International Journal of Infectious Diseases 15 (2011) e44–e47

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Effect of oral ribavirin treatment on the viral load and disease progression in Crimean-Congo hemorrhagic fever

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ARTICLE INFO

Article history:
Received 12 November 2008
Received in revised form 5 August 2010
Accepted 14 September 2010

Corresponding Editor: Mark Holodniy, California, USA

Keywords:
Ribavirin
Virus
Hemorrhagic fever

SUMMARY

Objectives: Crimean-Congo hemorrhagic fever (CCHF) is a lethal hemorrhagic disease. There is currently no specific antiviral therapy for CCHF approved for use in humans. In this study we aimed to investigate the effect of oral ribavirin treatment on the viral load and disease progression in CCHF.
Methods: The study population was composed of patients who had a definitive diagnosis of CCHF by means of clinical presentation plus detection of viral RNA by reverse transcriptase polymerase chain reaction (RT-PCR). Ten patients who received oral ribavirin for 10 days and 40 control patients who received supportive treatment only were included in the study. Ribavirin treatment consisted of oral ribavirin 4 g/day for 4 days and then 2.4 g/day for 6 days. Viral load and hematological and biochemical laboratory parameters, which were measured daily, were analyzed.
Results: Mean age (37.4 vs. 45.5, $p = 0.285$), gender (male 50% vs. 62.5%, $p = 0.470$), days from the appearance of symptoms to admission (4.3 vs.4.4 days, $p = 0.922$), and initial complaints were similar between the ribavirin group and the control group. Upon hospital admission, mean viral load was 8.2×10^8 copies/ml in the ribavirin group and 8.3×10^8 copies/ml in the control group ($p = 0.994$). During follow-up, no statistically significant differences were found between the groups with regard to the decrease in viral load, the reduction in alanine aminotransferase and aspartate aminotransferase levels, and the increase in platelet count. The case-fatality rate was 20% (2/10 patients) in the ribavirin group and 15% (6/40 patients) in the control group ($p = 0.509$).
Conclusion: In this study, oral ribavirin treatment in CCHF patients did not affect viral load or disease progression.

1. Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a potentially fatal infection caused by the CCHF virus belonging to the genus *Nairovirus* of the family *Bunyaviridae*. The CCHF virus is transmitted to humans by Hyalomma ticks or by direct contact with the blood of infected humans or domestic animals.[1] Presently, CCHF is a public health problem in more than 30 countries in Africa, Asia, Southeast Europe, and the Middle East.[2,3] Since 2002, a rapid emergence of CCHF has occurred in the central, northern, and eastern regions of Turkey.[4,5] By the end of 2007, there had been 1820 confirmed cases and 92 deaths (a case-fatality rate of 5%) in Turkey.[6]

Treatment options for CCHF are limited. There is currently no specific antiviral therapy for CCHF approved for use in humans.[1] The World Health Organization (WHO) currently recommends ribavirin as a potential therapeutic drug for CCHF.[3,7] Ribavirin has been found to be effective against CCHF virus in vitro,[8,9] but the efficacy of ribavirin remains controversial.[10,11]

For many viral diseases, including CCHF, viral load measurement has become an important part of disease management. Furthermore, increased severity of the CCHF disease has been shown to correlate with high viremia titers.[12,13]

In this study, we aimed to investigate the effect of oral ribavirin treatment on the viral load and disease progression in CCHF patients in Turkey.

2. Materials and methods

2.1. Patient population

This case–control study was conducted at the Ankara Numune Education and Research Hospital between 2006 and 2008. The

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doi:10.1016/j.ijid.2010.09.009

Table 1
Comparison of baseline characteristics and laboratory findings between the ribavirin group and the control group

Characteristic	Ribavirin group ($n=10$)	Control group ($n=40$)	p-Value
Age, years	37.4 ± 17.9	45.5 ± 19.3	0.285[a]
Male sex	5 (50%)	25 (62.5%)	0.470[b]
Duration of complaints until hospitalization, days	4.3 ± 1.4	4.4 ± 1.4	0.922[a]
Most common complaints			
Myalgia	10 (100%)	37 (92.5%)	0.504[c]
Fever	10 (100%)	38 (95%)	0.637[c]
Lack of appetite	10 (100%)	38 (95%)	0.637[c]
Headache	9 (90%)	31 (77.5%)	0.349[c]
Nausea and/or vomiting	8 (80%)	25 (62.5%)	0.257[c]
Bleeding (any kind)	3 (30%)	14 (35%)	0.539[c]
Physical findings			
Fever, temperature >38 °C	4 (40%)	19 (47.5%)	0.670[c]
Hepatomegaly	0 (0%)	3 (7.5%)	0.504[c]
Splenomegaly	1 (10%)	1 (2.5%)	0.363[c]
Rash			
Maculopapular	1 (10%)	7 (17.5%)	0.491[c]
Petechiae	3 (30%)	7 (17.5%)	0.314[c]
Bleeding			
Ecchymosis	2 (20%)	7 (17.5%)	0.584[c]
Epistaxis	2 (20%)	9 (22.5%)	0.618[c]
Hematemesis	2 (20%)	3 (7.5%)	0.258[c]
Melena	0 (0%)	5 (12.5%)	0.311[c]
Somnolence	2 (20%)	5 (12.5%)	0.429[c]
Laboratory findings			
Platelet count ($\times 10^9$/l)	52.8 ± 47.5	58.1 ± 41.3	0.544[d]
WBC count ($\times 10^9$/l)	2.320 ± 1.203	2.707 ± 1.210	0.444[d]
AST level, U/l (normal range 5–34 U/l)	305 ± 280	336 ± 331	0.780[d]
ALT level, U/l (normal range 0–55 U/l)	133 ± 92	166 ± 214	0.560[d]
LDH level, U/l (normal range 123–243 U/l)	737 ± 439	649 ± 416	0.559[d]
CPK, U/l (normal range 25–200 U/l)	512 ± 401	918 ± 1044	0.525[d]
PT, s (normal range 11.5–15.5 s)	12.4 ± 2	12.7 ± 2	0.653[a]
aPTT, s (normal range 20–34 s)	42.8 ± 19.5	40.9 ± 15	0.971[d]
INR (normal range 0.8–1.2)	0.98 ± 0.16	1.02 ± 0.16	0.551[a]
Fibrinogen, mg/dl (normal range 180–350 mg/dl)	251 ± 74	264 ± 73	0.734[d]

Data are n (%) of patients or mean value ± standard deviation.
WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; CPK, creatine phosphokinase; PT, prothrombin time; aPTT, activated partial thromboplastin time; INR, international normalized ratio.
[a] Student's t-test.
[b] Chi-square test.
[c] Fisher's exact test.
[d] Wilcoxon–Mann–Whitney test.

study population was composed of patients with a definitive diagnosis of CCHF by means of clinical presentation plus detection of viral RNA by reverse transcriptase polymerase chain reaction (RT-PCR). Patients with suspected cases of CCHF were offered oral ribavirin therapy upon hospital admission. Ribavirin treatment was initiated before laboratory confirmation of the CCHF diagnosis, as the results were only available a few days after obtaining the samples. If a patient agreed to use ribavirin treatment, they were treated with oral ribavirin 4 g/day for 4 days and then 2.4 g/day for 6 days. Only ribavirin-treated patients with a confirmed CCHF diagnosis by RT-PCR were included in the study. Control patients received supportive treatment only. All patients also received preparations of erythrocytes, platelets, and fresh frozen plasma, depending on their homeostatic state.

In total, this study included 10 case patients (patients who received oral ribavirin) and 40 control patients (patients who received supportive treatment only). For each ribavirin case, four control cases were randomly selected to match one ribavirin case with respect to similar initial viral loads and duration of symptoms. Matching ribavirin and control cases in a 1:4 ratio maximized the power of the analysis.

Written informed consent was obtained from the patient and/or their family members for all patients enrolled in this study. Also, for each patient, permission was obtained from the Turkish Ministry of Health to use ribavirin for an unlicensed indication. This study also followed procedures in accordance with the ethical standards of the Helsinki Declaration.

2.2. Laboratory assessments

Quantitative measurements of CCHF virus were performed daily. A TaqMan-based one-step RT-PCR assay was used for detection and quantification of CCHF virus RNA.[14] The assay was performed in a Perkin-Elmer 7700 Sequence Detection System by using the combination of reverse-transcriptase (MBI Fermentas) and Hot Start Taq DNA Polymerase (Birion GmBH) enzymes. Biochemical and hematological laboratory parameters were measured on a daily basis after hospital admission.

2.3. Statistical analysis

Viral loads and hematological and biochemical laboratory parameters, measured on a daily basis, were analyzed. Patient outcomes were also analyzed. The Chi-square test or Fisher's exact test was used when appropriate to compare proportions. Continuous variables were compared using an independent-groups t-test if normality assumptions were met; otherwise groups were compared using the Wilcoxon–Mann–Whitney test.

Table 2
Comparison of the duration of hospitalization, amounts of administered blood products, and outcome between the ribavirin group and the control group

Characteristic	Ribavirin group ($n = 10$)	Control group ($n = 40$)	p-Value
Duration of hospitalization (days)	6.6 ± 2.8	7.4 ± 2.5	0.398[a]
Mean thrombocyte suspension transfused (units)	26.3 ± 17.7	29.3 ± 14.6	0.616[b]
Mean fresh frozen plasma transfused (units)	20.4 ± 13.3	15.2 ± 13.9	0.239[b]
Mean erythrocyte suspensions transfused (units)	2 ± 1.8	2.63 ± 1.8	0.791[b]
Fatal outcome	2 (20%)	6 (15%)	0.509[c]

[a] Student's t-test.
[b] Wilcoxon–Mann–Whitney test.
[c] Fisher's exact test.

In order to compare the effect of ribavirin on viral load over time, a quadratic growth curve mixed effects model was performed. Daily viral load data were log-transformed to meet the normality assumption for use in the quadratic growth curve mixed effects model. p-Values of <0.05 were considered to be statistically significant. Software package Stata 9.0 (Stata Corp., College station, TX, USA) was used for the analysis.

3. Results

Between 2006 and 2008, 188 patients were diagnosed with CCHF and 141 of them had PCR results positive for CCHF virus RNA in blood samples. Quantitative measurement of CCHF virus was performed daily in 109 patients. Among these patients, 10 who had received oral ribavirin and 40 controls who had received supportive treatment only were included in the study.

Baseline clinical characteristics and laboratory values of the ribavirin and control groups are presented in Table 1, which shows that both groups had similar baseline and laboratory test results upon admission.

The efficacy of ribavirin therapy was evaluated with respect to viral load, supportive treatment required by the patients during the course of the disease, mean duration of hospitalization, and fatal outcome between the ribavirin group and the control group. No statistically significant differences between the two groups were determined for any of these parameters (Table 2).

Erythrocyte suspension infusions were needed in 40% and 20% ($p = 0.186$), fresh frozen plasma in 50% and 42.5% ($p = 0.669$), and platelet suspension in 60% and 45% ($p = 0.396$) of those in the ribavirin group and the control group, respectively. The quantities of blood or blood product infusions required per patient are compared in Table 2. Although the amount of fresh frozen plasma administered per patient was lower in the control group, amounts of other blood products were similar and no statistically significant differences were found.

No statistically significant difference was found between the ribavirin and control groups during daily follow-up with regard to the decrease in viral load, the reduction in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, and the increase in platelet count (Figure 1).

At the time of hospital admission, viral loads ranged from 1×10^3 to $\geq 9.9 \times 10^9$ copies/ml in the serum samples. The mean viral load was 8.2×10^8 copies/ml (range 8.4×10^3–7.2×10^9) in the ribavirin group and 8.3×10^8 copies/ml (range 6.1×10^3–9.9×10^9) in the control group ($p = 0.994$). Viral loads of $\geq 1 \times 10^9$ copies/ml were detected in one patient in the ribavirin group and in four patients in the control group. All of these patients had fatal outcomes. Furthermore, two patients in the control group and one patient in the ribavirin group had viral loads $\geq 5.5 \times 10^8$ copies/ml at admission. One of these control patients had a viral load of 5.5×10^8 copies/ml at admission, which was elevated to 3.9×10^9 copies/ml on the third day of hospitalization. With the exception of one control patient with a non-fatal outcome and a titer of 7.5×10^8 copies/ml at admission, all patients with fatal outcomes had viral loads $\geq 5.5 \times 10^8$ copies/ml at the time of hospital admission.

The number of patients with a positive viral load on each day over the total number tested for viral load in the ribavirin group and control group, respectively, were as follows: first day 10/10 and 40/40, second day 9/10 and 30/39, third day 5/9 and 22/39, fourth day 2/9 and 12/37, fifth day 1/9 and 4/34, and sixth day 1/8 and 0/34 (patients with negative viral loads were included in the analysis and patients who died were excluded from the analysis).

In patients with fatal outcomes, the mean duration of symptoms before hospitalization was 4.5 ± 0.7 days in the ribavirin group and 4.2 ± 0.9 days in the control group ($p = 0.680$). The case-fatality rate was 20% (2/10 patients) in the ribavirin group and 15% (6/40 patients) in the control group ($p = 0.509$; Table 2).

4. Discussion

Treatment for CCHF is primarily supportive.[1,3] Ribavirin has been used in the treatment of CCHF, but its efficacy in the treatment of this disease is controversial.[10,11,15–17] Ribavirin is a guanosine analog with a broad spectrum of activity in vitro against RNA viruses, and it has been shown to inhibit viral replication of the CCHF virus in vitro.[8,9,18,19]

The level of viremia has been shown to have prognostic significance in CCHF, and the duration of symptoms has been shown to have a significant effect on the disease stage. A high viral load tends to indicate a fatal outcome, and thus viral load is a useful predictor of clinical progress.[12,13] Specifically, a viral load of $\geq 10^8$ copies/ml is a strong factor for differentiating CCHF patients who

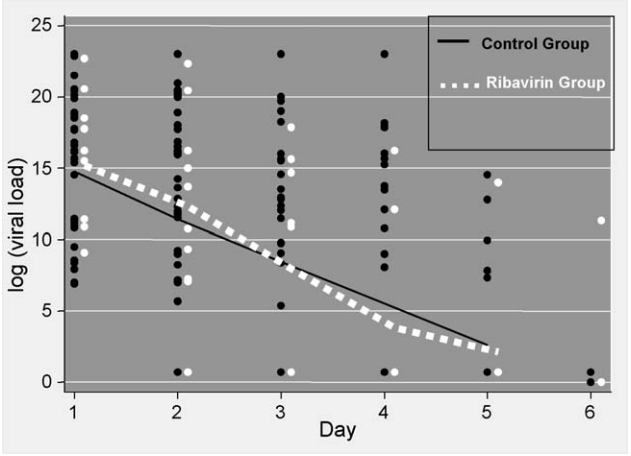

Figure 1. Ribavirin effect on viral load over time; $p > 0.05$ (data were log-transformed). White dots represent the ribavirin group and black dots represent the control group.

die from those who survive. Another use of the viral load is the systematic monitoring of patients receiving ribavirin therapy.[20]

There are several case reports and observational studies on the efficacy of ribavirin in CCHF.[10,11,15–17] The shared features of these observational studies are small sample sizes, retrospective nature, and, in most of them, unknown disease severity. These observational studies show conflicting results with regard to the efficacy of ribavirin. However, to date, no randomized controlled studies have been performed to rigorously confirm the efficacy of ribavirin for treating CCHF.

In order to investigate the effect of oral ribavirin treatment on viral load, we chose to perform a case–control study. As severity and stage of the disease and different time delays before treatment can affect the progression of CCHF, we randomly chose four control patients for each ribavirin-treated patient with the same viral load and the same duration of symptoms in order to prevent potential selection bias while composing the study groups.

There are some limitations to our study. Our sample size was small, as the study group consisted of 50 patients and there were only 10 patients in the ribavirin treatment group. A further limitation was the retrospective nature of the data collection.

In our study, comparison of baseline characteristics of the patients showed that both groups had similar findings and similar laboratory test results at admission. A quadratic growth curve mixed effects model was performed in order to compare the effect of ribavirin on viral load between the two groups simultaneously over time. There was no significant effect of ribavirin on the decrease in viral load. At follow-up, the decrease in ALT and AST levels and the increase in platelet count did not show any statistically significant difference on a daily basis between the ribavirin and control groups. The mean duration of hospitalization was 6.6 days in the ribavirin group and 7.4 days in the control group ($p = 0.398$). The case fatality rate was 20% in the ribavirin group and 15% in the control group ($p = 0.509$). All fatal cases had a viral load $\geq 5.5 \times 10^8$ copies/ml at admission, regardless of ribavirin use.

In conclusion, oral ribavirin treatment in CCHF did not show any effect on viral load or the disease progression in this study. It is very difficult to reach a consensus about the efficacy of ribavirin in the treatment of CCHF with these conflicting results in the literature. Therefore, this highlights the need for randomized controlled trials with larger sample sizes.

Conflict of interest: No conflict of interest and no funding source to declare. Ethical approval and informed consent were obtained.

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