dosed infected control ferrets.

tocol, they were replaced by vehicle, and in these cases, additional doses were then given twice daily at the end of the normal treatment period to ensure that a constant amount of compound, given in 13 doses, was given in the three treatment protocols used in the experiment. Subsequent experiments have shown that the day 21 serum antibody titers to influenza A/Mississippi/1/85 virus following infection were similar (>1:1,280) in vehicle-dosed and GG167-dosed ferrets (0.1/mg/kg per dose) by the standard 13-dose treatment procedure.

GG167 was not readily bioavailable following intranasal administration, with a urinary recovery of unchanged material (0 to 24 h) of 5.7%. In contrast, 73% of intraperitoneally administered material was recovered in the urine. Assuming that the 73% represents the maximum recovery following parenteral dosing, then the percent bioavailability following intranasal administration is 7.8%, which is lower than the 43% seen in the mouse (2) but similar to the 10% reported in humans (1). In our model, the disease induced in susceptible ferrets following the intranasal inoculation of influenza virus is similar to that described previously (3); thus in many respects, both virological and constitutional, the model is representative of the disease syndrome seen in humans. In summary, by using the nasal wash virus titer (AUC %) responses and the presence or absence of significant pyrexia as a measure of in vivo activity, it can be seen that GG167, when tested in parallel but at different doses, is at least 100 to 1,000 times more active than ribavirin and amantadine against influenza A/Singapore/1/57 virus, influenza A/Mississippi/1/85 virus, and influenza B/Victoria/102/85 virus when given by the intranasal route, which is in keeping with the relative in vitro sensitivities of the viruses to these agents (5). GG167 was also effective in significantly reducing nasal wash virus titers and abrogating the pyrexic response when given at a dose of five times the $ED_{AUC_{10}}$ dose even when the initial treatment was given approximately 1 day postinfection, a time at which there was some evidence of virus shedding but before there were overt signs of pyrexia. GG167 had no effect on the serum antibody response to infection. We conclude that, if these laboratory observations on efficacy are repeatable in humans, then GG167 should be extremely effective in treating and controlling influenza A and B virus infections in humans when administered by topical (respiratory) application.

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