Effects of Ribavirin on Severe Fever with Thrombocytopenia Syndrome Virus In Vitro

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SUMMARY: Severe fever with thrombocytopenia syndrome (SFTS) is a disease with a high case fatality rate that is caused by infection with the recently identified tick-borne SFTS virus (SFTSV), for which there are no specific countermeasures. We examined the effects of ribavirin and mizoribine, which are nucleoside analogue drugs with broad antiviral activities, on SFTSV proliferation in vitro. When 3 cell lines were treated with these drugs before and during infection with a Chinese SFTSV strain, the 99% effective concentrations (EC99) of ribavirin were 19–64 µg/ml (78–262 µM); in contrast, the EC99 of mizoribine was > 500 µg/ml (1,929 µM). Similar levels of inhibitory effects of ribavirin were observed with 4 Japanese SFTSV strains. However, when Vero cells were treated with ribavirin 3 days after inoculation, the inhibitory effect was dramatically decreased, indicating that ribavirin did not effectively reduce virus production in pre-infected cells. These results suggest that ribavirin could be used as post-exposure prophylaxis for the prevention of SFTS.

INTRODUCTION

Severe fever with thrombocytopenia syndrome (SFTS) is a recently-identified disease characterized by fever, gastrointestinal symptoms, thrombocytopenia, leukopenia, and elevated levels of liver enzymes in peripheral blood (1,2). Multiple organ failure and disseminated intravascular coagulation are often observed (1,2). Multiple organ failure and disseminated intravascular coagulation are often observed in severe cases (3). Its case fatality rate is approximately 12% (1). The causative agent of the disease is the SFTS virus (SFTSV) (family: Bunyaviridae, genus: Phlebovirus), the discovery of which was reported in 2011 (1,2). Although sporadic outbreaks of the disease have been recently found in Japan and South Korea, retrospective studies have indicated the emergence of SFTS in Japan in 2005 and in China in 2006 (4–6).

Epidemiology suggests that the transmission routes of SFTSV are tick bites and human-to-human transmission. The virus has been detected in several tick species including Haemaphysalis longicornis and Rhipicephalus microplus, which have been found in areas surrounding the places in which SFTS patients reside a substantial number of whom had a history of tick bites (1,2,7). Although not common, direct human-to-human transmission through close contact with virus-containing blood or excreta has also been reported (4,8–11).

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), a guanosine analogue with broad antiviral activities, is effective in the treatment of Lassa fever (12) and hepatitis C when administered in combination with other drugs (13). Ribavirin is also used in the treatment of Crimean–Congo hemorrhagic fever (CCHF) (14); however, its efficacy in CCHF treatment remains to be proven. Ribavirin exerts its antiviral effects through various mechanisms, including the reduction of viral RNA-dependent RNA polymerase activity, mutagenesis in the viral genome, inhibition of RNA capping, reduction of cellular inosine monophosphate dehydrogenase (IMPDH) activity, and modulation of the host immune response (15). The drug has been used in the treatment of some SFTS patients, but at the time of use, its effect was unknown (4,16). Mizoribine (4-carbamoyl-1-β-D-ribofuranosylimidazolium-5-olate) is an imidazole nucleoside that has been used as an immunosuppressive agent in Japan. Although mizoribine shows inhibitory effects in vitro against herpes simplex virus (17), respiratory syncytial virus (18), influenza virus (19), and severe acute respiratory syndrome virus (20), there have been no reports on mizoribine usage or its effects with regard to SFTS or SFTSV.

In the present study, we examined the effects of ribavirin and mizoribine on SFTSV in vitro to clarify the potential of these drugs as countermeasures against SFTS. When ribavirin treatment was initiated before virus inoculation, SFTSV proliferation dramatically reduced, suggesting the prospective use of ribavirin as a
Effects of ribavirin and mizoribine on SFTSV proliferation: Fig. 1 shows viral titers obtained from monkey Vero cells, which were inoculated with the Chinese SFTSV strain (HB29) in the presence of ribavirin or mizoribine (0, 4, 20, 100, and 500 μg/ml). Although statistically significant inhibitory effects (P < 0.05) of ribavirin and mizoribine were observed at 20–500 μg/ml and 100–500 μg/ml, respectively, ribavirin showed a greater reduction of viral titers than mizoribine (Fig. 1A, B). Ribavirin was also found to profoundly affect HB29 proliferation in 2 human cell lines, Huh7 and U2OS (Fig. 1C–F). Reduction curves were used to calculate 99% effective concentrations (EC99), the drug concentrations at which viral titers were reduced by 2 logs (Fig. 1). The EC99 of ribavirin, was 64 μg/ml (263 μM), 20 μg/ml (83 μM), and 19 μg/ml (78 μM) in Vero, Huh7, and U2OS cells, respectively. In contrast, no 99% inhibition was observed with mizoribine, even at the highest concentration of 500 μg/ml (1,929 μM; Table 1).

Similar results were obtained with the Japanese SFTSV strain SPL030 (Table 1). The 3 additional Japanese strains (SPL004, SPL010, and YG1) were tested to further confirm the effects of ribavirin. Huh7 cells were inoculated with 100 TCID50 of each strain in the presence of 20 μg/ml of ribavirin and cultured for 3 days. Titers of SPL004, SPL010, and YG1 strains were reduced by 1.83, 1.83, and 2.25 log(s), respectively.

Cytotoxicity was examined by measuring cell viability after cell culture in the presence of the drugs. As shown in Fig. 2, >60% viability was maintained in all 3 cell types, even at 500 μg/ml.

Effects of ribavirin on RVFV proliferation: To speculate on the potential effects of ribavirin on SFTSV/ RVFV in vivo, the drug’s inhibitory effects on RVFV proliferation were examined. RVFV is another member of the Phlebovirus genus, for which both the in vitro and in vivo effects of ribavirin have been reported (22–24). Ribavirin reduced RVFV proliferation in a dose-dependent manner (Fig. 1G), and the EC99 in Vero cells was 50 μg/ml (207 μM; Table 1).

Effects of ribavirin in SFTSV preinfected cells: We next examined the potential of ribavirin for reducing virus production from cells that had been previously infected with SFTSV. Vero cells were inoculated with SFTSV and cultured for 3 days (the culture period during which most inoculated cells became SFTSV-positive [data not shown]). Culture media were then replaced with ribavirin-containing media (0–500 μg/ml). After another 3 days of culture, supernatants were harvested and titrated. As shown in Fig. 3, ribavirin displayed statistically significant effects at 100 and 500 μg/ml; however, the reduction at 500 μg/ml was only about 1 log.

DISCUSSION

In the presence of ribavirin, SFTSV proliferation was moderately reduced, and the inhibitory effects in in vitro experiments were not strain-specific (Fig. 1; Table...
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Fig. 1. Effects of ribavirin and mizoribine on SFTSV/RVFV proliferation. Vero cells (A, B), Huh7 cells (C, D), and U2OS cells (E, F) were inoculated with SFTSV HB29 strain in the presence of indicated concentrations of ribavirin (A, C, E) or mizoribine (B, D, F) and titers of supernatants 3 days after inoculation are shown. Vero cells were inoculated with RVFV in the presence of indicated concentrations of ribavirin. Titers of supernatants at 3 days after inoculation are shown (G). Results were obtained from 3 independent experiments. *, $P < 0.05$.

1; data not shown). Diversity among the nucleotide sequences of SFTSV strains reported from China, Japan, and South Korea is less than 5% (5,6), which suggests that ribavirin would likely show similar inhibitory effects against all SFTSV strains, regardless of areas from where the viruses have been isolated. Although both ribavirin and mizoribine have been shown to inhibit several viruses including herpes simplex virus, respiratory syncytial virus, influenza virus, and severe acute respiratory syndrome virus (17–20), mizoribine did not show an efficient inhibitory effect on SFTSV proliferation. The finding that effective doses of ribavirin against SFTSV were higher in Vero cells than in the other 2 cell lines (Table 1) is consistent with previous reports by Huffman et al. (25) and Peters et al. (23), in which the impact of ribavirin on several DNA/RNA viruses was compared in several cell lines. Given that the known functions of mizoribine are the inhibition of inosine monophosphate synthetase and guanosine monophosphate synthetase (26), other functions of ribavirin, such as viral polymerase inhibition, mutagenesis, RNA capping inhibition, and/or reduction of cellular IMPDH, might be critical in its effects on SFTSV proliferation.

To speculate on the potential effects of ribavirin on SFTS/SFTSV in vivo, the drug’s inhibitory effects on RVFV proliferation (another member of the Phlebo-virus genus) were examined in our assays and compared with those on SFTSV. Peters et al. reported that ribavirin at 23 μg/ml showed 2-log inhibition against RVFV in Vero cells (23). In animal models, ribavirin treatment (75–100 mg/kg/day) led to a 60%–75% survival rate in RVFV-inoculated mice (22–24), while it suppressed viremia in RVFV-infected monkeys (30 mg/kg/day, more than 2-log reduction of viral titers) (23). In the present study, the EC99 of ribavirin in Vero cells against SFTSV

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Table 1. Effects of ribavirin and mizoribine on SFTSV/RVFV proliferation

<table>
<thead>
<tr>
<th>Virus</th>
<th>Cell (origin)</th>
<th>Ribavirin, EC₉₀, µg/ml (µM)¹</th>
<th>Mizoribine, EC₉₀, µg/ml (µM)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFTSV HB29</td>
<td>Vero (monkey)</td>
<td>64 ± 17 (263 ± 68)</td>
<td>&gt;500 (1,929)</td>
</tr>
<tr>
<td></td>
<td>Huh7 (human)</td>
<td>20 ± 5 (82 ± 20)</td>
<td>&gt;500 (1,929)</td>
</tr>
<tr>
<td></td>
<td>U2OS (human)</td>
<td>19 ± 2 (78 ± 6)</td>
<td>&gt;500 (1,929)</td>
</tr>
<tr>
<td>SFTSV SPL030</td>
<td>Vero (monkey)</td>
<td>104 ± 22 (424 ± 88)</td>
<td>&gt;500 (1,929)</td>
</tr>
<tr>
<td></td>
<td>Huh7 (human)</td>
<td>15 ± 2 (63 ± 7)</td>
<td>&gt;500 (1,929)</td>
</tr>
<tr>
<td></td>
<td>U2OS (human)</td>
<td>19 ± 4 (73 ± 15)</td>
<td>&gt;500 (1,929)</td>
</tr>
<tr>
<td>RVFV MP-12</td>
<td>Vero (monkey)</td>
<td>50 ± 12 (207 ± 68)</td>
<td>ND²</td>
</tr>
</tbody>
</table>

¹: Data are mean ± SD from 3 independent experiments.
²: Not done.

Fig. 2. Cytotoxicity of ribavirin and mizoribine. Viability of Vero cells cultured in the presence of ribavirin (A) or mizoribine (B) was measured. Cell viability was calculated as follows: (absorbance of cells in the presence of the drug - absorbance of no cells in the presence of the drug)/(absorbance of cells in the absence of the drug - absorbance of no cells in the absence of the drug) × 100 (%). Experiments were performed in triplicate and means ± standard deviation are shown.

Fig. 3. Effects of ribavirin on SFTSV production from SFTSV-pre-infected cells. Vero cells were inoculated with SFTSV and cultured for 3 days. Culture media were then replaced with ribavirin-containing media. After a further 3 days culture, supernatants were harvested and viral titers were determined. Results were obtained from 3 independent experiments.

* P < 0.05.

(64–104 µg/ml, 2-log inhibition) suggests that SFTSV may be slightly less sensitive to ribavirin than RVFV (Table 1). However, because the impact of ribavirin in human cell lines occurred at 15–20 µg/ml (Table 1) and a single dose of ribavirin at 600–2,400 mg reached peak serum concentrations of 47–161 µM (equivalent to approximately 11–39 µg/ml) (27,28), the effects of ribavirin in vivo could be substantial. Recently, an animal model using interferon α/β receptor-knockout mice was reported in which SFTSV infection was fatal (29). The model might be useful for understanding the pathogenesis of SFTS and for evaluating the in vivo effects of antiviral drugs, including ribavirin, against SFTS/SFTSV.

In the present study, ribavirin added before virus inoculation inhibited SFTSV proliferation. In some SFTS cases, exposure to SFTSV can be recognized immediately (4,8–11). Thus, suggesting that ribavirin could be an effective post-exposure prophylaxis that will minimize the severity of SFTS in cases where individuals are deemed to be at a high risk of SFTSV infection, such as in cases in which contaminated sharp instruments penetrate skin or in which an individual comes into close contact with the patient’s blood or excreta.

Ribavirin did not show an effective reduction of virus production in pre-infected cells, when added 3 days after inoculation (Fig. 3). This suggests that ribavirin has no effect on fully-infected cells or in patients in whom SFTSV has already expanded systemically. In SFTS patients, serum viral loads are very high on hospital admission, particularly in fatal cases (30,31). It is therefore unlikely that ribavirin would be effective in the treatment of patients who are already showing symptoms of SFTS. This corresponds with a report by Liu et al., which noted that neither fatality ratios nor blood laboratory test results of SFTS patients improved with ribavirin therapy (32). However, in hepatitis C, combination therapies that include ribavirin (along with interferon and/or specific antiviral drugs) have been shown to be very effective, while the usage of ribavirin alone is less profound (13,33). As such, the possibility exists that ribavirin could be effective as a part of combination therapy in the treatment of SFTS patients.

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Conflict of interest None to declare.
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