

The Pharmacokinetics, Antigenicity, and Fusion-Inhibition Activity of RSHZ19, a Humanized Monoclonal Antibody to Respiratory Syncytial Virus, in Healthy Volunteers

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Single ascending doses of RSHZ19 (also known as SB 209763), a humanized monoclonal antibody (MAb) directed to the fusion protein of respiratory syncytial virus, were administered to healthy men to evaluate the safety, pharmacokinetics, antigenicity, and fusion inhibition (FI) activity of RSHZ19. Doses of RSHZ19 (0.025–10.0 mg/kg) or placebo were infused over 30 min, and subjects were followed for 10 weeks. Plasma concentrations of RSHZ19 and RSHZ19-specific antibodies were determined by ELISAs. FI titers were used to evaluate the ability of plasma to inhibit virus-induced fusion of VERO cells previously infected with RS Long strain virus. Twenty-six subjects, mean age 24, completed the study. RSHZ19 was safe and well tolerated, and no subject developed antibodies to RSHZ19 during follow-up. RSHZ19 had low plasma clearance and a half-life of ~23 days, similar to native IgG. Increases in FI titers relative to pretreatment levels were seen 24 h after MAb administration in all 4 subjects given 10 mg/kg and in 2 of 4 given 5 mg/kg.

Respiratory syncytial virus (RSV), a pneumovirus of the family Paramyxoviridae, is a common respiratory pathogen in all age groups [1]. Infection with RSV tends to be seasonal with peaks in the winter in areas with temperate climates and during the rainy season in warmer regions. There are two subtypes, which differ primarily in the G surface glycoprotein. Although both subtypes, designated A and B, may circulate concomitantly during a single RSV season, one subtype usually predominates. Currently ribavirin, a synthetic nucleoside, is the only therapy approved for treatment of RSV infection. Pathologic changes associated with RSV infection include inflammation and necrosis of bronchiolar epithelium [2]. Airway obstruction leading to hyperinflation and atelectasis may be caused by mucous plugging that results from the inflammatory response to the virus. In some patients, wheezing may persist for years after presentation with bronchiolitis. The cause is unknown but may involve mediators such as RSV-specific IgE and leukotriene C4 [3].

The role of cell-mediated and humoral immunity in the pathogenesis of this disease is not completely understood. Cell-mediated immune responses may cause expression of the respiratory manifestations of RSV infection. Local immune mechanisms within the lung may also be responsible for the pulmonary component of clinical disease [3]. Infection with RSV provides incomplete immunity of limited duration, as demonstrated by the high rates of reinfection reported in studies in day care settings and in studies using nasal challenge techniques. Repeated infection may provide some reduction in the severity of disease over time [3–7].

Although there is no good animal model of clinical RSV disease, human RSV replicates in the upper and lower respiratory tracts of mice and cotton rats, and these species have been extensively used to evaluate RSV vaccines and antiviral agents. Monoclonal antibodies (MAbs) to the fusion (F) protein have been highly effective in preventing or treating RSV infection in this animal model [8, 9]. The F protein of RSV, which mediates fusion of the virus with cell membranes and subsequent cell-to-cell spread of the virus, is an important antigen in inducing cross-protective immunity [6, 7]. RSHZ19, a humanized MAb directed to the F protein, is currently under investigation for the treatment and prophylaxis of RSV infection in infants and young children. RSHZ19 has potent and equivalent neutralization and fusion-inhibition activity against RSV isolates *in vitro* and has been protective in animal models of RSV infection [10, 11]. With the exception of a small number of amino acid residues of mouse origin, the human framework of RSHZ19 MAb has been kept intact.

Based on currently available animal data and *in vitro* work, it appears that RSHZ19 may be effective in the treatment and prevention of RSV infection without inducing a clinically significant antigenic response, which sometimes accompanies the

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This study conformed with Title 21, US Code of Federal Regulations, and the Declaration of Helsinki, as amended. The protocol was reviewed and approved by the Institutional Review Board, Presbyterian Medical Center, University of Pennsylvania Health System. The nature of the study was fully explained to each subject, and written informed consent was obtained; subjects could withdraw from the study at any time.

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administration of a foreign protein. This study describes the first administration of RSHZ19 to humans. The objectives of the study were to demonstrate the safety and tolerability of single intravenous doses of RSHZ19 in healthy adult men and to evaluate the pharmacokinetics of RSHZ19, the development of antibodies to RSHZ19, and changes in fusion inhibition activity in plasma.

Materials and Methods

Study design. This was a single-blind, randomized, placebo-controlled, single-dose, parallel group, intravenous dose-rising study. Subjects were allocated at random to receive active or placebo doses. Active doses of RSHZ19 were 0.025, 0.25, 1.25, 5, and 10 mg/kg. There were 4 subjects in each of the 2 low-dose groups (0.025 and 0.25 mg/kg): 2 were actively treated with RSHZ19 and 2 received placebo (0.9% saline infusion). There were 6 subjects in the other dose groups (1.25, 5, and 10 mg/kg): 4 subjects per group received RSHZ19 and 2 were given placebo. Placebo was allocated within each dose group according to a randomization schedule. In this study, higher doses of RSHZ19 were not administered until lower doses had been safely administered and their effects observed in 3 subjects.

RSHZ19 study drug. RSHZ19 is a humanized MAb in development by SmithKline Beecham Pharmaceuticals under a license agreement with Scotgen Biopharmaceuticals (Aberdeen, UK). This MAb is a humanized version of a murine MAb, RSMU19, that is directed against the conserved fusion protein of RSV. RSHZ19 was constructed using molecular techniques to insert the antigen-binding regions (complementarity determining regions) from an F protein-specific murine MAb (RSMU19) into a human variable framework domain [9]. The functional protein is made up of two heavy (IgG1) and two light (κ) chains with a predicted native molecular mass of 146 kDa. In an effort to reduce the potential for immunogenicity in humans, changes to the human framework of RSHZ19 have been kept to the minimum consistent with retention of activity.

Study subjects. Twenty-six male volunteers were enrolled in the study. Subjects were required to be healthy, nonsmoking men, 18–45 years old, weighing ≥ 50 kg (and within 10% of ideal weight based on height). Subjects could not have had any clinically relevant abnormalities by screening history or physical or laboratory examinations (noted below). Excluded from the study were subjects who used prescription or nonprescription drugs (including vitamins) within 2 weeks of dosing and persons with a history of alcohol or drug abuse in the previous year. All subjects had negative urine drug screens within 30 days of enrollment.

Study procedure. Subjects were screened within 30 days before enrollment to confirm they met the study entrance criteria. The screening visit included a complete medical history, physical examination, chest radiograph, and standard 12-lead electrocardiogram (ECG). Blood and urine specimens were obtained for clinical laboratory tests, including complete blood cell and platelet counts, prothrombin time, activated partial thromboplastin time, serum chemistry tests to evaluate electrolytes, renal and hepatic function tests, and urinalysis.

On the day of drug (MAb or placebo) administration, subjects reported to the Clinical Pharmacology Unit (CPU) after fasting

and had a brief physical examination and a 12-lead ECG. Blood and urine specimens were obtained for clinical laboratory studies (as above) before drug administration. The appropriate dose of RSHZ19 or a 0.9% sodium chloride placebo injection was administered intravenously via syringe pump (Baxter, Deerfield, IL) over 30 min. Using sodium chloride as a diluent, all doses were prepared to a standard volume of 100 mL. Subjects remained in the CPU for 24 h after dosing. No food or fluid was permitted for 5 h after dosing except for water, which was allowed ad libitum. Water, soft drinks without caffeine, or fruit juices were permitted ad libitum beginning 5 h after dosing. Lunch and dinner, respectively, were given ~ 5 and 9–10 h after dosing. An evening snack was permitted until 10:00 P.M.

Subjects were to abstain from ingestion of xanthine-containing drinks and alcohol and from strenuous exercise from 24 h before and until 48 h after study drug administration.

Single-lead ECG (SpaceLabs, Redmond, WA) was monitored continuously from before drug administration until 8 h after dosing to aid in the interpretation of any potential clinical event. Supine blood pressure and pulse rate were measured before and 15 and 30 min after the start of the infusion and at 0.25, 0.05, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12 and 24 h after the infusion ended. Tolerability was monitored by nursing assessment and spontaneous reporting of symptoms.

Blood (15 mL) and urine specimens were obtained ~ 24 h after the study medication dose to repeat safety clinical laboratory tests (as described previously). After a brief physical examination and 12-lead ECG were done 24 h after the study medication dose, subjects were allowed to leave the CPU.

Sampling for RSHZ19 concentrations, anti-RSHZ19 antibodies, and fusion inhibition. For each dose of RSHZ19, a series of blood samples (~ 5 mL each) was obtained from each subject. For pharmacokinetic analysis, blood was collected before the infusion began (predose), during the infusion at 15 and 30 min, and after the infusion at 5 min and 1, 2, 4, 8, 12, 24, 48, and 72 h and 1, 2, 4, 6, 8, and 10 weeks after dosing.

Before the infusion and at 2, 4, and 10 weeks after dosing, additional blood samples (5 mL) were obtained for detection of antibody to RSHZ19. Blood (5 mL) was obtained before study drug administration and at 5 min, 24 h, and 1, 4, and 10 weeks after completion of the infusion for assessment of plasma RSV fusion inhibition titers.

All blood samples were collected into tubes containing sodium citrate to prevent coagulation. The blood samples were centrifuged, and plasma was separated into multiple aliquots and transferred to polypropylene tubes. The plasma samples were frozen on dry ice, protected from light, and stored at about -70°C until analysis.

Clinical follow-up. Subjects returned to the CPU as scheduled (see above). At the 1- and 4-week and final visits (10 weeks after infusion), blood (15 mL) and urine specimens were obtained for repeat safety clinical laboratory tests. A brief physical examination and a 12-lead ECG were done at the 1-week visit.

RSHZ19 assay. Plasma samples were analyzed by ELISA for RSHZ19. The B11/B12 ELISA is based on the simultaneous binding of RSHZ19 to two bovine antiidiotypic MAbs, B11 and B12. Details of the assay have been published (rat and monkey plasma) [11]. In human plasma, variable background assay response has been observed in a fraction of control samples tested. However, in the current study, predose plasma from subjects given 0.025–

5 mg/kg dose had no cross-reaction (relative to pooled low background control human plasma). Low predose background assay response noted in 1 subject in the 10 mg/kg group had no effect on the data analysis, since the RSHZ19 concentrations observed after dosing were ≥ 150 times greater than the apparent predose level. In the absence of this background signal, the lower limit of quantification of the B11/B12 ELISA was 1 ng/mL in human plasma (100- μ L sample).

Anti-RSHZ19 antibodies. Serum samples from subjects were analyzed for anti-RSHZ19 antibodies by sandwich ELISA. In brief, RSHZ19 was used to coat a microtiter plate to bind anti-RSHZ19 in serially diluted serum samples. The “sandwiched” anti-RSHZ19 was detected by binding to biotin-labeled RSHZ19, followed by use of an avidin/biotinylated alkaline-phosphatase system. Bovine antiidiotypic B12 MAb and rabbit polyclonal anti-RSHZ19 MAb were included in each assay as positive controls.

RSV fusion-inhibition titers. The ability of test samples to inhibit virus-induced cell fusion was determined using a modification of the in vitro microneutralization assay described by Beeler and Coelingh [12]. Two-fold dilutions of plasma samples were added to VERO cells infected 4 h earlier with RS Long strain virus, followed by a 6-day incubation at 37°C. Virus growth in the presence or absence of test sample was then determined by ELISA using biotin-labeled anti-F protein antibody. In neutralization assays, serial dilutions of plasma were premixed with virus for 2 h before the addition of VERO cells. Titers are expressed as the reciprocal of the dilution that caused a 50% reduction in ELISA signal (equivalent to >90% reduction in virus) based on regression analysis of the sample titration. The virus infectivity titer was determined in each experiment and ranged from 63 to 139 TCID₅₀.

Data analysis. Pharmacokinetic parameters after intravenous administration of MAb or placebo were estimated using MODFIT (version 5.0) data analysis software [13]. Pharmacokinetic analysis used actual blood sampling times. Data were fit to a two-compartment model by weighted (1/year) nonlinear regression analysis. The reported half-lives were obtained from the fitted data. The systemic plasma clearance (CL), and the volume of distribution at steady state (V_{ss}) were estimated by noncompartmental methods [14] using an in-house computer software program. The area under the plasma concentration time curve (AUC_{0-t}) was determined using the log-linear trapezoidal rule. The AUC from the last data point to infinity (AUC_{t-inf}) was extrapolated by dividing the last data point by the terminal rate constant of elimination obtained from the fitted data. The total AUC (AUC_{0-inf}) was calculated as the sum of AUC_{0-t} and AUC_{t-inf} . The percentage of total AUC in the λ_1 and λ_2 disposition phases (%AUC λ_1 and %AUC λ_2) was calculated by the method of separate exponentials [15].

Data for anti-RSHZ19 antibodies are reported as mean optical density (OD; average of duplicate or triplicate wells) of each control or test sera. Antibody titer is defined as the next higher dilution past which a positive response is detected.

For analysis of fusion inhibition titers, pre- and postdose plasma samples collected from 2 RSHZ19-treated and 2 placebo-treated subjects were included in each experiment. Results are expressed as increases in fusion-inhibition titer in plasma obtained 5 min or 24 h after dosing with 1.25, 5, or 10 mg/kg of RSHZ19, over individual predose titers. A positive increase in titer was arbitrarily defined as ≥ 2 -fold increase over individual predose titer. An RSHZ19 reference standard was included in each experiment, and

the fusion-inhibition titer (ED₅₀) was determined to be 0.65 ± 0.46 μ g/mL ($n = 6$ experiments), which is comparable to the activity in previous studies (unpublished data).

Statistical analysis. We used descriptive statistics to summarize demographic, vital sign, pharmacokinetic, and anti-RSHZ19 antibody data. Fusion-inhibition titers were defined as the reciprocal dilution of test sample or concentration of RSHZ19 reference standard, which caused a 50% reduction in ELISA signal (ED₅₀) compared with virus controls. Based on the curve generated in the ELISA by the standard virus titration, a 50% reduction in OD₄₅₀ in wells containing 10–100 TCID₅₀ virus/well corresponds to >90% reduction in virus. To determine the ED₅₀, mean absorbance for replicate cultures was plotted against dilution of sample. Calculation of the 50% point, defined as (mean absorbance of virus – infected cells \pm mean absorbance of uninfected cells)/2, was based on regression analysis designed for use with the RS/1 statistics software package (RS/1 release 3; BBN Software Products, Cambridge, MA).

Results

Study population. The 26 healthy men enrolled in the study all met the eligibility requirements specified by the protocol. Subjects were 19–29 years old (mean, 24; SD = 3) and weighed 61.5–96.3 kg (mean, 75.6; SD = 8.8). No subject withdrew prematurely from the study. Two subjects took medication within the 4 weeks after dosing in violation of the protocol. One took enteric-coated aspirin and ibuprofen 1 day after he received placebo for a fever that was thought to be related to a viral infection. Another subject took acetaminophen for a headache 9 days after he received 0.25 mg/kg of RSHZ19. It is unlikely that these violations had a significant effect on the study results. The protocol allowed concomitant medications after the 4-week postdosing follow-up visit. Five subjects took medication during postdosing weeks 5–10 for conditions that included a tinea infection, musculoskeletal pain, a dental procedure, allergies, and sinusitis.

Safety and tolerability. No serious adverse experiences (AEs) were reported in association with this study. Ten AEs were reported in 6 of 10 subjects who received placebo. Five AEs were reported in 5 of the 16 subjects who received RSHZ19. There did not appear to be an increase in frequency of AEs related to increased dosage of RSHZ19. Reported AEs were considered to be unrelated or probably unrelated to study medication. None of the 26 subjects in the study had changes in heart rate or systolic blood pressure measurements that were considered related to the study drug.

No clinically relevant changes were observed in 12-lead ECG tracings of any subject after dosing. Single-lead continuous ECG recordings were normal, except in 1 28-year-old white man, who had episodic unifocal premature ventricular contractions (≤ 16 /h during the 8-h monitoring after infusion of 10 mg/kg RSHZ19). These and subsequent periventricular contractions in this subject were asymptomatic and occurred during periods when his heart rate was 40–50 beats/min. A cardiolo-

Table 1. Mean (SD) pharmacokinetic parameters for monoclonal antibody RSHZ19.

Dose, mg/kg (n)	CL, mL/h/kg	V _{ss} , mL/kg	T _{1/2} -λ ₁ , h	T _{1/2} -λ ₂ , h	%AUCλ ₂
0.025 (2)	0.170	117	26.3	480	96.9
0.25 (2)	0.142	87.5	60.6	493	88.9
1.25 (4)	0.122 (0.018)	104 (7.5)	32.2 (10.6)	603 (115)	96.3 (1.4)
5.0 (4)	0.142 (0.030)	104 (8.4)	20.2 (10.1)	521 (107)	97.2 (1.4)
10.0 (4)	0.126 (0.012)	109 (4.1)	18.4 (8.9)	617 (46)	98.1 (1.3)

NOTE. CL, plasma clearance; V_{ss}, volume of distribution at steady state; T_{1/2}-λ₁, apparent half-life in initial phase; T_{1/2}-λ₂, apparent half-life in secondary phase; %AUCλ₂, % area under plasma concentration vs. time curve during secondary phase.

gist who did an independent review considered the contractions to be benign and not unexpected in a healthy young man with a high resting vagal tone and sinus bradycardia.

One subject had a serum glucose concentration of 55.2 mg/dL 1 week after receiving 5 mg/kg of RSHZ19. This appeared to be an isolated observation, as serum glucose measurements at all other evaluations were normal. This same subject had 12,700 white blood cells/mm³ at week 10 (upper limit of reference range, 9600 cells/mm³). Minor elevations in white blood cell counts were also observed at weeks 1 and 4. Urinalysis revealed 20 red blood cells/high-power field (hpf; upper limit of reference range, 3) at week 10. This subject had 1+ hemoglobinuria before dosing; red blood cells in the urine were within the reference range at all other time points, except for 10 cells/hpf at 4 weeks, which decreased to 3 cells/hpf 2 weeks later. Bacteria and white blood cells in urine were within the reference range for this subject throughout the study. The subject could not be located for further follow-up after the 10-week study period.

Pharmacokinetics. Mean pharmacokinetic parameters estimated from the B11/B12 double-antiidiotype sandwich ELISA are summarized in table 1. Mean plasma concentration versus time profiles for the 3 highest dose groups (1.25–10 mg/kg) are shown in figure 1.

After the intravenous administration, a biphasic decline in the plasma concentration was observed. For all subjects, the dominant terminal elimination phase (accounting for ≥81% of AUC_{0-t}) was characterized by a half-life of 557 ± 98 h (23 ± 4 days; range, 17–30). A relatively shorter disposition phase (accounting for at most 19% of the AUC_{0-t}) was characterized by a 29 ± 18 h half-life. Very low plasma clearances were observed (0.101–0.195 mL/h/kg) as were low steady-state volumes of distribution (88–142 mL/kg). As judged by the low SDs in the 3 highest dose groups (coefficients of variation, ≤21%), intersubject variability in total plasma clearance, V_{ss}, and elimination half-life was low.

Although only 2 volunteers were evaluated in each of the 2 lowest dose groups (0.025 and 0.25 mg/kg), dose-normalized AUC was not markedly dose-dependent, suggesting that the pharmacokinetics of RSHZ19 was essentially proportional over the 400-fold dose range investigated. Thus, calculation of the

mean CL and V_{ss} for all subjects was warranted. For doses of 0.025–10 mg/kg, the mean ± SD CL and V_{ss} were 0.136 ± 0.027 mL/h/kg and 105 ± 13 mL/kg, respectively.

Anti-RSHZ19 antibodies. The ODs from the positive control antibodies showed that the antiidiotypic MAb B12 (titer, >1:5400) and the polyclonal rabbit anti-RSHZ19 sera (titer, 1:540) bound specifically to RSHZ19. OD readings of subject sera did not indicate the presence of an anti-RSHZ19 antibody response up to 10 weeks after a single intravenous administration of RSHZ19.

RSV fusion-inhibition titers. A pilot analysis of RSV titers in normal adults indicated that fusion-inhibition titers were 10- to 50-fold lower than corresponding neutralization titers for a given individual (range, 1:10–1:545; data not shown). In contrast, the EC₅₀ of RSHZ19 was similar in neutralization and fusion-inhibition assays (1.1 vs. 0.8 μg/mL, respectively). Therefore, to minimize interference by existing anti-RSV titers, we used the fusion-inhibition assay to monitor RSHZ19 activity in plasma from study subjects.

Fusion-inhibition titers in predose plasma samples varied considerably between subjects (range, 1:11–1:2072) against RS Long strain virus for the 26 persons studied. Increases in fusion-inhibition titer at either 5 min or 24 h after dosing with RSHZ19 were seen in plasma of 4 of 4 subjects in the 10 mg/kg group, 2 of 4 in the 5 mg/kg group, and in none of 4 in the 1.25 mg/kg group (table 2). Lower doses of RSHZ19 were not examined for fusion inhibition. Samples beyond 24 h were not assayed, because it was considered unlikely that measurable increases over predose titers would be detected at the later time points. No increases in fusion-inhibition titer were observed in plasma from subjects given placebo. Higher predose titers were observed in subjects in the 5 mg/kg group than in the 10 mg/kg group and may have compromised the ability to detect increases in titer due to RSHZ19 administration in this group. Predose titers in 3 subjects from the 1.25 mg/kg group were relatively low (<1:118), but no additional antiviral activity was detectable in these subjects after dosing. In general, increases in antiviral titer were relatively small (~3-fold differences) and were comparable whether subjects received 5 or 10 mg/kg of RSHZ19. Differences in the predose titers of treated subjects (as well as the variability inherent to the fusion-inhibition

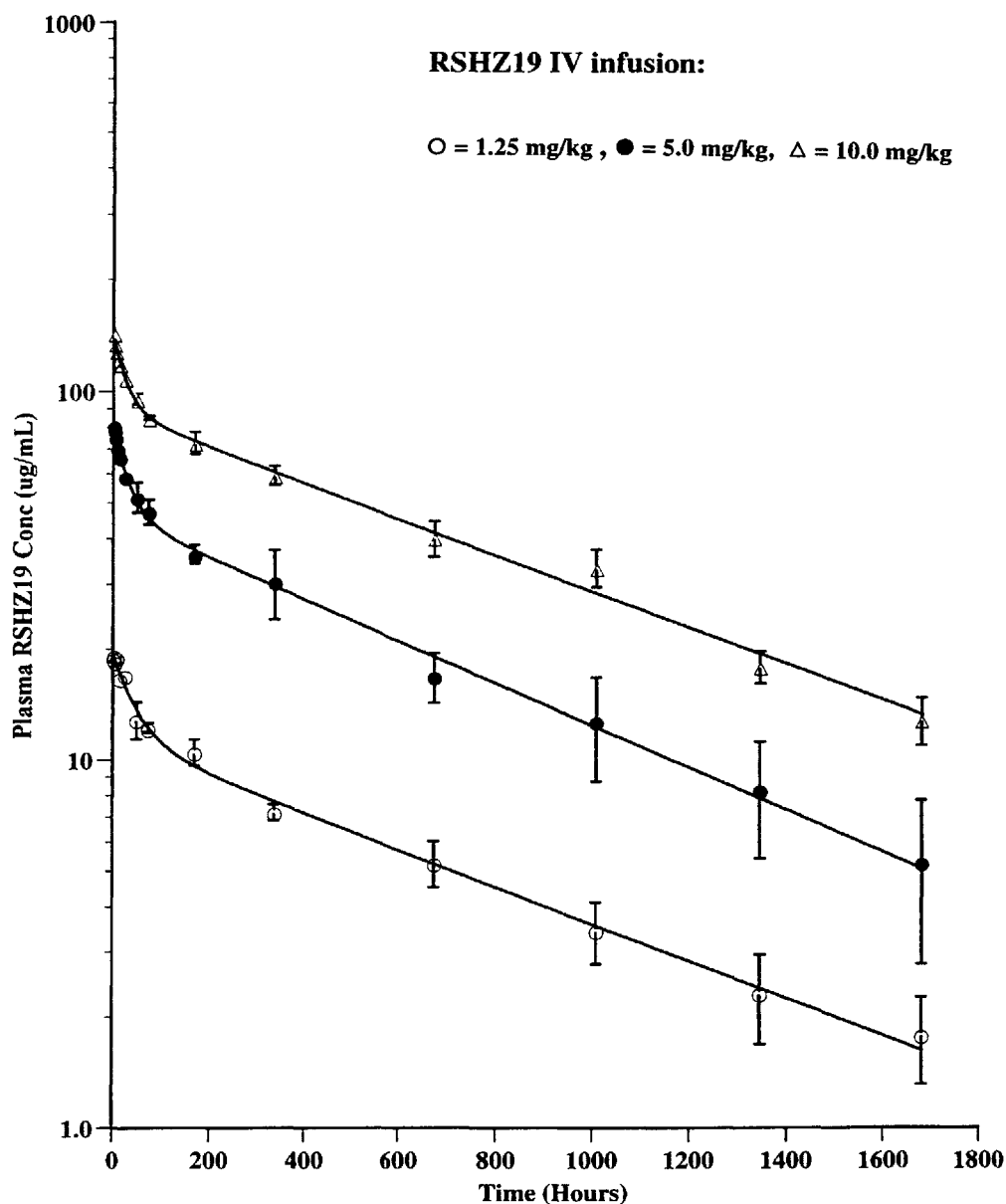


Figure 1. Mean (\pm SD) plasma RSHZ19 concentration vs. time profiles after single intravenous (IV) infusion to healthy adult men ($n = 4$ /group).

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Table 2. Increases in anti-RSV fusion-inhibition titers in normal subjects treated with SB 209763.

Treatment group (mg/kg)	Increase in titer* by time after dose				RSHZ19 concentration (μ g/mL) [†] at 24 h
	5 min		24 h		
	No. positive/total	Fold increase	No. positive/total	Fold increase	
10	4/4	3.0–6.2	4/4	2.0–3.5	82–131
5	1/4	4.2	2/4	3.3–4.6	43–69
1.25	0/4	<2.0	0/4	<2.0	14–17
Placebo	0/4	<2.0	0/4	<2.0	Not tested

* No. of increases detected/total subjects/group. Response was considered positive if increase in titer over individual predose titer was ≥ 2.0 .

[†] Concentrations were determined using B11/B12 antiidiotypic sandwich ELISA.

assay) disallowed association of specific plasma titers with specific doses of RSHZ19. In contrast to these results, circulating concentrations of RSHZ19 were readily detected in all subjects at all doses by use of the antiidiotype ELISA.

Discussion

RSV is a major cause of serious lower respiratory tract disease in children. It is estimated that 40%–50% of children hospitalized with bronchiolitis and 25% of children hospitalized with pneumonia are hospitalized as a direct result of RSV infections. The only specific antiviral drug currently licensed for the treatment of RSV infection in the United States is ribavirin, which has achieved only limited acceptance in the medical community. A polyclonal RSV hyperimmune globulin (IVIG; from pooled human sera) for the prophylaxis of infants at high risk has been reported [16]. This product was recently approved by the Food and Drug Administration for the prophylaxis of infants and young children at risk for serious RSV disease.

A MAb, such as RSHZ19, could be an ideal therapy for both prophylaxis and treatment of RSV infection if it is safe, well tolerated, has a long half-life similar to native IgG, and if the potent fusion-inhibiting property seen *in vitro* and the virus-reducing effect seen in animal studies translate into effectiveness in human infection. Such a MAb would have the advantage of a small administration volume. In the current study, RSHZ19 was safe and well tolerated in healthy adult men when given as a single intravenous infusion in doses up to 10 mg/kg. Subjects tolerated the infusions well. The AEs reported over the 10 weeks of follow-up were more prevalent in subjects who received placebo than in those given RSHZ19, and no laboratory abnormalities or vital sign changes were considered related to RSHZ19.

In this study, the pharmacokinetics of RSHZ19 was characterized at all dose levels. A low total plasma clearance and long elimination half-life similar to native IgG were observed. The V_{ss} was greater than blood volume but significantly less than total extracellular water [17].

While the half-lives of native IgG subclasses range from ~16 to 30 days [18], the clearance and half-life of MAbs or antibody fragments vary considerably. Single-chain antibody fragments are filtered in the kidney and have the shortest half-lives, measured in minutes, while MAbs have increasing half-lives as the composition of the antibody progresses from pure murine to chimeric to fully human [19, 20]. Fully murine MAbs have half-lives of ~1–2 days [21], while mouse/human chimeric MAbs have somewhat longer half-lives (~5–10 days) [22]. A fully human MAb against cytomegalovirus had a mean half-life of 24 days in healthy volunteers [23]. In one study of a humanized IgG1 antibody to a glycoprotein on lymphocytes, CAMPATH-1H, an antibody half-life of <7 days was described [24]; this shorter half-life may have been due to multiple factors, including incomplete saturation of binding sites,

an antiglobulin response, and the presence of a large amount of endogenous antigen (the cell glycoprotein).

Murine MAbs commonly elicit an immune response that limits the ability to give repeat dose therapy. Up to 80% of patients develop antibodies to muromonab-CD3 (OKT3), a murine antibody approved for use in transplant patients [21]. This immune response may neutralize subsequently administered muromonab-CD3 and render further therapy less effective in some patients. Repeat doses of the humanized MAb CAMPATH-1H have elicited an immune response in some patients [24], and thus the antigenicity of even humanized antibodies remains a concern. In this study, there was no evidence of an antibody response to RSHZ19 up to 10 weeks after healthy volunteers received a single intravenous dose.

Studies in animal models of RSV infection and epidemiologic observations in full-term infants indicate that the maintenance of serum neutralization titers of 1:200–1:400 prevents lower respiratory tract RSV infection [25–28]. These data were used to define a target RSV antibody titer in recent clinical trials evaluating the efficacy of human serum IVIG for prevention of RSV infection in children. Clinical trials evaluating RSV IVIG (human) (RSVIG) prescreened for high-titer anti-RSV activity resulted in serum titers >1:200 in children given 750 mg/kg and indications of effective prophylaxis in treated subjects [16]. However, the proportion and specificity of neutralizing antibodies measurable in human serum that contribute to protection from RSV infection is not known. Animal studies suggest that only a subset of those antibodies that are neutralizing *in vitro* is also protective *in vivo* [29]. In an analysis of F protein-specific MAbs, *in vitro* fusion-inhibition activity was the best correlate of protective efficacy in a murine RSV model [30].

In contrast to studies with IVIG, studies in cotton rats that evaluated the protective efficacy of RSHZ19 (5–10 mg/kg) indicated that protection was associated with serum neutralization titers of $\geq 1:32$ and circulating MAb concentrations of ~50 $\mu\text{g}/\text{mL}$ [31]. These studies support the hypothesis that lower antibody doses accompanied by lower serum antibody titers of a highly specific protective MAb should be required for effective antiviral activity as compared to a polyclonal preparation in which only a subset of the neutralizing antibodies may actually be protective. In light of this, the ability to detect increases in titer associated with administration of a MAb could be compromised by background antibody titers in persons previously exposed to RSV. One of the objectives of the present study was to determine if the antiviral activity of RSHZ19 could be monitored in plasma from normal healthy adults given RSHZ19.

Increases in titer were observed in plasma from 4 of 4 subjects given 10 mg/kg of RSHZ19 and in 2 of 4 who received 5 mg/kg of RSHZ19. No increases in anti-RSV fusion-inhibition activity were observed in plasma samples from subjects given 1.25 mg/kg of the drug or in the placebo group. Corresponding plasma concentrations of RSHZ19 (determined by antiidiotype ELISA) for the 10, 5, and 1.25 mg/kg dose groups 24 h after dosing were 82–131, 43–69, and 16–18 $\mu\text{g}/\text{mL}$, respectively. In the absence

of preexisting anti-RSV antibody in the plasma, the expected titers of RSHZ19 in plasma would correspond to the plasma dilution yielding $\sim 0.65 \pm 0.46 \mu\text{g/mL}$ RSHZ19, based on the ED_{50} of the RSHZ19 reference standard. This corresponds to a serum titer of 1:126–1:201 in persons in the 10 mg/kg group (high dose). Modest (~ 3 -fold) increases in fusion-inhibition activity were consistently detected in the presence of preexisting anti-RSV titers when circulating concentrations of RSHZ19 exceeded 80 $\mu\text{g/mL}$. In contrast, fusion-inhibition titers were readily detectable in seronegative cynomolgus macaques at circulating concentrations of $\geq 2 \mu\text{g/mL}$ RSHZ19 [11].

This first study of the humanized MAb RSHZ19 in humans has given preliminary evidence that the MAb is safe and well tolerated when given as single intravenous doses up to 10 mg/kg to healthy volunteers. RSHZ19 did not elicit any antibody response to itself, and its pharmacokinetics appear similar to native IgG. These properties plus detection of modest increases in fusion-inhibition titers in the volunteers with variable pre-dose titers make RSHZ19 an ideal candidate for further testing in the target population of infants and young children with serious RSV infection or at risk of serious infection. Future studies must evaluate the effectiveness of RSHZ19 in preventing or altering the course of clinical RSV infection.

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