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Evaluation of Lassa antiviral compound ST-193 in a guinea pig model

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ABSTRACT

Lassa virus (LASV), a member of the *Arenaviridae* family, causes a viral hemorrhagic fever endemic to West Africa, where as many as 300,000 infections occur per year. Presently, there are no FDA-approved LASV-specific vaccines or antiviral agents, although the antiviral drug ribavirin has shown some efficacy. A recently identified small-molecule inhibitor of arenavirus entry, ST-193, exhibits submicromolar antiviral activity *in vitro*. To determine the antiviral utility of ST-193 *in vivo*, we tested the efficacy of this compound in the LASV guinea pig model. Four groups of strain 13 guinea pigs were administered 25 or 80 mg/kg ST-193, 25 mg/kg of ribavirin, or the vehicle by the intraperitoneal (i.p.) route before infection with a lethal dose of LASV, strain Josiah, and continuing once daily for 14 days. Control animals exhibited severe disease, becoming moribund between days 10 and 15 postinfection. ST-193-treated animals exhibited fewer signs of disease and enhanced survival when compared to the ribavirin or vehicle groups. Body temperatures in all groups were elevated by day 9, but returned to normal by day 19 postinfection in the majority of ST-193-treated animals. ST-193 treatment mediated a 2–3-log reduction in viremia relative to vehicle-treated controls. The overall survival rate for the ST-193-treated guinea pigs was 62.5% (10/16) compared with 0% in the ribavirin (0/8) and vehicle (0/7) groups. These data suggest that ST-193 may serve as an improved candidate for the treatment of Lassa fever.

1. Introduction

Lassa fever is an acute and often fatal illness endemic to regions of West Africa, including the countries of Liberia, Guinea, Nigeria and Sierra Leone. Recently, the importation of cases into non-endemic areas has been of increasing concern. Imported cases of Lassa fever have been reported in the United States, Europe and Canada (Lassa fever, 2000a,b,c; Imported Lassa fever, 2004; Atkin et al., 2009; Kitching et al., 2009; Mahdy et al., 1989). It is estimated that 300,000–500,000 cases of Lassa fever occur each year (Fisher-Hoch and McCormick, 2004; McCormick, 1987; McCormick et al., 1987b), with mortality rates of 15%–20% in hospitalized patients (Buchmeier et al., 2007). The etiologic agent of Lassa fever is Lassa virus (LASV), a member of the *Arenaviridae* family of RNA viruses. This family is divided into the Old World viruses (e.g., LASV and lymphocytic choriomeningitis virus, or LCMV) and the New World viruses (e.g., Junín, Machupo, Guanarito and Sabiá). The natural host for Lassa virus is the peridomestic multimammate rat (*Mastomys natalensis*). Transmission to humans occurs most often through contact with infected rodent excreta. Lassa fever poses a particular threat to health care workers in endemic areas, as nosocomial infections have occurred in nearly every recorded outbreak (Fisher-Hoch et al., 1995; Isaäcson, 2001). The incubation period for Lassa fever is approximately 10 days (with a range of 3–21 days) (Bausch et al., 2001; Frame et al., 1970; McCormick et al., 1987a; Mertens et al., 1973; Monath et al., 1974). The early stages of disease are characterized by the onset of general flu-like symptoms (headache, malaise, etc.), thus making a specific early diagnosis of Lassa fever difficult, as many diseases present in a similar manner in the endemic areas. Gastrointestinal manifestations are common and sore throat typically accompanied by inflammatory or exudative pharyngitis is observed in two out of three of patients (Buchmeier et al., 2007; McCormick et al., 1987a). Complications of Lassa fever have been reported in the literature and include mucosal bleeding, and pleural or pericardial effusion (McCormick et al., 1987a). Bleeding tendencies are observed in less than one third of patients but are an

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indicator of poor outcome, as is the development of severe edema (Buchmeier et al., 2007; Enria et al., 2006; Frame, 1989). Unilateral or bilateral deafness associated with infection-related damage to the eighth cranial nerve is a lasting complication in approximately one-third of patients released from the hospital after recovering from Lassa fever (Cummins, 1992; Cummins et al., 1990; Liao et al., 1992).

There are currently no FDA-licensed vaccines, therapeutics or immunotherapies developed specifically for preventing or treating Lassa fever. In addition to supportive care, the primary treatment option is administration of intravenous ribavirin, a nucleoside analogue antiviral drug that has shown efficacy in high-risk patients when administered early in the disease (McCormick et al., 1986). Typically, patients with Lassa fever in the endemic areas do not present to a hospital until late in disease progression, diminishing the likelihood of successful ribavirin treatment. Ribavirin causes hemolytic anemia which is often dose-limiting (Fuster et al., 2005) and is extremely teratogenic in animal models. In addition, ribarivin combination therapies have been associated with acute hematological, mitochondrial, pulmonary, hepatocellular and bone marrow toxicity, especially in patients who are co-infected with hepatitis C and human immunodeficiency virus (Bani-Sadr et al., 2005; Drapeau et al., 2008; Fuster et al., 2005; Kumar et al., 2002; Mira et al., 2007). Frequently, these complications are severe enough to force dose reduction or cessation of treatment, thus viable alternative treatments are necessary (Fuster et al., 2005).

A variety of approaches have been taken for the development of vaccines and therapeutics against arenavirus infections. Vaccine candidates include live attenuated, reassortant, yellow fever virus-based, and vesicular stomatitis virus-based platforms (Bredenbeek et al., 2006; Carrion et al., 2007b; Fisher-Hoch and McCormick, 2004; Geisbert et al., 2005; Lukashevich et al., 2005). Potential therapeutics against arenavirus infections include such novel therapies as small interfering RNA (siRNA) particles, antiviral compounds, and small-molecule inhibitors of virus entry and/or replication. Müller et al. recently demonstrated that siRNA particles were able to inhibit a LASV replicon system *in vitro* (Müller and Günther, 2007). A pyrazine derivative, T-705, is currently in clinical trials against influenza virus and has shown efficacy against arenavirus infection *in vitro* and *in vivo* (Gowen et al., 2007, 2008). This novel broad-spectrum antiviral demonstrated late-stage efficacy in a Pichinde virus hamster model (a biosafety level-2 surrogate for LASV infection), though the mechanism is unknown (Gowen and Holbrook, 2008; Gowen et al., 2008). T-705 has yet to be tested against LASV.

Another strategy is to target viral entry into cells, a process mediated by the virus-encoded envelope glycoprotein. The characteristics of the arenavirus glycoprotein are consistent with a class I envelope protein, which is typified by the influenza hemagglutinin and found also in retroviruses, paramyxoviruses, coronaviruses, and filoviruses (Colman and Lawrence, 2003; Eschli et al., 2006; York et al., 2005). The glycoprotein precursor (GPC) is processed initially by a signal peptidase to produce an unusual stable signal peptide and subsequent posttranslational cleavage by the host cell protease SKI-1/S1P yields glycoprotein 1 (GP1) and glycoprotein 2 (GP2) subunits (Agnihothram et al., 2007; Lenz et al., 2001). GP1 serves a role in receptor binding, while GP2 contains structural motifs consistent with the fusogenic subunit of other class I envelope proteins that facilitate host cell entry via acid-dependent membrane fusion (Borrow and Oldstone, 1992; Burns and Buchmeier, 1991; Di Simone and Buchmeier, 1995; Gallaher et al., 2001; Wright et al., 1990). Attempting to interfere with LASV GPC processing or viral entry mediated by the GP1 or GP2 subunits represents a viable antiviral strategy. Two groups have recently described small-molecule inhibitors of LASV entry (Larson et al., 2008; Lee et al., 2008). At least one of these compounds, ST-193, appears to target the GP2 subunit of the envelope glycoprotein (Larson et al., 2008). In this study, we assessed the *in vivo* efficacy of ST-193 in a guinea pig model of Lassa fever.

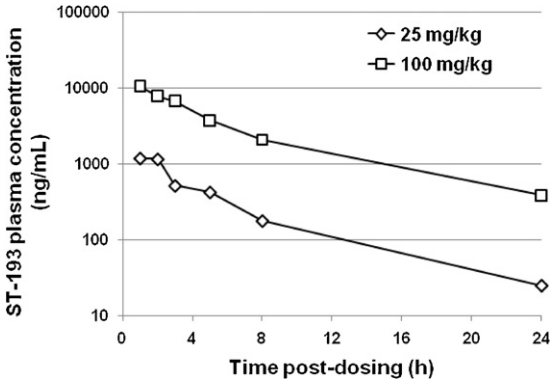

Fig. 1. ST-193 pharmacokinetics in guinea pigs. Guinea pigs were injected intraperitoneally with ST-193 at a dose of either 25 mg/kg or 100 mg/kg of body weight. Plasma samples were obtained at the indicated time points (two animals per dose per time point) and the average concentration for each time point is shown.

The guinea pig is the most practical small animal disease model for Lassa fever. In-bred strain 13 guinea pigs infected with between 2 and 240,000 plaque-forming units (pfu)/ml of LASV develop a severe disease characterized by interstitial pneumonia, pulmonary edema and pleural effusion, necrosis of the kidney and spleen, and myocarditis resulting in 100% mortality between days 14 and 17 postinfection (range 11–22) (Jahrling et al., 1982). The resulting disease course did not differ significantly in animals given different doses of LASV above 2 pfu (Jahrling et al., 1982). LASV titers are detectable in most tissues, with high titers of LASV found in blood, lung, spleen, pancreas, lymph nodes, adrenal gland, kidneys, liver and heart when virus is inoculated subcutaneously (Jahrling et al., 1982; Walker et al., 1975).

Here, we evaluated the utility of ST-193 against a lethal LASV infection in the strain 13 guinea pig model as well as compared the performance of this novel therapeutic to ribavirin, the current standard of care.

2. Materials and methods

2.1. Pharmacokinetic analysis

ST-193 was formulated as a solution in 32% (w/v) 2-hydroxypropyl-β-cyclodextrin (HP-β-CD). Compound was dissolved in 40% HP-β-CD by acidification with the addition of 10% final volume 1 M HCl, and subsequently was neutralized by the addition of 10% final volume 1 M NaOH (final ratio of 0.8:0.1:0.1 of the respective HP-β-CD, HCl, and NaOH solutions). Female Hartley guinea pigs were injected intraperitoneally with a 10 mg/ml solution of ST-193 at a volume of either 2.5 ml per kg of body weight (25 mg/kg) or 10 ml per kg (100 mg/kg). Four animals per dose were used, with blood samples collected from two animals per dose at each time point in alternating fashion. Blood samples were obtained at the indicated time points (Fig. 1) through a jugular cannula and plasma isolated at 15,000 × *g* for 2 min. at 4 °C in plasma separation tubes containing lithium heparin (BD Microtainer #365958, Franklin Lakes, NJ). Samples were stored at −80 °C, then shipped on dry ice to Absorption Systems (Exton, PA), extracted on a Tomtec Quadra 96-Model liquid handling system, and diluted 4-fold in acetonitrile containing 100 ng/ml ritonavir as the internal standard on a Sirocco Protein Precipitation Plate (Waters Corporation). Resulting suspensions were mixed by air aspiration, filtered into a 96-well collection plate by vacuum, spun briefly at low speed, and filtrates were transferred to Chro-

macol vials for analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a PE Sciex API 3000 system. Chromatography was performed with a Keystone Hypersil BDS C_{18} column (3-μm particle size; 30 mm × 2.1 mm) at a flow rate of 0.3 ml/min with a mobile phase containing 25 mM NH_4OH (to pH 3.5 with 88% formic acid) using a PerkinElmer Series 200 Micro Pump with autosampler. Quantitation was performed against calibration curves generated by spiking blank guinea pig plasma with ST-193; quality control samples, prepared from independent weighings, were prepared and quantitated in the same manner to ensure acceptable precision and accuracy.

2.2. Animal study

Strain 13 guinea pigs (*Cavia porcellus*) 6–12 weeks old were divided into four groups, with each group consisting of seven (four male and three female vehicle controls) or eight (four male and four female) animals for the treatment groups. Both ST-193 and ribavirin were solubilized in 32% (w/v) 2-hydroxypropyl-β-cyclodextrin, which was also used without drug as the vehicle control. Animals were anesthetized with an injectable ketamine-acepromazine cocktail and then administered a vehicle control, ribavirin (25 mg/kg), or ST-193 (25 mg/kg or 80 mg/kg) by intraperitoneal (i.p.) injection beginning 1 h before LASV infection and continuing once daily for 14 days, as follows: one group ($n = 7$) received 6.4 ml/kg of the vehicle control (formulation buffer alone); one group ($n = 8$) received 25 mg/kg (6.4 ml/kg of a 3.9 mg/ml formulation) of ribavirin; one group ($n = 8$) received 25 mg/kg ST-193 (2 ml/kg of a 12.5 mg/ml formulation); and one group ($n = 8$) received 80 mg/kg ST-193 (6.4 ml/kg of a 12.5 mg/ml formulation). Viral infection was carried out under biosafety level 4 conditions. Each animal was administered subcutaneously (s.c.) a single dose of 1000 pfu of LASV (strain Josiah) in a total volume of 100 μl of physiological saline. Animals were observed twice daily and were assigned a morbidity score. Morbidity scores were assessed by assigning symptoms of illness a numeric value. Symptoms evaluated included: piloerection, visible weight loss, dehydration, orbital exudates, dyspnea, paralysis or hypothermia. The sum of the numbers associated with symptoms were added each day and the average of the morbidity score was tabulated daily for each treatment group. Blood samples were taken on days −7, 7, 14, 21 and 29 postinfection. Animals were euthanized when non-ambulatory and/or moribund, and surviving animals were euthanized and terminal blood samples collected on day 30 of the study (day 29 postinfection). Serum samples were analyzed for viremia, LASV-specific antibodies, neutralizing antibodies, and blood chemistry values. Necropsies were performed on each animal, and tissues were analyzed for LASV-specific histopathological and immunohistochemical analysis.

Research was conducted under an IACUC approved protocol in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

2.3. Analysis of LASV-specific antibody production

Serum samples collected before viral infection and at days 7, 14, 21, and 29 postinfection were analyzed for LASV-specific antibody production by ELISA. A preparation of purified, irradiated LASV was used as antigen. Cell culture supernatant containing LASV was gradient purified (unpublished method of P. Jahrling), then exposed to cobalt (γ) irradiation, 1×10^7 rads total dose. Viral inactivation was confirmed by performing two rounds of plaque assays and an immunofluorescence assay. Wells of 96-well microtiter plates were coated with 2 mg/ml of the purified, irradiated LASV preparation and incubated overnight at 4 °C. Plates were washed three times with phosphate-buffered saline plus 0.1% Tween 20 (PBST) then blocked for 1 h at 37 °C with a 5% lowfat milk solution. After washing three times as before, 100 μl of each of a twofold serial dilution series ranging from 1:2 to 1:128 of serum in cell culture medium was added to the wells and incubated for 1 h at 37 °C. Bound antibodies were subsequently detected with guinea pig-specific anti-IgG1 or anti-IgG2 HRP-conjugated secondary antibodies (Immunological Consultants, Inc.). A colorimetric assay using SureBlue TMB (KPL Laboratories) as substrate was performed and absorbance values at A_{450} obtained using a microtiter plate reader. Experiments using serum collected from LASV-infected animals were performed in biosafety level-4 conditions.

2.4. Analysis of viremia

Serum samples collected pre- and postinfection were assayed for viral titers via a standard plaque assay with some modifications (Tomori et al., 1987). Briefly, Vero cells seeded in 6-well cell culture plates were adsorbed with gentle rotation at 37 °C, 5% CO_2 with 10-fold serial dilutions of serum for 1 h, then an overlay of 0.8% molecular grade agarose in EBME (basal medium Eagle with Earle's salts) fortified with 10% fetal bovine serum and 20 μg/ml gentamicin was applied to each well and allowed to solidify. After the overlay solidified, cells were incubated at 37 °C, 5% CO_2 for 4 days, then stained with neutral red. After an overnight incubation at 37 °C in the stain, plaques were counted and recorded.

2.5. Plaque-reduction neutralization test (PRNT)

Neutralizing capabilities of antibodies in the serum were analyzed by a standard plaque-reduction/neutralization test with some modifications (Hooper et al., 1999). Briefly, twofold dilutions of serum (in 100 μl volumes) were incubated for 1 h at 37 °C with LASV diluted to approximately 100 pfu per serum dilution. After incubation, each serum dilution/virus mixture was then added to Vero cells seeded in 6-well cell culture plates. The remainder of the procedure was as described above for the plaque assay. Plaques were counted and compared to control wells containing cells infected with 100 pfu LASV pre-incubated with naïve guinea pig serum. Neutralizing antibody titers yielding a 50% or 80% reduction in plaques were determined.

2.6. Blood chemistry analysis

Serum samples collected pre- and postinfection were diluted 1:3, then analyzed for glucose, blood urea nitrogen, creatinine, uric acid, calcium, albumin, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), total bilirubin, gamma glutamyl transferase, and amylase. Approximately 100 μl of serum diluted 1:3 was applied to a General Chemistry 13-panel rotor and evaluated in a Piccolo point-of-care blood chemistry analyzer (Abaxis). Values for each serum sample were normalized for the dilution, recorded and compiled.

2.7. Pathological analysis of tissues

Tissues were collected from all animals at necropsy and stored in a 10% buffered formalin solution and held in biocontainment for 21 days. Tissues for histopathology underwent routine histological processing, were embedded in paraffin, sectioned and were stained with hematoxylin and eosin. Immunohistochemistry

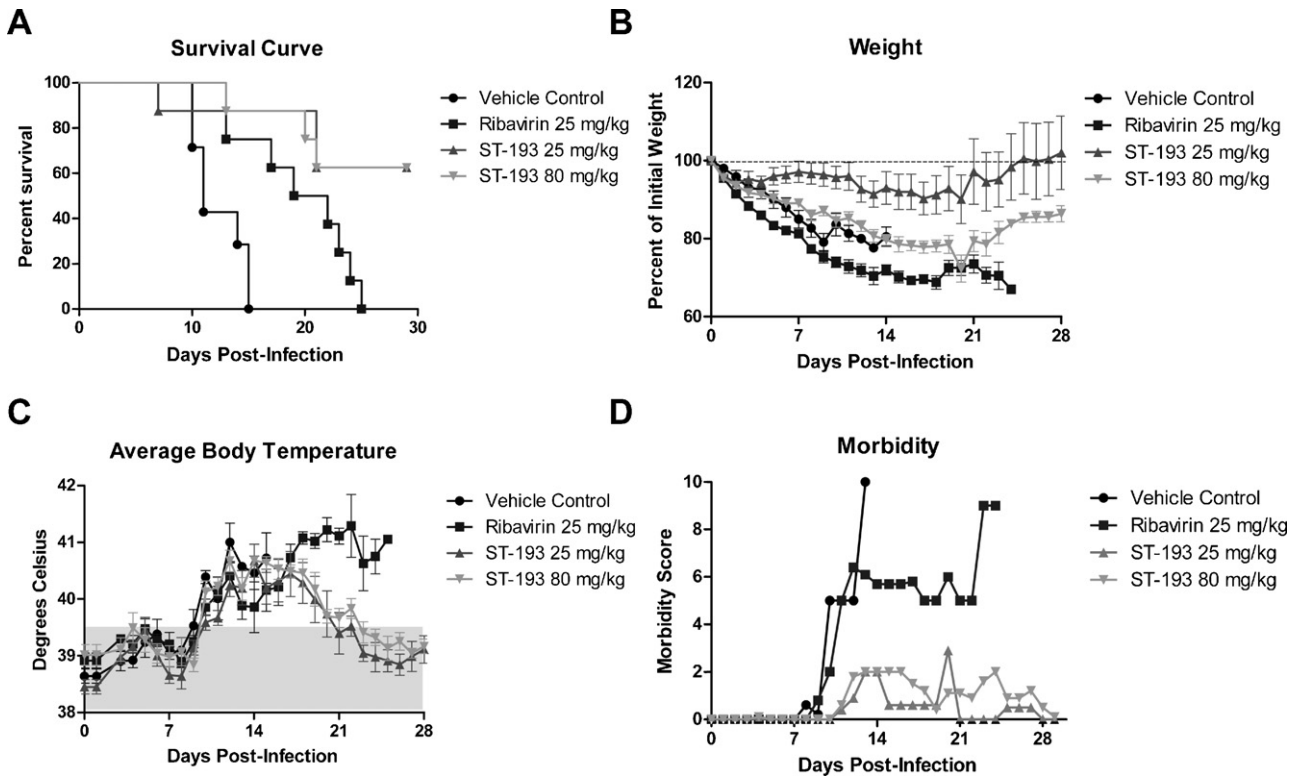

Fig. 2. Outcome of the different treatment groups post-LASV infection. Animals administered the vehicle control are indicated by the closed circles. The closed squares indicate the ribavirin-treated group. The ST-193 treatment groups are indicated by the solid triangles (black triangle indicates the 25 mg/kg treatment group and the gray triangle indicates the 80 mg/kg treatment group). (A) Survival curve. (B) Average weight postinfection. (C) Average temperature postinfection. The gray band indicates the normal body temperature range for guinea pigs. (D) Morbidity score.

was performed on all tissue sections using a mouse monoclonal antibody against LASV at a 1:15,000 dilution and a commercially available immunoperoxidase kit (EnVision System; DAKO, Carpinteria, CA). After deparaffinization and peroxidase blocking, tissue sections were incubated with the primary antibody at room temperature for 1 h, then with a secondary antibody and incubated as before, then counterstained with hematoxylin.

3. Results

3.1. In vivo characterization of ST-193

Initial *in vivo* testing of ST-193 was performed in guinea pigs. ST-193 was found to be tolerated well when administered daily as an intraperitoneal injection of either 25 or 100 mg/kg/day for 14 days, as assessed by daily observation, body weight, and terminal necropsy (data not shown). Blood plasma obtained after the first and last (14th) doses were analyzed by liquid chromatography-mass spectrometry (LC/MS) to assess ST-193 levels. Fig. 1 shows the pharmacokinetics of ST-193 after the initial dose. ST-193 concentrations were similar after the 14th and final dose, indicating no substantial accumulation (data not shown). Plasma concentrations of ST-193 were maintained well in excess of the *in vitro* effective concentration (EC_{50}) of 1.6 nM or the equivalent of 0.6 ng/ml, providing support for a valid assessment of *in vivo* efficacy with these dosing schemes (Larson et al., 2008).

3.2. LASV infection

Thirty-one strain 13 guinea pigs were divided into four treatment groups: low-dose ST-193 (25 mg/kg), high-dose ST-193 (80 mg/kg), ribavirin (25 mg/kg) and vehicle alone. ST-193 doses were chosen so as not to exceed maximum recommended volumes for daily i.p. injections. The same buffer formulation (vehicle) was used for all four groups. One hour after the initial treatment dose, all animals were exposed subcutaneously with 1000 pfu of LASV, Josiah strain. This infection dose is uniformly lethal in strain 13 guinea pigs and represents greater than $10^3 \times LD_{50}$ (Jahrling et al., 1982). All surviving animals continued to receive daily treatment until 13 days postinfection (14 daily doses total).

All vehicle-treated animals succumbed to LASV infection between days 10 and 15 postinfection, and all ribavirin-treated animals succumbed between days 13 and 25 postinfection (Fig. 2A). One animal from the ST-193 25 mg/kg treatment group died much earlier (day 7 postinfection) than expected for LASV-infected guinea pigs (Bredenbeek et al., 2006; Carrion et al., 2007b; Jahrling, 1983; Jahrling et al., 1982). This animal developed an infected wound in its right hind foot 3 days postinfection which became ulcerated by day four. Although administered topical antibiotics, it could not be ruled out that this injury contributed to the death of this guinea pig. Unlike all the other animals in the study, this animal never became febrile. Daily body weight monitoring revealed progressive weight loss in all groups but the ST-193 25 mg/kg group (Fig. 2B). The ST-193 80 mg/kg group lost body weight initially, but weight loss stabilized by day 14 postinfection and guinea pigs began to gain weight starting at approximately day 21 postinfection. Vehicle and ribavirin-treated groups lost body weight at a steady rate until they were euthanized when moribund. Animals in all treatment groups experienced elevated body temperatures starting day 9 postinfection (Fig. 2C). However, fevers (body temperature > 39.5 °C) in both ST-193 treated groups resolved in the majority of animals by day 19 postinfection. Morbidity scores were observed and recorded daily for each animal (Fig. 2D). Scores were consistently higher for the vehicle and ribavirin groups than for

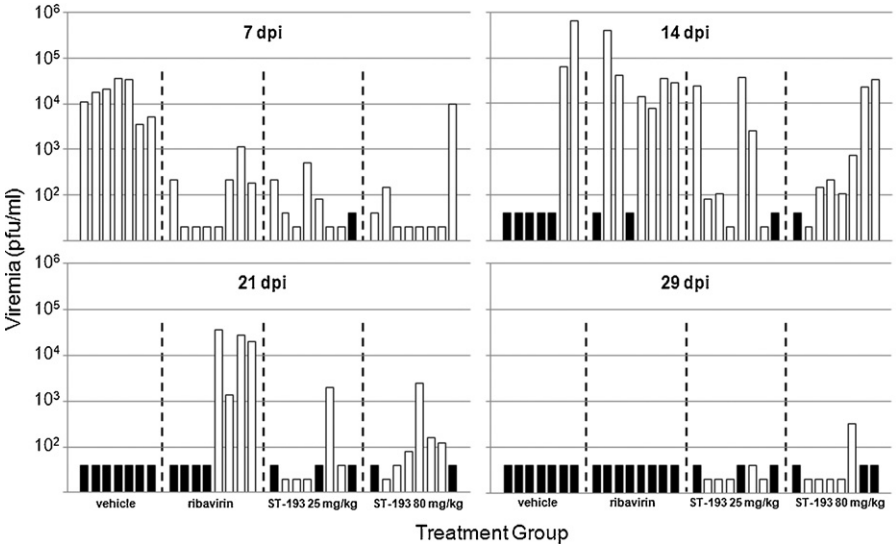

Fig. 3. Serum viremia in animals postinfection. White bars indicate titers obtained from live animals, presented in the same order for each time point and organized in groups as indicated; black bars indicate animal did not survive to that time point. The limit of detection for all samples is 40 pfu/ml, shown by the height of the black bars; bars shown below 40 pfu/ml indicate no plaques were obtained from that sample.

the ST-193-treated groups. Morbidity scores for the ST-193-treated groups returned to preinfection levels before the study endpoint. Data presented in Fig. 2 represent the average values observed for each group.

3.3. Viremia

All groups eventually became viremic postinfection (Fig. 3). However, at 7 days postinfection, only half (four of eight) of the ribavirin-treated and seven of 15 total ST-193-treated animals exhibited detectable serum titers (≥40 pfu/ml), while all vehicle-treated animals had titers of 10^3–10^4 pfu/ml. At day 14 postinfection, average viremia in ST-193 treated guinea pigs ($n = 14$) was 4×10^2 pfu/ml as compared to 3.5×10^4 in ribavirin treated animals ($n = 6$) and 2×10^5 pfu/ml in animals receiving the vehicle control ($n = 2$ survivors at 14 days postinfection). ST-193 treated animals began to clear viremia by day 21 postinfection, and viremia was undetectable in the majority of animals by day 29 postinfection.

3.4. LASV-specific IgG1 and IgG2 responses pre- and post LASV infection

LASV-specific IgG2 was detected in the ribavirin-treated and both ST-193-treated groups starting at the 21 days postinfection timepoint (Fig. 4B). No LASV-specific IgG1 responses were detectable in either the control, ribavirin-treated or ST-193-treated groups. Data are the average values acquired for each sampling time for each group.

3.5. Pre- and postinfection LASV neutralizing antibody responses in vehicle and antiviral-treated guinea pigs

The presence of neutralizing antibodies was evaluated in available sera from treated and control groups. Neutralizing antibody was not detectable in either the control or ribavirin-treated groups using the methods described. Evaluation of the ST-193-treated groups showed the presence of neutralizing activity as early as day 7. Given the presence of circulating ST-193 during the 14-day treatment regimen, this neutralizing activity could well be mediated by ST-193. By 21 days postinfection, however, no interfering residual ST-193 was expected to remain (the terminal elimination half-life of ST-193 in guinea pigs is approximately 4 h). At 21 or 29 days postinfection, the serum from the two ST-193 treatment groups showed significant neutralizing ability. The titers demonstrating either 50% reduction ($PRNT_{50}$) or 80% reduction ($PRNT_{80}$) in plaques are presented in Table 1.

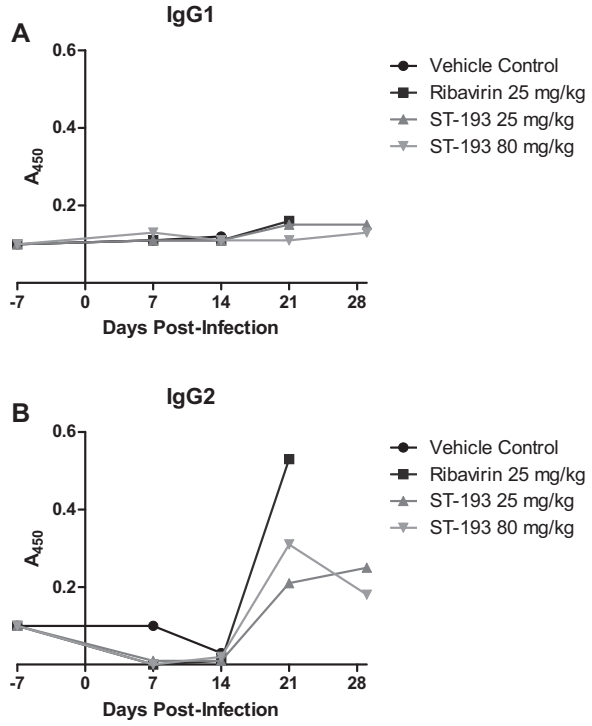

Fig. 4. Analysis of LASV-specific antibody production postinfection. Animals administered the vehicle control are indicated by the closed circles. The closed squares indicate the ribavirin-treated group. The ST-193 treatment groups are indicated by the solid triangles (black triangle indicates the 25 mg/kg treatment group and the gray triangle indicates the 80 mg/kg treatment group). Two-fold serial dilutions of serum were assayed for LASV-specific antibody production. Data from the 1:4 dilution of serum are shown.

Table 1
Plaque-reduction neutralization test titers of surviving animals on days 21 or 29 post-infection[a].

Treatment group	Day 21 postinfection		Day 29 postinfection	
	$PRNT_{50}$	$PRNT_{80}$	$PRNT_{50}$	$PRNT_{80}$
Vehicle control	ND	ND	ND	ND
Ribavirin	ND	ND	ND	ND
ST-193 25 mg/kg	64	4	128	16
ST-193 80 mg/kg	128	16	128	16

[a] Titers are listed as the reciprocal of the dilution resulting in either 50% or 80% reduction in plaques compared to control. ND = Not detected.

3.6. Blood chemistry analysis

Of the 13 blood chemistry analytes tested, only three displayed statistically significant differences between treatment groups and the vehicle control. Of the analytes tested, ALT, AST and AP (Fig. 5A–C, respectively) were highly elevated in the vehicle control group relative to the other treatment groups. The ribavirin-treated group also displayed elevated ALT and AST, but to a lesser degree than the control. Surviving animals in the ST-193-treated groups did not display elevated levels of ALT, AST, or ALP relative to normal levels during the course of the study, but experienced increases in ALT and AST at the study endpoint.

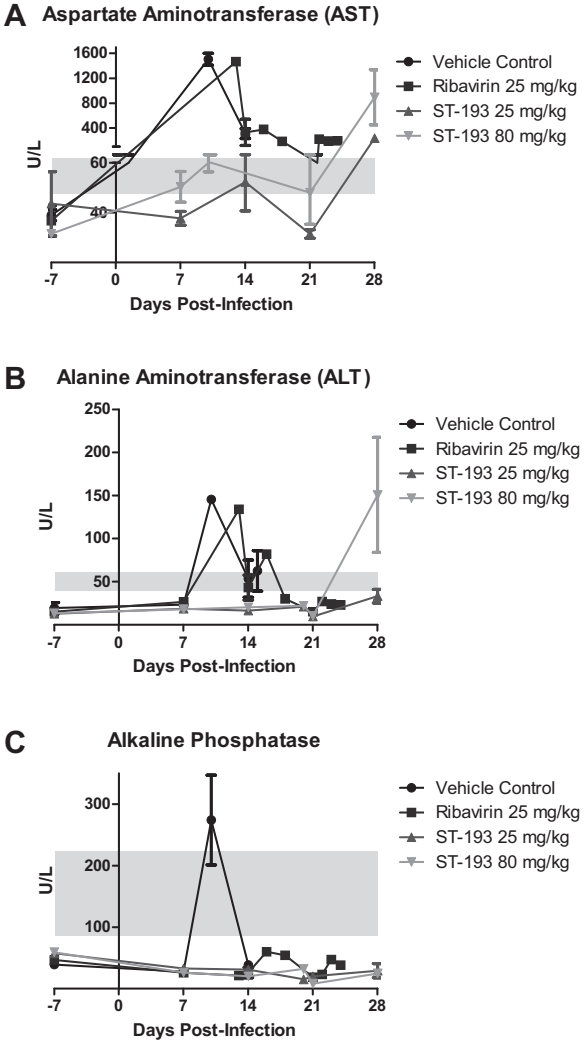

Fig. 5. Blood chemistry analysis. Animals administered the vehicle control are indicated by the closed circles. The closed squares indicate the ribavirin-treated group. The ST-193 treatment groups are indicated by the solid triangles (black triangle indicates the 25 mg/kg treatment group and the gray triangle indicates the 80 mg/kg treatment group). The gray bands denote normal levels of AST, ALT or AP in the guinea pig. (A) Changes in aspartate aminotransferase (AST), a liver enzyme, after LASV infection. (B) Changes in alanine aminotransferase (ALT), a liver enzyme, after LASV infection. (C) Changes in alkaline phosphatase after LASV infection.

3.7. Pathology analysis of guinea pig tissues post-LASV infection

Table 2 summarizes the results of the pathology analysis. The time to death after infection was 10–15 days postinfection in vehicle control group, and 13–25 days postinfection in the ribavirin-treated group. Animals that did not survive in the ST-193 treatment groups succumbed between 7 and 21 days postinfection. Histological changes were similar in all three groups but varied in severity. The most common histological observations included hepatic necrosis, interstitial pneumonia and lymphadenopathy (Fig. 6). Other less consistently noted lesions included endocarditis and pancarditis, renal tubular degeneration and peritonitis. Animals within the ST-193-treated groups had the least severe changes and those animals in the ribavirin group had the most severe lesions. The vehicle control group animals had the most acute course of disease (shortest time to death) with generally moderate to occasionally severe lesions. Immunohistochemical reactivity was found primarily with macrophages of the lung, liver and spleen in all groups (Fig. 7). Surviving animals were evaluated and generally found to be unremarkable. Evidence of interstitial pneumonia and mild hepatocellular degeneration or necrosis was noted in several animals; however, immunohistochemistry was negative for the presence of virus in all tissues examined in the surviving animals.

4. Discussion

LASV is a significant human pathogen for which new treatment options are needed. This pilot study provides the proof of concept that ST-193, a small-molecule inhibitor of arenavirus entry, can protect animals from a lethal LASV infection. Although all infected animals became viremic, developed fevers, and exhibited clinical signs of disease (such as piloerection, reduced appetite and weight loss), treatment with ST-193 significantly limited the disease and prevented a fatal outcome in a majority of animals. Viremia in particular, as measured by serum titer, was dramatically suppressed by ST-193 treatment. In human patient populations, LASV viremia correlates directly with clinical outcome (Johnson et al., 1987; McCormick, 1986). Similar observations have been made in monkey (Baize et al., 2009; Jahrling et al., 1980) and guinea pig models (Jahrling et al., 1982).

Although weight loss is a prominent symptom in LASV animal models, it typically does not manifest itself until several days postinfection (Carrion et al., 2007a; Lukashevich et al., 2005). Here, weights declined steadily throughout the first two weeks of the study. Three of the four groups experienced average weight loss of more than 20%, while the 25 mg/kg ST-193 group did not exceed 10%. This suggests that weight loss might be exacerbated by the daily injection volume of test article, as the three high-loss groups all received 3.2 times as much volume as the 25 mg/kg ST-193 group. This effect appears to correlate with the vehicle (32%, w/v, 2-hydroxypropyl-β-cyclodextrin) and not ST-193, ribavirin or the animal-handling protocol.

Ribavirin has demonstrated antiviral activity against a broad range of virus families, including arenaviruses (Stephen et al., 1980), and was used here as a positive control for *in vivo* antiviral activity. The ribavirin dosing scheme was chosen for convenience as one within the tolerable range for guinea pigs (Kenyon et al., 1986), yet one with reported efficacy against arenavirus infection

Table 2
Pathologic findings in guinea pigs subcutaneously infected with 1000 pfu LASV.

	Vehicle control	Ribavirin 25 mg/kg	ST-193 25 mg/kg Survivors	ST-193 25 mg/kg Non-survivors	ST-193 80 mg/kg Survivors	ST-193 80 mg/kg Non-survivors
Average time to death[a] (range) in days	12 (10–15)	20 (13–25)	n/a[b]	16 (7–21)	n/a[b]	18 (14–21)
Lung: Interstitial pneumonia[c]	++	+++	++	++	+	++
Liver: Necrosis and/or Hepatitis[c]	++	+++	++	++	+	++
IHC positive cells[d]	Liver-3; Spleen-3; Lung-3	Liver-3; Spleen-1; Lung-3	Liver-0; Spleen-0; Lung-0	Liver-3; Spleen-1; Lung-3	Liver-0; Spleen-0; Lung-0	Liver-3; Spleen-3; Lung-3
Additional lesions		Endocarditis; Meningitis; Lymph node hyperplasia		Pyelonephritis; Endocarditis; Lymph node hyperplasia		Lymph node hyperplasia

[a] Time-to-death of animals that did not survive the entire 29-day study.
[b] These animals survived to the end of the study (29 days post-infection), at which point they were euthanized for pathology analysis.
[c] (+)—Minimal to Mild; (++)—Moderate; (+++)—Severe to Marked.
[d] Immunohistochemical (IHC) grading was based on the average number of positive cells per high-powered field: (1) (minimal): 1–3 cells labeled. (2) (mild): 3–10 cells labeled. (3) (moderate): 10–15 cells labeled. (4) (severe): >15 cells labeled.

(Lucia et al., 1989). It may not necessarily be an optimal dosing regimen. However, this dose clearly demonstrated antiviral activity in this study as shown by reduced viremia and prolonged survival. Although extending ribavirin treatment beyond the 14 days used in this study might have improved survival, it is noteworthy that viremia was higher in ribavirin-treated animals than in ST-193 animals even during the dosing period. Cursory reports in the literature with respect to ribavirin efficacy against LASV in guinea pigs are mixed. While it has been described as protective (Jahrling and Peters, 1986; Peters et al., 1987; Stephen et al., 1980), it has also been reported that i.p.-administered ribavirin extends time-to-death in LASV-exposed guinea pigs with little or no effect on survival rates (Huggins, 1989), consistent with the observations reported here. Likewise, ribavirin administered s.c. at 45 mg/kg/day has been shown to prolong death in Junín virus-exposed Hartley guinea pigs without enhancing survival (Kenyon et al., 1986). Ribavirin-treated animals in this study, though experiencing enhanced survival compared to the vehicle control group, exhibited a higher morbidity score (Fig. 2C) than the ST-193 treatment groups. In nonhuman primate models, however, ribavirin has shown clear efficacy against LASV (Jahrling et al., 1980, 1984).

Though ST-193 did not completely protect surviving animals from symptoms of illness, including fever and weight loss, the dramatic reduction in serum viral titer observed in the surviving animals at peak viremia may have been sufficient to protect them until the development of a productive immune response. Serum from surviving animals collected at all time points postinfection had the ability to neutralize LASV in an *in vitro* PRNT test. Though the ribavirin-treated animals experienced prolonged survival compared to the vehicle control group, none of these animals developed a neutralizing antibody titer at any time point, despite the fact that this group had the most robust anti-LASV IgG2 response, as measured by ELISA (Fig. 4). Interestingly, one animal in the 80 mg/kg ST-193 treatment group succumbed on day 21 postinfection, yet had only a minimum viral titer (1.2×10^2 pfu/ml) in the terminal serum and immunohistochemistry failed to identify virus in the tissue samples examined. Histological analysis revealed interstitial pneumonia, lymph node hyperplasia and splenic depletion to a degree similar to that observed in surviving animals. However, this animal did exhibit significant viremia (>10^4 pfu/ml) in the day 14 postinfection sample. The terminal serum from this animal had a neutralizing titer similar to that of the surviving animals.

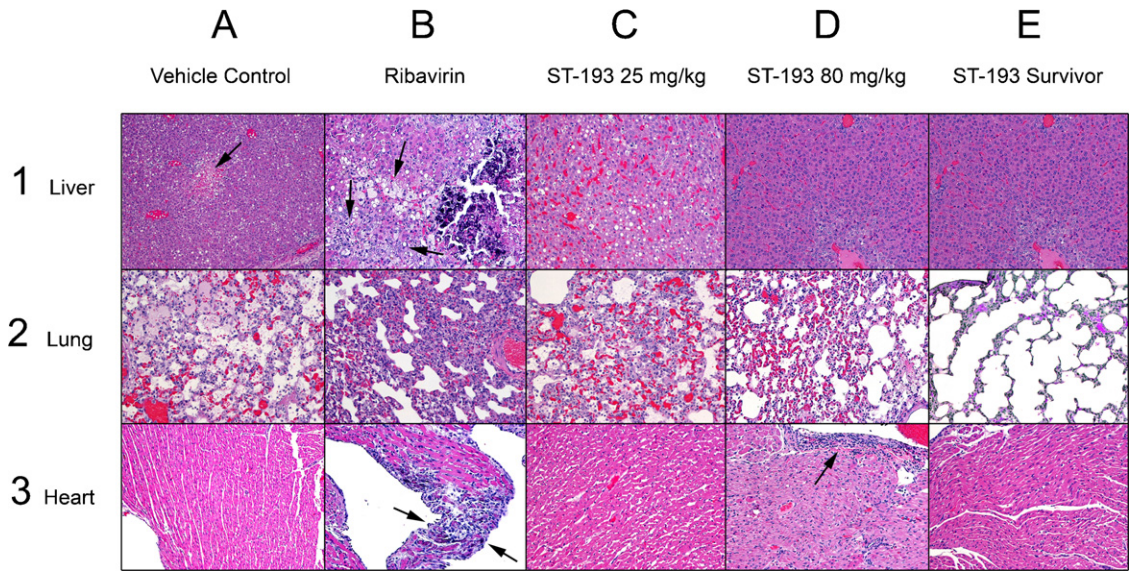

Fig. 6. Comparative histopathologic analysis of vehicle control (Column A), ribavirin (Column B), ST-193 low-dose (Column C), ST-193 high-dose (Column D), and ST-193 survivor (Column E) (20×). Representative examples from liver (row 1), lung (row 2), and heart (row 3) are shown. Necrosis in the liver (arrows) was most severe in the vehicle control and ribavirin groups. In the ST-193 25 mg/kg, 80 mg/kg, and survivor groups, hepatic necrosis was present but to a much lesser degree. In the lung, interstitial pneumonia with thickened alveolar walls, congestion, and alveolar edema and inflammation was present in all groups, with ribavirin, and ST-193 groups being the most severe. Within the heart, inflammation (endocarditis) was present (arrows) in the ribavirin and ST-193 80 mg/kg groups.

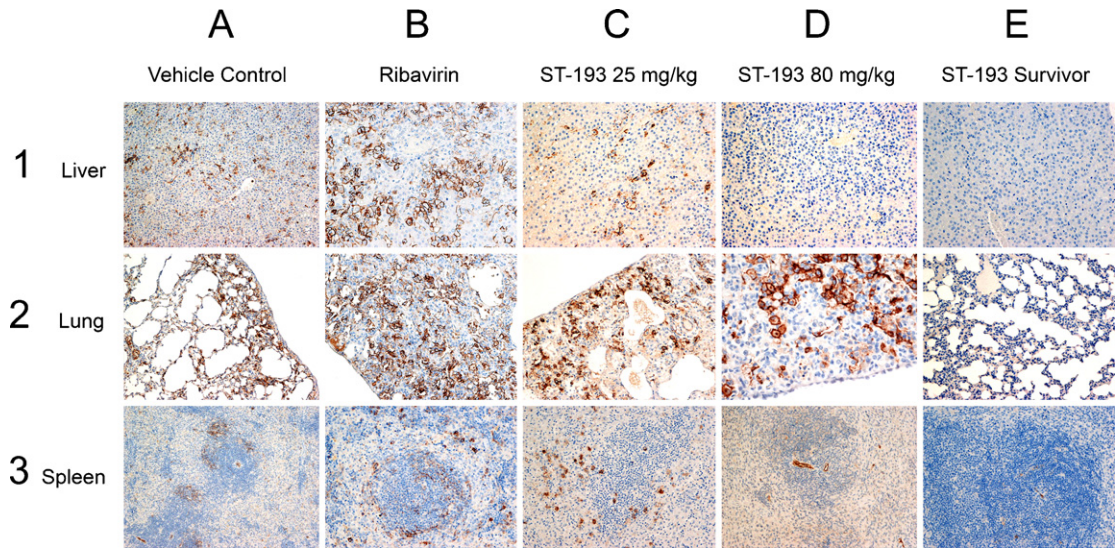

Fig. 7. Comparative immunohistochemical analysis of vehicle control (Column A), ribavirin (Column B), ST-193 low-dose (Column C), ST-193 high-dose (Column D), and ST-193 survivor (Column E) (20×). Representative samples of liver (row 1), lung (row 2), and spleen (row 3) are shown. Within the liver, strongly positive cells are found in the vehicle control, ribavirin, and ST-193 25 mg/kg groups and less strongly in the ST-193 80 mg/kg group. There was no positivity in the survivor group. Within the lung, there are strongly positive cells within all groups except the ST-193 survivor group. There are multifocal strongly positive cells within the spleens of all groups except the ST-193 survivor group.

The neutralizing ability of serum samples from surviving animals was an unexpected result. Previous reports have shown that neutralizing antibodies only appear later (>32 days postinfection) in surviving LASV-infected guinea pigs (Jahrling et al., 1982) and do not appear to play a significant recovery role in human cases (Johnson et al., 1987). Although the formal possibility exists that residual compound present in the serum mediated neutralization of LASV, this explanation seems unlikely as the day 21 and day 29 samples were drawn 8 and 16 days after the last compound dose was delivered. Thus these results suggest that a specific immune response against LASV was produced in the survivors. One possibility is that ST-193 treatment may mitigate LASV-associated lymphopenia (Jahrling et al., 1982) and thus allow for a more timely humoral immune response. The lack of strong production of LASV-specific IgG1 or IgG2 in treated animals as measured by ELISA may be due to several factors. Because these animals all became viremic to varying degrees during the study, it is possible that LASV-specific antibodies present in the serum were bound to free antigen, thus preventing binding to the immobilized antigen in the ELISA. Additionally, it is unknown what effect irradiation has on the antigenic epitopes of LASV, thus the low specific antibody titer observed may also be due to weakened LASV antigens.

Both doses of ST-193, 25 and 80 mg/kg/day, were sufficient to provide a level of protection from a lethal LASV infection. Differences between these two treatment groups were not remarkable, although neither was fully protective. Possibly the lower dose is sufficient to yield the maximum antiviral effect, or perhaps the group sizes are too small to detect differences in survival rates between the two doses. Another consideration is that within the context of this experiment, while the lower dose provided less ST-193 to the animals, the higher dose exposed animals to higher volumes of the vehicle. Weight loss associated with the vehicle volume may exacerbate the disease and offset the additional quantity of antiviral compound. Such an effect might contribute to the poor survival rate of the ribavirin-treated group as well, as that group received the same vehicle volume as the 80 mg/kg ST-193 group.

Unexpectedly, some surviving animals in the ST-193 treatment groups experienced elevated liver enzymes (AST and ALT) at the study endpoint. Levels of AST and ALT were elevated in surviving animals, but had returned in the normal range by day 21 postinfection, only to become elevated again at the study endpoint. One out of five surviving animals in the ST-193 25 mg/kg treatment group and three out of five in the ST-193 80 mg/kg treatment group experienced this late increase in ALT levels. All of the surviving animals experienced a late increase in AST levels to varying degrees. This late increase in liver enzymes occurred despite survivors having no clinical signs of illness and little or no detectable viremia at the study endpoint. Repeat analyses were not possible due to limited sample volumes. Histologically, the surviving animals with elevated liver enzymes also had evidence of mild hepatocellular degeneration and/or necrosis at necropsy. Other laboratories have reported multifocal hepatic necrosis in the Hartley guinea pig that is unrelated to age or overall health (Maeda et al., 2000). Maeda et al. observed that in 202 guinea pigs between 4 and 23-weeks old, 111 of these animals displayed multiple necrotic lesions, most commonly coagulative necrosis, in the absence of any known disease (Maeda et al., 2000). Indeed, we have observed this phenomenon in our laboratory as well in uninfected strain 13 guinea pigs and at early stages of LASV infection before observable liver pathology with corresponding elevated enzyme levels. Another potential explanation for the elevated liver enzymes could be hemolysis of the samples, a well known cause of overestimated AST and ALT (Sonntag, 1986). In fact, another symptom of hemolysis, depressed alkaline phosphatase (Lippi et al., 2006; Sonntag, 1986) is seen in parallel with those samples with elevated AST and ALT. Alkaline phosphatase levels have been reported to actually increase in LASV-infected guinea pigs (Lukashevich et al., 2005), suggesting that the depressed levels seen here may be more consistent with hemolysis than with liver damage. Finally, a subsequent LASV infection study in strain 13 guinea pigs did not exhibit this phenomenon (R. Carrion, personal communication with. S.A.); in this follow-up study, ST-193-treated survivors displayed normal liver enzyme values. Ultimately, this finding may be incidental and unrelated to either LASV infection or treatment with ST-193. Additional studies should help resolve this.

This work demonstrates that ST-193 can effectively limit viral replication in an animal model of Lassa fever and protect the majority of animals from lethal disease. Future studies will examine different dosing regimens (e.g., amount, frequency, and duration), drug combinations, postinfection treatment and acquisition of pro-

tective immunity among treated survivors. Given the potency of ST-193 and its ability to inhibit other hemorrhagic arenaviruses *in vitro* (Larson et al., 2008), this *in vivo* validation bolsters its prospects as a candidate for further development.

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References

Agnihothram, S.S., York, J., Trahey, M., Nunberg, J.H., 2007. Bitopic membrane topology of the stable signal peptide in the tripartite Junín virus GP-C envelope glycoprotein complex. J. Virol. 81, 4331–4337.

Atkin, S., Anaraki, S., Gothard, P., Walsh, A., Brown, D., Gopal, R., Hand, J., Morgan, D., 2009. The first case of Lassa fever imported from Mali to the United Kingdom, February 2009. Euro Surveill. 14 (10), pii=19145.

Baize, S., Marianneau, P., Loth, P., Reynard, S., Journeaux, A., Chevallier, M., Tordo, N., Deubel, V., Contamin, H., 2009. Early and strong immune responses are associated with control of viral replication and recovery in Lassa virus-infected cynomolgus monkeys. J. Virol. 83, 5890–5903.

Bani-Sadr, F., Carrat, F., Pol, S., Hor, R., Rosenthal, E., Goujard, C., Morand, P., Lunel-Fabiani, F., Salmon-Ceron, D., Piroth, L., Pialoux, G., Bentata, M., Cacoub, P., Perronne, C., ANRS HC02-Ribavic Study Team, 2005. Risk factors for symptomatic mitochondrial toxicity in HIV/hepatitis C virus-coinfected patients during interferon plus ribavirin-based therapy. J. Acquir. Immune Defic. Syndr. 40, 47–52.

Bausch, D.G., Demby, A.H., Coulibaly, M., Kanu, J., Goba, A., Bah, A., Condé, N., Wurtzel, H.L., Cavallaro, K.F., Lloyd, E., Baldet, F.B., Cissé, S.D., Fofona, D., Savané, I.K., Tolno, R.T., Mahy, B., Wagoner, K.D., Ksiazek, T.G., Peters, C.J., Rollin, P.E., 2001. Lassa fever in Guinea: I. Epidemiology of human disease and clinical observations. Vector Borne Zoonotic Dis. 1, 269–281.

Borrow, P., Oldstone, M.B.A., 1992. Characterization of lymphocytic choriomeningitis virus-binding protein(s): a candidate cellular receptor for the virus. J. Virol. 66, 7270–7281.

Bredenbeek, P.J., Molenkamp, R., Spaan, W.J.M., Deubel, V., Marianneau, P., Salvato, M.S., Moshkoff, D., Zapata, J., Tikhonov, I., Patterson, J., Carrion, R., Ticer, A., Brasky, K., Lukashevich, I.S., 2006. A recombinant Yellow Fever 17D vaccine expressing Lassa virus glycoproteins. Virology 345, 299–304.

Buchmeier, M.J., de la Torre, J.-C., Peters, C.J., 2007. *Arenaviridae*: the viruses and their replication. In: Knipe, D.M., Howley, P.M., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, B., Straus, S.E. (Eds.), Fields Virology. Lippincott, Williams & Wilkins, New York, pp. 1792–1827.

Burns, J.W., Buchmeier, M.J., 1991. Protein-protein interactions in lymphocytic choriomeningitis virus. Virology 183, 620–629.

Carrion Jr., R., Brasky, K., Mansfield, K., Johnson, C., Gonzales, M., Ticer, A., Lukashevich, I., Tardif, S., Patterson, J., 2007a. Lassa virus infection in experimentally infected marmosets: liver pathology and immunophenotypic alterations in target tissues. J. Virol. 81, 6482–6490.

Carrion Jr., R., Patterson, J.L., Johnson, C., Gonzales, M., Moreira, C.R., Ticer, A., Brasky, K., Hubbard, G.B., Moshkoff, D., Zapata, J., Salvato, M.S., Lukashevich, I.S., 2007b. A ML29 reassortant virus protects guinea pigs against a distantly related Nigerian strain of Lassa virus and can provide sterilizing immunity. Vaccine 25, 4093–4102.

Colman, P.M., Lawrence, M.C., 2003. The structural biology of type I viral membrane fusion. Nat. Rev. Mol. Cell Biol. 4, 309–319.

Cummins, D., 1992. Rats, fever and sudden deafness in Sierra Leone. Trop. Doct. 22, 83–84.

Cummins, D., McCormick, J.B., Bennett, D., Samba, J.A., Farrar, B., Machin, S.J., Fisher-Hoch, S.P., 1990. Acute sensorineural deafness in Lassa fever. JAMA 264, 2093–2096.

Di Simone, C., Buchmeier, M.J., 1995. Kinetics and pH dependence of acid-induced structural changes in the lymphocytic choriomeningitis virus glycoprotein complex. Virology 209, 3–9.

Drapeau, C.M., Remotti, D., Noto, P., Capone, A., Boumis, E., Petrosillo, N., 2008. Bone marrow toxicity in HCV genotype 5a-infected patient after peg-IFN alpha-2a and ribavirin therapy. J. Chemother. 20, 648–651.

Enria, D., Mills, J.N., Flick, R., Bowen, M.D., Bausch, D., Shieh, W.J., Peters, C.J., 2006. Arenavirus infections. In: Guerrant, R.L., Walker, D.H., Weller, P.F. (Eds.), Tropical Infectious Diseases: Principles, Pathogens & Practice. , 2nd ed. Elsevier, Philadelphia, pp. 734–755.

Eschli, B., Quirin, K., Wepf, A., Weber, J., Zinkernagel, R., Hengartner, H., 2006. Identification of an N-terminal trimeric coiled-coil core within arenavirus glycoprotein 2 permits assignment to class I viral fusion proteins. J. Virol. 80, 5897–5907.

Fisher-Hoch, S.P., McCormick, J.B., 2004. Lassa fever vaccine. Expert Rev. Vaccines 3, 189–197.

Fisher-Hoch, S.P., Tomori, O., Nasidi, A., Perez-Oronoz, G.I., Fakile, Y., Hutwagner, L., McCormick, J.B., 1995. Review of cases of nosocomial Lassa fever in Nigeria: the high price of poor medical practice. BMJ 311, 857–859.

Frame, J.D., 1989. Clinical features of Lassa fever in Liberia. Rev. Infect. Dis. 11 (Suppl. 4), S783–789.

Frame, J.D., Baldwin Jr., J.M., Gocke, D.J., Troup, J.M., 1970. Lassa fever, a new virus disease of man from West Africa. I. Clinical description and pathological findings. Am. J. Trop. Med. Hyg. 19, 670–676.

Fuster, D., Huertas, J.A., Gómez, G., Solà, R., González García, J., Vilaró, J., Pedrol, E., Force, L., Tor, J., Sirera, G., Videla, S., Planas, R., Clotet, B., Tural, C., for the PEG-TOX Research Group, 2005. Short communication. Baseline factors associated with haematological toxicity that leads to a dosage reduction of pegylated interferon-α2a and ribavirin in HIV- and HCV-coinfected patients on HCV antiviral therapy. Antivir. Ther. 10, 841–847.

Gallaher, W.R., DiSimone, C., Buchmeier, M.J., 2001. The viral transmembrane superfamily: possible divergence of Arenavirus and Filovirus glycoproteins from a common RNA virus ancestor. BMC Microbiol. 1, 1.

Geisbert, T.W., Jones, S., Fritz, E.A., Shurtleff, A.C., Geisbert, J.B., Liebscher, R., Grolla, A., Ströher, U., Fernando, L., Daddario, K.M., Guttieri, M.C., Mothé, B.R., Larsen, T., Hensley, L.E., Jahrling, P.B., Feldmann, H., 2005. Development of a new vaccine for the prevention of Lassa fever. PLoS Med. 2, e183.

Gowen, B.B., Holbrook, M.R., 2008. Animal models of highly pathogenic RNA viral infections: hemorrhagic fever viruses. Antiviral Res. 78, 79–90.

Gowen, B.B., Wong, M.-H., Jung, K.-H., Sanders, A.B., Mendenhall, M., Bailey, K.W., Furuta, Y., Sidwell, R.W., 2007. In vitro and in vivo activities of T-705 against arenavirus and bunyavirus infections. Antimicrob. Agents Chemother. 51, 3168–3176.

Gowen, B.B., Smee, D.F., Wong, M.-H., Hall, J.O., Jung, K.-H., Bailey, K.W., Stevens, J.R., Furuta, Y., Morrey, J.D., 2008. Treatment of late stage disease in a model of arenaviral hemorrhagic fever: T-705 efficacy and reduced toxicity suggests an alternative to ribavirin. PLoS One 3, e3725.

Hooper, J.W., Kamrud, K.I., Elgh, F., Custer, D., Schmaljohn, C.S., 1999. DNA vaccination with hantavirus M segment elicits neutralizing antibodies and protects against Seoul virus infection. Virology 255, 269–278.

Huggins, J.W., 1989. Prospects for treatment of viral hemorrhagic fevers with ribavirin, a broad-spectrum antiviral drug. Rev. Infect. Dis. 11 (Suppl. 4), S750–761.

Imported Lassa fever—New Jersey, 2004. MMWR Morb. Mortal. Wkly Rep. 53, 894–897.

Isaäcson, M., 2001. Viral hemorrhagic fever hazards for travelers in Africa. Clin. Infect. Dis. 33, 1707–1712.

Jahrling, P.B., 1983. Protection of Lassa virus-infected guinea pigs with Lassa-immune plasma of guinea pig, primate, and human origin. J. Med. Virol. 12, 93–102.

Jahrling, P.B., Peters, C.J., 1986. Serology and virulence diversity among Old-World arenaviruses, and the relevance to vaccine development. Med. Microbiol. Immunol. 175, 165–167.

Jahrling, P.B., Hesse, R.A., Eddy, G.A., Johnson, K.M., Callis, R.T., Stephen, E.L., 1980. Lassa virus infection of rhesus monkeys: pathogenesis and treatment with ribavirin. J. Infect. Dis. 141, 580–589.

Jahrling, P.B., Smith, S., Hesse, R.A., Rhoderick, J.B., 1982. Pathogenesis of Lassa virus infection in guinea pigs. Infect. Immun. 37, 771–778.

Jahrling, P.B., Peters, C.J., Stephen, E.L., 1984. Enhanced treatment of Lassa fever by immune plasma combined with ribavirin in cynomolgus monkeys. J. Infect. Dis. 149, 420–427.

Johnson, K.M., McCormick, J.B., Webb, P.A., Smith, E.S., Elliott, L.H., King, I.J., 1987. Clinical virology of Lassa fever in hospitalized patients. J. Infect. Dis. 155, 456–464.

Kenyon, R.H., Canonico, P.G., Green, D.E., Peters, C.J., 1986. Effect of ribavirin and tributylribavirin on Argentine hemorrhagic fever (Junin virus) in guinea pigs. Antimicrob. Agents Chemother. 29, 521–523.

Kitching, A., Addiman, S., Cathcart, S., Bischop, L., Krahé, D., Nicholas, M., Coakley, J., Lloyd, G., Brooks, T., Morgan, D., Turbitt, D., 2009. A fatal case of Lassa fever in London, January 2009. Euro Surveill. 14 (6), pii=19117.

Kumar, K.S., Russo, M.W., Borczuk, A.C., Brown, M., Esposito, S.P., Lobritto, S.J., Jacobson, I.M., Brown Jr., R.S., 2002. Significant pulmonary toxicity associated with interferon and ribavirin therapy for hepatitis C. Am. J. Gastroenterol. 97, 2432–2440.

Larson, R.A., Dai, D., Hosack, V.T., Tan, Y., Bolken, T.C., Hruby, D.E., Amberg, S.M., 2008. Identification of a broad-spectrum arenavirus entry inhibitor. J. Virol. 82, 10768–10775.

Lassa Fever Imported to England, 2000a. Commun. Dis. Rep. CDR Wkly 10, 99.

Lassa Fever, 2000b. Case Imported to Germany. Wkly Epidemiol. Rec. 75, 17–18.

Lassa Fever, 2000c. Imported Case, Netherlands. Wkly Epidemiol. Rec. 75, 265.

Lee, A.M., Rojek, J.M., Spiropoulou, C.F., Gundersen, A.T., Jin, W., Shaginian, A., York, J., Nunberg, J.H., Boger, D.L., Oldstone, M.B.A., Kunz, S., 2008. Unique small molecule entry inhibitors of hemorrhagic fever arenaviruses. J. Biol. Chem. 283, 18734–18742.

Lenz, O., ter Meulen, J., Klenk, H.-D., Seidah, N.G., Garten, W., 2001. The Lassa virus glycoprotein precursor GP-C is proteolytically processed by subtilase SKI-1/S1P. Proc. Natl. Acad. Sci. USA 98, 12701–12705.

Liao, B.S., Byl, F.M., Adour, K.K., 1992. Audiometric comparison of Lassa fever hearing loss and idiopathic sudden hearing loss: evidence for viral cause. Otolaryngol. Head Neck Surg. 106, 226–229.

Lippi, G., Salvagno, G.L., Montagnana, M., Brocco, G., Guidi, G.C., 2006. Influence of hemolysis on routine clinical chemistry testing. Clin. Chem. Lab. Med. 44, 311–316.

Lucia, H.L., Coppenhaver, D.H., Baron, S., 1989. Arenavirus infection in the guinea pig model: antiviral therapy with recombinant interferon-alpha, the immunomodulator CL246,738 and ribavirin. Antiviral Res. 12, 279–292.

Lukashevich, I.S., Patterson, J., Carrion, R., Moshkoff, D., Ticer, A., Zapata, J., Brasky, K., Geiger, R., Hubbard, G.B., Bryant, J., Salvato, M.S., 2005. A live attenuated vaccine for Lassa fever made by reassortment of Lassa and Mopeia viruses. J. Virol. 79, 13934–13942.

Maeda, H., Kawaguchi, H., Sato, K., Satake, S., Izumi, H., Miyajima, H., 2000. Spontaneous multiple focal to massive hepatic necrosis in guinea pigs. J. Toxicol. Pathol. 13, 207–212.

Mahdy, M.S., Chiang, W., McLaughlin, B., Derksen, K., Truxton, B.H., Neg, K., 1989. Lassa fever: the first confirmed case imported into Canada. Can. Dis. Wkly Rep. 15, 193–198.

McCormick, J.B., 1986. Clinical, epidemiologic, and therapeutic aspects of Lassa fever. Med. Microbiol. Immunol. 175, 153–155.

McCormick, J.B., 1987. Epidemiology and control of Lassa fever. Curr. Top. Microbiol. Immunol. 134, 69–78.

McCormick, J.B., King, I.J., Webb, P.A., Scribner, C.L., Craven, R.B., Johnson, K.M., Elliott, L.H., Belmont-Williams, R., 1986. Lassa fever. Effective therapy with ribavirin. N. Engl. J. Med. 314, 20–26.

McCormick, J.B., King, I.J., Webb, P.A., Johnson, K.M., O'Sullivan, R., Smith, E.S., Trippel, S., Tong, T.C., 1987a. A case-control study of the clinical diagnosis and course of Lassa fever. J. Infect. Dis. 155, 445–455.

McCormick, J.B., Webb, P.A., Krebs, J.W., Johnson, K.M., Smith, E.S., 1987b. A prospective study of the epidemiology and ecology of Lassa fever. J. Infect. Dis. 155, 437–444.

Mertens, P.E., Patton, R., Baum, J.J., Monath, T.P., 1973. Clinical presentation of Lassa fever cases during the hospital epidemic at Zorzor, Liberia, March-April 1972. Am. J. Trop. Med. Hyg. 22, 780–784.

Mira, J.A., López-Cortés, L.F., Merino, D., Arizcorreta-Yarza, A., Rivero, A., Collado, A., Ríos-Villegas, M.J., González-Serrano, M., Torres-Tortoso, M., Macías, J., Valera-Bestard, B., Fernández-Fuertes, E., Girón-González, J.A., Lozano, F., Pineda, J.A., Grupo para el Estudio de las Hepatitis Viricas de la Sociedad Andaluza de Enfermedades Infecciosas, 2007. Predictors of severe haematological toxicity secondary to pegylated interferon plus ribavirin treatment in HIV-HCV-coinfected patients. Antivir. Ther. 12, 1225–1235.

Monath, T.P., Maher, M., Casals, J., Kissling, R.E., Cacciapuoti, A., 1974. Lassa fever in the Eastern Province of Sierra Leone, 1970-1972. II. Clinical observations and virological studies on selected hospital cases. Am. J. Trop. Med. Hyg. 23, 1140–1149.

Müller, S., Günther, S., 2007. Broad-spectrum antiviral activity of small interfering RNA targeting the conserved RNA termini of Lassa virus. Antimicrob. Agents Chemother. 51, 2215–2218.

Peters, C.J., Jahrling, P.B., Liu, C.T., Kenyon, R.H., McKee Jr., K.T., Barrera Oro, J.G., 1987. Experimental studies of arenaviral hemorrhagic fevers. Curr. Top. Microbiol. Immunol. 134, 5–68.

Sonntag, O., 1986. Haemolysis as an interference factor in clinical chemistry. J. Clin. Chem. Clin. Biochem. 24, 127–139.

Stephen, E.L., Jones, D.E., Peters, C.J., Eddy, G.A., Loizeaux, P.S., Jahrling, P.B., 1980. Ribavirin treatment of toga-, arena- and bunyavirus infections in subhuman primates and other laboratory animal species. In: Smith, R.A., Kirkpatrick, W. (Eds.), Ribavirin: A Broad Spectrum Antiviral Agent. Academic Press, New York, pp. 169–183.

Tomori, O., Johnson, K.M., Kiley, M.P., Elliott, L.H., 1987. Standardization of a plaque assay for Lassa virus. J. Med. Virol. 22, 77–89.

Walker, D.H., Wulff, H., Lange, J.V., Murphy, F.A., 1975. Comparative pathology of Lassa virus infection in monkeys, guinea-pigs, and *Mastomys natalensis*. Bull. World Health Organ. 52, 523–534.

Wright, K.E., Spiro, R.C., Burns, J.W., Buchmeier, M.J., 1990. Post-translational processing of the glycoproteins of lymphocytic choriomeningitis virus. Virology 177, 175–183.

York, J., Agnihothram, S.S., Romanowski, V., Nunberg, J.H., 2005. Genetic analysis of heptad-repeat regions in the G2 fusion subunit of the Junín arenavirus envelope glycoprotein. Virology 343, 267–274.