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Comparison of the inhibitory effects of interferon alfacon-1 and ribavirin on yellow fever virus infection in a hamster model

Justin G. Julander [a,*], John D. Morrey [a], Lawrence M. Blatt [b], Kristiina Shafer [a], Robert W. Sidwell [a]

[a] *Institute for Antiviral Research, Utah State University, Logan, UT 84322-5600, United States*
[b] *InterMune Inc., Brisbane, CA, United States*

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Abstract

Antiviral compounds were evaluated for efficacy against yellow fever virus (YFV) in a hamster model of YFV-induced liver disease. Challenge with a 10^2 50% cell culture infectious doses of YFV resulted in a 50–80% mortality rate in female hamsters. Virus was detected by quantitative real-time RT-PCR (QRT-PCR) in liver, kidney, spleen and serum with peak titers on 4–6 days post-viral challenge (dpi). Serum levels of alkaline phosphatase, alanine aminotransferase (ALT), bilirubin, blood urea nitrogen, potassium and creatinine were significantly elevated, while serum levels of albumin, amylase, glucose, calcium, globulin, phosphorus, sodium and total protein were significantly reduced. Packed cell volume and white blood cell count were significantly elevated during the course of the infection. Intraperitoneal treatment of hamsters with 0.5–5 μg/kg/day interferon (IFN) alfacon-1, 100 mg/kg/day viramidine or 50 mg/kg/day ribavirin, initiated 4 h prior to YFV challenge, resulted in significant improvement in survival and serum ALT levels. Treatment with IFN alfacon-1 or ribavirin starting 2 dpi, also significantly improved survival and serum ALT levels in hamsters challenged with YFV. Pre- and post-virus exposure treatment with IFN alfacon-1 was efficacious in improving disease in YFV-infected hamsters.

Keywords: Yellow fever virus; Flavivirus; Interferon; Infergen; Ribavirin; Antiviral; Hamster; Alanine aminotransferase; Treatment

1. Introduction

There are no specific antiviral agents for the treatment of yellow fever virus (YFV), and despite a commercial YFV vaccine, there are still approximately 30,000 deaths worldwide each year and cases have been increasing in the last 20 years (Tomori, 2004). The virus is endemic in Africa and South America, but cases of YFV have been reported in non-endemic areas after travel (Bae et al., 2003; McFarland et al., 1997). Viscerotropic infections have also occurred with vaccination, so there is also risk of infection with vaccine strains (Anon., 2002; Martin et al., 2001; Vasconcelos et al., 2001). Therefore, the development of therapies for treatment of YFV disease is not only important for those in endemic areas, but also for those that may travel to endemic areas or those who may experience disease after vaccination.

YFV is a single-stranded RNA virus of the Flaviviridae family, and is related to hepatitis C, dengue, West Nile and other viruses of human concern. Mosquito species of *Aedes* and *Haemogogus* transmit YFV and serve as a reservoir for the virus; humans and monkeys are the primary hosts for viral infection (Lourenco-de-Oliveira et al., 2004; Vasconcelos et al., 2003). The disease may be limited to a mild febrile illness or may be more severe, including jaundice, renal failure, vascular instability and shock (Monath and Barrett, 2003). There is an approximately 50% case fatality rate in severe YFV cases (Tomori, 2004).

Until recently, non-human primates such as macaques and squirrel monkeys, have been the only available animal models for YFV-induced liver disease (Arroyo et al., 1988; Guirakhoo et al., 2002; Monath et al., 2002). Development of a hamster model for YFV-induced liver disease has been recently described (Tesh et al., 2001; Xiao et al., 2001; Sbrana et al., 2006). The availability of such a model has made it possible to screen potential antiviral agents for activity against the virus in a small animal model. The purpose of this study was to further characterize the

* Corresponding author. Tel.: +1 435 797 7215; fax: +1 435 797 3959.
E-mail address: jgjulander@cc.usu.edu (J.G. Julander).

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hamster YFV for use in the evaluation of antiviral compounds for efficacy against the virus.

2. Materials and methods

2.1. Virus

Jimenez, hamster-adapted (HA), YFV was kindly provided by Robert Tesh (University of Texas Medical Branch, Galveston, TX, USA). Stock virus was prepared by intraperitoneal (i.p.) inoculation of five adult female hamsters. The livers of the infected hamsters were removed 3 days post-virus injection (dpi), and homogenized in a 2× volume of sterile PBS. The homogenate was clarified by allowing tissue to settle for 15 min, and the supernatant was collected and frozen in aliquots at −80 °C. The virus was titrated in hamsters, and a 10^{-4} dilution was used in subsequent studies, which dose corresponded with 10^2 50% cell culture infectious doses ($CCID_{50}$)/0.1 ml as determined by infectious cell culture assay.

2.2. Animals

Six- to eight-week-old (110–140 g) female Syrian golden hamsters were used (Simonsen Laboratories, Gilroy, CA, USA). Animals were challenged intraperitoneally with 10-fold dilutions of virus stock to determine an acceptable infectious dose. The hamsters were randomly assigned to cages, and given food and water ad libitum. All work with these animals was performed in the Biosafety Level 3 (BL-3) area of the AAALAC-accredited Laboratory Animal Research Center (LARC) at Utah State University (USU).

2.3. Compounds

Interferon alfacon-1 (IFN alfacon-1, infergen), a consensus-type interferon, was provided by InterMune Inc. (Brisbane, CA, USA) as an aqueous solution. Animals were treated with 5 μg/kg/day for 7 days. IFN alfacon-1 has a specific activity of 1×10^9 U/mg of protein. In the present study, hamsters were dosed at 5 μg/kg/day IFN alfacon-1, which converts to 5 MU/kg/day. Ribavirin was provided by Valeant Pharmaceuticals (Costa Mesa, CA, USA) and was used at 50 mg/kg/day. Viramidine (ribamidine) was also provided by Valeant Pharmaceuticals and was used at 100 mg/kg/day. All of the compounds were prepared in sterile physiological saline just prior to use, and stored at 4 °C thereafter.

2.4. Quantitative real-time RT-PCR (QRT-PCR)

Primer-pairs (forward AGTTGATTCCATCTTGGGCTTC, reverse ACCTCTTCCTCTCCATCCCATC) and Taq-man probe (5′-6-carboxyfluorescein-CCTATGGTGGCTCATGGAAGTT-GGAAGG-6-carboxy-*N*,*N*,*N*′,*N*′-tetramethylrhodamine-3′) specific for nucleotides 4767–4860 of the Asibi YFV strain (AY640589.1) were used (Qiagen, Valencia, CA, USA). The one-step Brilliant QRT-PCR master mix 1-step kit (Stratagene, La Jolla, CA, USA) was used for RT and amplification of YFV RNA with primers and probe at 0.2 μM. RNA was isolated from tissue using Trizol (Invitrogen, Carlsbad, CA, USA) according to manufactures instructions and pelleted RNA was reconstituted in 100 μl of DEPC-treated water and stored at −20 °C. One microliter of total cellular RNA (from a total of 100 μl), extracted from infected or control tissues was used. Samples were run on a DNA Engine Opticon 2 (MJ Research Inc., Waltham, MA, USA). Reverse transcription of cellular RNA was performed for 30 min at 50 °C followed by PCR, which consisted of 40 cycles of 15 s at 95 °C and 60 s at 61 °C. Results were given in terms of relative equivalents (re), reflecting the amount of YFV present in the sample as extrapolated from a standard curve obtained from amplification of a dilution of total RNA obtained 2 dpi from Vero cells infected with YFV.

2.5. VetScan diagnostics

A volume of 100 μl of serum, obtained at necropsy, was loaded on a VetScan Comprehensive Diagnostic Profile reagent rotor and run on the VetScan Chemistry Analyzer following manufacturer instructions. Serum levels of alanine aminotransferase (ALT), albumin, alkaline phosphatase, amylase, calcium, creatinine, globulin, glucose, phosphorus, potassium, sodium, total bilirubin, total protein and blood urea nitrogen (BUN), were measured.

2.6. Serum ALT assay

Serum ALT was determined using an ALT reagent kit (Teco Diagnostics, Anaheim, CA, USA). A volume of 50 μl of ALT substrate was placed in each well of a 96-well plate. To the ALT substrate, 10 μl of serum, obtained at necropsy and stored at −80 °C until use, was added at timed intervals. Following the same time interval, and after incubation at 37 °C for 30 min, 50 μl of color reagent was added to each well. After another incubation at 37 °C for 10 min, 200 μl of ALT color developer was added and mixed, after which the samples were incubated at 37 °C for an additional 5 min. The plate was read on a SpectraMax Plus384 spectrophotometer (Molecular Devices, Sunnyvale, CA, USA) at 505 nm. Normal and abnormal control sera with known ALT concentrations were used as positive controls.

2.7. Blood parameters

Blood samples (approximately 1 ml) were taken at necropsy and parameters, including packed cell volume (PCV) and white blood cell (WBCC) count, were recorded for each day after virus challenge. Whole blood was loaded into a capillary tube, plugged at one end, and spun for 5 min on a capillary centrifuge and the PCV was measured. For WBCC, 10 μl of whole blood was added to 190 μl working solution (0.2% crystal violet and 0.4% glacial acetic acid) for 5 min and then counted on a hemocytometer.

2.8. Experimental design for titration of YFV in Syrian golden hamsters

Stock YFV was serial diluted 10-fold from undilute to a 10^{-5} dilution in minimal essential media. Each dilution was injected i.p. into a group of five animals and mortality was recorded through 21 dpi. Daily weight change was also measured in individually marked animals. This experiment was repeated with 10 animals per experimental group, and the resulting data were combined with the results from the first titration experiment.

2.9. Experimental design for characterization of YFV infection in Syrian golden hamsters

Hamsters were challenged i.p. with a 10^{-4} dilution of stock YFV. On each day for 8 days after virus challenge, liver, kidney, spleen and serum samples were obtained from 15 animals. These tissues were analyzed for various parameters, including tissue virus titers, serum ions and proteins as measured by the VetScan instrument, PCV and WBCC.

2.10. Experimental design for treatment of YFV

Hamsters, challenged i.p. with a 10^{-4} dilution of stock YFV, were treated i.p. with various dilutions (0.5, 1.6 or 5 μg/kg/day) of IFN alfacon-1 or (5 or 50 mg/kg/day) ribavirin starting 4 h prior to virus challenge and continued through 6 dpi. IFN alfacon-1 treatment was given once daily, while ribavirin treatment was administered twice daily, 12 h apart. Serum samples were analyzed for ALT levels 6 dpi, which was the time that ALT reaches peak values. Weight change was measured on 3 and 6 dpi, which time-points allow for the maximum statistical difference in weight change to be observed in YFV-infected hamsters, and is prior to highest death rates in infected animals.

In a second study, viramidine (100 mg/kg/day) was administered i.p. twice daily from 4 h prior to virus challenge to 6 dpi, and serum samples were taken as above for ALT measurement and mortality was observed through 21 dpi. Weight change was also measured between 3 and 6 dpi. In a third experiment, treatment with IFN alfacon-1 or ribavirin was initiated 2 dpi, and animals were treated following the same dosing schedule as above from 2 to 8 dpi to see if treatment initiated later in the infection would be effective in increasing survival, reducing weight loss and reducing serum ALT levels. Mortality was observed up to 21 dpi and mean day to death was calculated.

2.11. Statistical analysis

The *t*-test was used to determine statistical differences in all VetScan assay results and serum ALT levels between test groups and placebo-treated controls. Wilcoxon and log-rank tests were used for analysis of survival data.

3. Results

3.1. Titration of YFV in hamsters

Groups of hamsters were inoculated with serial dilutions of virus stock. Mortality occurred in varying degrees in all virus challenge groups, but mortality and mean day to death (MDD) did not correlate with virus challenge (Table 1). Significant weight loss was also observed in animals infected with undiluted stock YFV and weight change correlated with the dilution of YFV stock that groups were challenged with. There was weight gain in the group challenged with a 10^{-5} dilution of stock virus, but weight loss was observed in all other virus dilution groups, with greatest weight loss in undiluted virus stock (Table 1). A highly significant difference in survival and a significant difference in weight gain between 3 and 6 dpi was observed in the groups of hamsters challenged with a 10^{-4} dilution (10^2 $CCID_{50}$/0.1 ml) as compared with sham-infected animals. Because of the significant differences in these parameters with the 10^{-4} dilution, this virus challenge was used in all subsequent experiments.

3.2. Characterization of YFV infection in Syrian golden hamsters

Groups of 20 hamsters were challenged with 10^{-4} dilution of virus stock, and tissue samples were obtained from 15 hamsters. Of the remaining hamsters challenged with YFV and not used for sample collection, 23 of 35 (66%) animals died prior to 8 dpi. Tissue samples, including liver, kidney, spleen and serum, were obtained each day after virus challenge, and assayed for biochemical and viral parameters. Tissue YFV titers were detected by QRT-PCR in liver, spleen, kidney and serum and reached peak levels on approximately 4–6 dpi (Fig. 1), respectively. The highest titer (4.8 $\log_{10}$) of virus was detected in the liver, which was not unexpected.

Serum parameters were measured using VetScan Comprehensive Diagnostic Profile cartridges. Serum levels of alkaline phosphatase, ALT, bilirubin, BUN, potassium and creatinine

Table 1
Survival, mean day to death and mean weight change of Syrian golden hamsters challenged with various serial dilutions of Jimenez hamster-adapted yellow fever virus

Virus challenge	Survival/total[a]	MDD[b]	Weight change[c] (g)
Undilute stock	7/20**	6.3 ± 1.0	−16.0 ± 4.1***
10^{-1}	7/15*	8.5 ± 1.7	−5.6 ± 10.7*
10^{-2}	9/15*	7.2 ± 1.5	−6.9 ± 8.1*
10^{-3}	7/15*	7.5 ± 1.6	−5.1 ± 8.4**
10^{-4}	3/15***	8.5 ± 1.9	−4.3 ± 7.7**
10^{-5}	11/15	8.3 ± 1.0	6.4 ± 3.6
Sham infection	8/8	>21 ± 0.0	5.4 ± 2.4

Results compiled from two separate titration experiments. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ as compared with sham infection.

[a] Number of animals surviving to 21 days post-virus inoculation (dpi) per total number of animals challenged.

[b] Mean day to death of animals dying before 21 dpi.

[c] Mean weight change of animals between 3 and 6 dpi.

Table 2
Time-course of various blood parameters of hamsters infected with hamster-adapted yellow fever virus ($^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared with sham injection)

Parameter[a]	Units	Days post-virus challenge								Controls[b]
		1	2	3	4	5	6	7	8	
ALT (Teco)	IU/L	–	67 ± 14	63 ± 9	77 ± 17	164 ± 34***	196 ± 89***	174 ± 96**	117 ± 52*	79 ± 15
ALT[c]	U/L	96 ± 35	59 ± 23	75 ± 16	109 ± 33	ADL[d]	ADL	ADL	ADL	77 ± 39
Albumin	g/dL	4.8 ± 0.6	4.3 ± 0.2	4.1 ± 0.1	4.5 ± 0.3	3.5 ± 0.5**	2.5 ± 1.6**	2.9 ± 1.1*	2.5 ± 1.0**	4.5 ± 0.3
Alk phos[e]	U/L	173 ± 44	152 ± 11	151 ± 17	173 ± 19	253 ± 160	730 ± 470**	628 ± 297**	864 ± 891**	151 ± 21
Amylase	U/L	1843 ± 515	1678 ± 351	1723 ± 126	1179 ± 475*	1024 ± 1034*	917 ± 535**	821 ± 809*	600 ± 536**	1723 ± 367
Calcium	mg/dL	14 ± 1.3	12 ± 0.4	12 ± 0.3	13.3 ± 1.2	11.6 ± 1.6	9.7 ± 2.3**	11 ± 2.3*	11 ± 0.9**	13 ± 1.2
Creatinine	mg/dL	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.2*	ADL	ADL	0.3 ± 0.1
Globulin	g/dL	2.2 ± 0.4	1.8 ± 0.0	1.7 ± 0.1	2.2 ± 0.3	1.7 ± 0.3*	1.3 ± 0.8	1.4 ± 0.9	1.2 ± 0.7**	2.1 ± 0.4
Glucose	mg/dL	163 ± 46	123 ± 19	126 ± 15	144 ± 13	119 ± 115	58 ± 45**	63 ± 58*	76 ± 38**	144 ± 25
Phosphorus	mg/dL	10 ± 1.4**	8.0 ± 0.8	6.9 ± 0.6**	8.8 ± 0.8	9.9 ± 1.9	8.2 ± 1.5	8.7 ± 1.6	7.5 ± 2.5	8.5 ± 1.2
Potassium	mmol/L	7.5 ± 0.7	7.1 ± 0.8	6.2 ± 0.3	6.9 ± 0.8	8.1 ± 0.6**	5.3 ± 3.0	6.0 ± 3.4	7.5 ± 0.6	6.6 ± 0.8
Sodium	mmol/L	159 ± 13	140 ± 2.4**	138 ± 3.2**	152 ± 9.2	147 ± 3.5	142 ± 4.3	145 ± 9.4	150 ± 7.4	149 ± 11
Bilirubin	mg/dL	0.2 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	2.1 ± 2.5	4.8 ± 3.3**	7.1 ± 4.2**	8.0 ± 5.0**	0.3 ± 0.0
Total prot[f]	g/dL	7.0 ± 1.0	6.1 ± 0.2	5.8 ± 0.1	6.7 ± 0.6	5.1 ± 0.8**	4.4 ± 1.2**	4.8 ± 1.5*	4.0 ± 1.2**	6.6 ± 0.7
BUN[g]	mg/Dl	27 ± 6.0	26 ± 2.0	21 ± 1.3	23 ± 2.6	26 ± 3.6	32 ± 7.0*	31 ± 16	32 ± 25	23 ± 3.2
WBCC	$\log_{10}$	6.8 ± 0.10	6.9 ± 0.12	6.7 ± 0.18	6.9 ± 0.16	6.7 ± 0.16	6.9 ± 0.13*	7.0 ± 0.24**	7.0 ± 0.32	6.8 ± 0.1
PCV	%	51.2	54.3	54.1	52.8	56.8**	52.9	53.4	50.3	53.3

[a]Parameters included with VetScan Comprehensive Diagnostic Profile cartridge; [b]controls consisted of sham infected hamsters; [c]alanine aminotransferase; [d]above detection limits; [e]alkaline phosphatase; [f]total protein; [g]blood urea nitrogen.

were significantly elevated at some point during the course of infection, while serum levels of albumin, amylase, glucose, calcium, globulin, phosphorus, sodium and total protein were significantly reduced (Table 2). Approximately one-half of the animals were observed to have discolored icteric livers, which were noticed 6 dpi, and correlated with icteric serum (data not shown). WBCC ($\log_{10}$) was significantly elevated 6 dpi (7.0 ± 0.1) and 7 dpi (7.1 ± 0.2) as compared with sham-infected controls (6.8 ± 0.1) ($P \leq 0.05$, $P \leq 0.01$, respectively). Red blood cell PCV was also significantly increased 5 dpi ($57 \pm 3.2\%$) as compared with sham-infected controls ($53 \pm 1.9\%$) ($P \leq 0.01$).

3.3. Treatment of YFV infection

In the first treatment study, animals challenged with 10^{-4} dilution of YFV stock were treated with IFN alfacon-1 or ribavirin from −4 h to 6 dpi. There was complete survival in animals treated with 5 μg/kg/day IFN alfacon-1, which was highly significant as compared with placebo treatment (Table 3). Treatment of hamsters with 1.6 or 0.5 mg/kg/day of IFN alfacon-1 also resulted in a significant improvement in survival, although some mortality was observed. Ribavirin treatment, at 50 mg/kg/day, also resulted in a significant improvement in survival, although 5 mg/kg/day did not improve any disease parameters. Weight loss was also markedly reduced in all IFN alfacon-1 treatment groups as well as with 50 mg/kg/day of ribavirin (Table 3). Serum ALT levels were significantly lower in IFN alfacon-1-treated and 50 mg/kg/day ribavirin-treated groups as compared to placebo. All of the serum samples obtained from 5 μg/kg/day IFN alfacon-1-treated animals were clear, while sera from 1 of 10 from 50 mg/kg/day ribavirin-treated animals, 1 of 10 from the 1.6 and 0.5 μg/kg/day IFN alfacon-1, and 4 of 10 from both 5 mg/kg/day ribavirin and placebo-treated animals were icteric, and correlated with high serum ALT values.

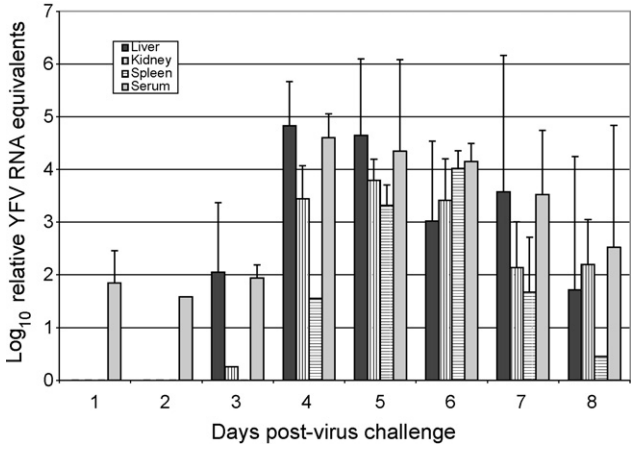

Fig. 1. Time-course of virus titer in various organs taken from hamsters challenged with Jimenez yellow fever virus and measured by QRT-PCR.

An additional study was conducted using viramidine, a derivative of ribavirin, in treatment of YFV. Viramidine also significantly improved survival and reduced weight loss in animals challenged with YFV (Table 3). Treatment with viramidine also significantly reduced serum ALT levels ($P \leq 0.001$) to normal, pre-treatment levels (Table 3). There was also a difference in the serum from some of the mice obtained from viramidine-treated animals, which was clear, while 6 of 9 serum samples obtained from placebo-treated animals were icteric and yellow in color. Icteric serum also correlated with death and high ALT values (Table 4).

In a third study, treatment was initiated 2 dpi with IFN alfacon-1 or ribavirin. This later treatment was also effective in significantly improving survival. There was a highly significant decrease in serum ALT levels in animals treated with ribavirin and a slightly significance in serum ALT decrease in hamsters

Table 3
Effect of i.p. treatment with interferon alfacon-1, ribavirin and viramidine in hamsters infected with hamster-adapted yellow fever virus

Treatment	Dose, schedule	Toxicity controls		YFV-infected			
		Survival/total[a]	Weight change (g)[b]	Survival/total	MDD[c]	ALT (6 dpi)[d]	Weight change (g)[b]
IFN alfacon-1	5 μg/kg/day, −4 h to 6 dpi, qd	3/3	7.0	$10/10^{***}$	>21 ± 0.0	$60 \pm 6.0^{***}$	$5.1 \pm 3.3^{***}$
IFN alfacon-1	1.6 μg/kg/day, −4 h to 6 dpi, qd	–	–	$9/10^{**}$	8.0 ± 0.0	$89 \pm 60^{**}$	$2.7 \pm 5.5^{**}$
IFN alfacon-1	0.5 μg/kg/day, −4 h to 6 dpi, qd	–	–	$8/10^{**}$	8.5 ± 2.1	$77 \pm 51^{**}$	$4.2 \pm 4.1^{**}$
Ribavirin	50 mg/kg/day, −4 h to 6 dpi, bid	3/3	4.4	$9/10^{**}$	7.0 ± 0.0	$71 \pm 46^{***}$	0.0 ± 5.2
Ribavirin	5 mg/kg/day, −4 h to 6 dpi, bid	–	–	5/9	7.3 ± 1.3	168 ± 70	−4.3 ± 3.6
Saline (study 1)	−4 h to 6 dpi, bid	3/3	8.7	2/10	7.6 ± 1.8	228 ± 75	−6.6 ± 5.4
Viramidine	100 mg/kg/day, −4 h to 6 dpi, bid	3/3	6.7	$9/10^{**}$	13 ± 0.0	$62 \pm 28^{***}$	$3.0 \pm 2.9^{**}$
Saline (study 2)	0–6 dpi, bid	3/3	5.3	3/10	7.3 ± 5.7	252 ± 106	−7.4 ± 6.8
IFN alfacon-1	5 μg/kg/day, 2–8 dpi, qd	3/3	1.0	$9/10^{*}$	8.0 ± 0.0	$100 \pm 52^{*}$	$4.1 \pm 3.8^{*}$
Ribavirin	50 mg/kg/day, 2–8 dpi, bid	3/3	4.4	$10/10^{**}$	>21.0 ± 0.0	$53 \pm 4^{***}$	3.7 ± 2.2
Saline (study 3)	2–8 dpi, qd	3/3	5.3	4/10	7.5 ± 0.8	230 ± 169	−3.0 ± 7.5
Normal controls	–	3/3	5.0	–	–	–	–

Data combined from several experiments. $^{***}P \leq 0.001$, $^{**}P \leq 0.01$ and $^{*}P \leq 0.05$ as compared with placebo. [a]Number of animals surviving past 21 dpi per number of total animals challenged with virus; [b]weight change between 3 and 6 dpi, correlating with the peak weight change in placebo-treated animals; [c]mean day to death; [d]serum alanine aminotransferase (IU/L).

treated with IFN alfacon-1 (Table 3). There was also a significant reduction of weight loss in IFN alfacon-1-treated animals, but not in animals treated with ribavirin (Table 3).

4. Discussion

In addition to alterations of various parameters previously described in this hamster model (Tesh et al., 2001; Sbrana et al., 2006), we also identified other altered parameters. Tissue titers, as measured by QRT-PCR, peaked between 4 and 6 dpi, and were reduced 7 dpi in liver, kidney, spleen and serum. High titers are found in the liver and spleen of YFV-infected patients, and correlate with the rapid disease progression (Bae et al., 2005; De Brito et al., 1992). Weight change also appeared to be a good indicator of drug efficacy in treatment, with treated animals generally losing less weight or gaining weight, as compared with animals treated with placebo. Further serum parameters, i.e. alkaline phosphatase, calcium, creatinine, globulin, glucose, phosphorus, sodium and total protein were measured and results gave an overall picture of sever liver and kidney disease. Each of these serum parameters was significantly reduced, and could potentially be used to determine the efficacy of an antiviral drug. Serum ALT was used because of availability of reagents, cost and importance to liver disease measurement.

Table 4
Correlation of survival with serum alanine aminotransferase levels and serum appearance in viramidine- and placebo-treated animals

Treatment	Animal number	ALT[a]	Survival or death	Serum appearance
Viramidine	1	60	Survival	Normal
	2	60	Survival	Normal
	3	53	Survival	Normal
	4	54	Survival	Normal
	5	55	Survival	Normal
	6	53	Survival	Normal
	7	142	Death	Normal
	8	48	Survival	Normal
	9	45	Survival	Normal
	10	47	Survival	Normal
Placebo	1	55	Survival	Normal
	2	143	Death	Normal
	3	160	Survival	Normal
	4	270	Survival	Icteric
	5	309	Death	Icteric
	6	337	Death	Icteric
	8	309	Death	Icteric
	9	330	Death	Icteric
	10	351	Death	Icteric

[a] Serum alanine aminotransferase levels (IU/L).

Titration of virus stock in hamsters revealed that hamsters were susceptible to a 10^{-4} dilution of virus stock, which correlated with 10^2 $CCID_{50}$. It was interesting that lower mortality was observed with higher virus challenge doses, and infection seemed not to be dose dependent. This is likely due to many different factors in the process of infection in an animal model such as defective interfering particles, initial replication in lymph nodes, immune recognition and others (Barrett et al., 1990; Vlaycheva et al., 2004; Melhop and Diamond, 2006). High variability was observed in these studies, which is a limitation of the model. Mortality rates between 50 and 80% are observed with the same inoculum and serum ALT is highly variable. To determine the number of animals needed to generate statistical significance in light of the variability observed, a statistical power analysis was performed on data from characterization experiments, which indicated that experiments using these parameters for the evaluation of antiviral compounds would need to include at least 10 animals per group (Julander, unpublished data).

Other parameters tested in this study were similar in many respects to published results (Tesh et al., 2001; Sbrana et al., 2006). Mortality in 6–8-week-old hamsters ranged from 50 to 80% in placebo-treated control animals, which allowed for significant differences between antiviral and placebo treatment to be observed. Serum ALT, bilirubin, BUN, potassium, albumin

and amylase had similar peaks to those published previously. Viremia followed a similar time-course as previously reported data with a peak titer 4 dpi. A statistically significant elevation in WBC count was observed 6 and 7 dpi and in PCV on 5 dpi, which correlated with the elevated WBC count and PCV previously reported by Tesh et al. (2001).

We demonstrated that treatment with IFN alfacon-1 (0.5, 1.6 and 5 μg/kg/day) reduced mortality, weight loss and serum ALT levels, even when treatment was initiated 2 days after virus challenge. Treatment with IFN alfacon-1 appeared to be dose responsive with higher survival and greater improvement in other disease parameters at the highest dose, but with diminished effect at lower doses of this cytokine. These results were not surprising as IFN alfacon-1 has been shown to have efficacy against different flaviviruses, as well as other groups of viruses (Gowen et al., 2005; Loutfy et al., 2003; Morrey et al., 2004a,b; Sjogren et al., 2005). IFN alfacon-1 treatment was effective when administered 2 dpi and further studies should be aimed at determining how late treatment may be initiated with this compound to reduce mortality and viral parameters. The relative biological potency of IFN alfacon-1 in hamsters compared to humans is approximately 40-fold less (Hu et al., 1995). Therefore, an estimate of the biological potency (IFN units) given to the hamsters in this study is 125,000 U/kg/day. The FDA approved human dose of IFN alfacon-1 for the treatment of chronic hepatitis C in humans is 9 μg or 9 MU. On average, chronic hepatitis C patients weigh approximately 75 kg resulting in the approved dose of IFN alfacon-1 as approximately 120,000 U/kg. Thus, hamsters in this study were treated with therapeutically relevant doses of IFN alfacon-1.

Viramidine is a prodrug of ribavirin that is converted to ribavirin in liver cells after uptake and has reduced toxicity and better liver-targeting properties as compared with ribavirin treatment (Lin et al., 2003; Sidwell et al., 2005). Viramidine treatment resulted in a highly significant reduction in serum ALT ($P < 0.001$) as compared with placebo. These and previous studies demonstrated the utility of ribavirin treatment in improving survival and reducing serum ALT (Sbrana et al., 2004). Ribavirin did not improve weight change with either treatment regimen, which may be due to slight toxic effects of this compound.

Serum ALT levels seemed to be a good indicator if treatments were successful. There appeared to be a correlation between ALT level and mortality, and animals with ALT levels three times greater than normal generally died. Icteric serum correlated with high ALT levels as well as fatty livers, and could serve as an indicator of which animals would potentially die of infection. Higher mortality has been observed in hepatitis C virus-infected patients with chronically elevated ALT levels as compared with patients that had no elevation (Bestard et al., 2005). A similar reduction of ALT and mortality was also seen in mice infected with murine cytomegalovirus and treated with HPMPC or gancyclovir (Bolger et al., 1999). Serum ALT levels may serve as a marker of death in YFV-infected hamsters.

With measurable and quantifiable parameters, such as serum ALT, tissue titers, mortality and weight change, this hamster model of YFV infection will be useful for experiments evaluating antiviral compounds for efficacy against YFV. There is a need for antiviral agents that are effective in the treatment of YFV infection and this model may be instrumental in determining possible treatments that may reduce morbidity and mortality caused by YFV.

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