Effectiveness of the ribavirin in treatment of hantavirus infections in the Americas and Eurasia: a meta-analysis

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Abstract Hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) are transmitted to humans through infection with the old- and new-world hantaviruses, respectively. Together these diseases affect tens of thousands of people every year, and no specific treatment is available. To investigate whether ribavirin treatment for hantaviruses infections decreases disease severity, we conducted a meta-analysis involving human and animal studies. After defining the research protocol and criteria for inclusion/exclusion, we identified seven studies. We found that in groups with HPS who were treated with ribavirin, there was no significant reduction in mortality (RR 0.99, 95 % CI 0.60–1.61, $I^2 = 0$ %). On the other hand, for animal group with HPS-like disease, there was significant increase in survival (RR 0.05, 95 % CI 0.01–0.34, $I^2 = 0$ %). For animal group infected with the old-world hantaviruses, treated with ribavirin, there was a statistically significant increase in survival (RR 0.56, 95 % CI 0.42–0.76, $I^2 = 64$ %). Similarly, for humans with HFRS treated, there was increase in survival (RR 0.28, 95 % CI 0.08–1), although only a study exist. Our meta-analysis provides data that should be interpreted with caution, partly due to the limited number of studies available. Additionally, the results of the application of ribavirin

in the population with HPS could not be determined, particularly in patients in the end stage of this disease.

Keywords Hantavirus · Hantavirus pulmonary syndrome · Emerging pathogens · Zoonoses · Meta-analysis

Infections by viruses of the genus *Hantavirus*, family Bunyaviridae, occur through inhalation of the aerosols from the excreta of infected wild rodents [19]. Hantavirus has a cosmopolitan distribution, and it can cause two distinct diseases in humans: hemorrhagic fever with renal syndrome (HFRS) in Europe and Asia, with a mortality rate of up to 15 % in severe cases; and hantavirus pulmonary syndrome (HPS) in the Americas, reported in 1993 in the United States, with a mortality rate that can reach 50 % [7, 9, 12].

HPS is an emerging disease in the Americas, and HFRS is considered a major public health problem [17, 19]. In spite of the important advances in the laboratory diagnosis of hantavirus infection through recombinant DNA technology, there is still no specific treatment for HPS apart from the application of various drugs, such as vasoactive amines, corticosteroids and antibiotics [16]. Existing support measures include mechanical ventilation, which should be administered promptly in serious cases.

Ribavirin (1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), which was first synthesized in 1970s [18], is a drug that has been used in the treatment of HPS and HFRS [2, 6]. This antiviral has broad spectrum action, acting in vitro and in vivo against the genetic material of various viruses.

Ribavirin is a synthetic nucleotide analog that easily diffuses into eukaryotic cells and is converted into mono-, di-, and triphosphate forms via cellular enzymes. Accordingly,

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several mechanisms for the action this compound have been suggested [18]: (1) the induction of mutations into the viral genetic material leading to fatal errors in viral replication, as shown in Hantaan virus; (2) inhibition of viral mRNA capping; (3) direct inhibition of RNA-dependent RNA viral polymerase, resulting in a delay in the initiation and elongation phases of mRNA transcript; (4) inhibition of IMP dehydrogenase, an enzyme important in the synthesis of GTP; (5) and partial suppression of cytokines through the inhibition of RANTES mRNA in Andes virus infections [8].

Although ribavirin has been shown to have clinical efficacy in infections by hepatitis C virus (<http://www.hepatitiscentral.com/hcv/ribavirin/info.html>), and perhaps in HFRS, it remains unknown whether it is effective for the treatment of HPS. Given the lethality of the disease [7, 9, 19], it is necessary to understand the effects of the drugs that are applied in HPS to develop better treatments that will improve the survival of patients. In this context, a systematic meta-analysis is useful to clarify a specific clinical question based on statistical tests that combine or integrate the results of several independent studies aimed at a topic of interest. Therefore, the objective of this study was to perform a meta-analysis on the real efficacy of the ribavirin in the treatment of hantavirus infections in the Americas. Additionally, this meta-analysis was conducted to more specifically determine the precise effects of the use this antiviral drug in hantavirus infections that cause HFRS.

To investigate whether treatment with ribavirin in HFRS and HPS can increase the survival of these patients, and also of animals, we conducted a meta-analysis according to the recommendations of the Cochrane Collaboration (<http://www.cochrane-handbook.org>). First, we searched the databases of PubMed, the Cochrane Library, LILACS, SciELO, Google Scholar, and MEDLINE. Second, the selection of the articles of interest in these databases was based in the following descriptors: hantavirus OR HPS OR HFRS AND Ribavirin OR treatment. We did not limit the period of publication, and we did restrict the language of the publications; English, Spanish and Portuguese. The exclusion criteria were related to the type of study design. Accordingly, we excluded revisions, letters, editorial, and in vitro studies. Additionally, we did not select studies that reported insufficient data to estimate the risk ratio. The selection of the studies was carried out by three authors. Moreover, extracted data related to the first author's name, year of publication, study design, gender, age and population size, method of diagnosis, new- or old-world hantavirus infection, whether the study population was composed of humans or animals and, finally, the risk ratio (RR). Third, for statistical analysis, RR with a confidence interval (CI) of 95 % was estimated. The results of the individual and aggregate RR analyses were expressed in a

type graph forest plot. Heterogeneity between the different studies was assessed by the I^2 statistic (ranges from 0 to 100 %). For the analysis of publication bias, we prepared a funnel plot of the joint distribution of the studies included in the meta-analysis. All statistical analyses were performed using the STATA 13 (STATA Corporation, College Station, TX, USA).

Searches in the databases mentioned above were conducted with the initial use of descriptors in combination, yielding a total of 518 studies. Finally, after applying of the selection and exclusion criteria, seven studies were included in this meta-analysis (Fig. S1) [2, 3, 5, 6, 11, 14, 15], and they were divided into four groups according to whether they were investigating new or old- world hantavirus infections involving humans or animals. A total of 506 clinically similar cases of HPS (124 cases) [2, 11, 14, 15] or HFSR (382) were represented [3, 5, 6]. In the Fig. 1 is shown a graphic of type forest plot with the RR for each study as well as the pooled RR is shown.

In the meta-analysis related to the hantaviruses that causes HPS, only a study was a randomized clinical trial [11], and three were prospective cohort [2, 14, 15]. As for the effectiveness of ribavirin for the treatment of HPS-like disease in animal, this group presented reduction in mortality, i.e., in the treatment group, the survival of this population was higher compared to the control group (RR 0.05, 95 % CI 0.01–0.34, $I^2 = 0$ %). However, when we conducted meta-analysis including studies in humans, we observed the opposite, i.e., there was no observed significant difference of RR between treated and no treated groups (RR 0.99, 95 % CI 0.60–1.61, $I^2 = 0$ %). Additionally, in treatment groups for studies involving humans, approximately 40 % of the cases resulted in death, while in the other group, which did not use this antiviral, there was a similar mortality rate (39 %).

For the meta-analysis of studies that applied ribavirin for the treatment of old- world hantavirus infections, three studies were included [3, 5, 6]. The drug dosage applied in animal ranged from 0 to 100 mg/kg (Table S1), with considerable reduction in the mortality rate (RR 0.56, 95 % CI 0.42–0.76, $I^2 = 64$ %). Regarding the source of heterogeneity was not possible to determine it, but is possible that difference in study design is the cause of divergence between the results.

The values presented in the forest plot (Fig. 1) (RR 0.28, 95 % CI 0.08–1) related to infection by the old- world hantaviruses in humans [6] show that the established treatment resulted in a 72 % reduction in the occurrence of the outcome (death) when compared to the control group. The mortality was 2.5 % in the treatment group, while in the control group (no drug), the fatality rate was at least three times higher (8.5 %). In addition, initially we planned to use the Egger funnel plot to assess possible publication

Fig. 1 Forest plot showing the RR (95 % CI) for treatment of hantaviruses infections with ribavirin. The drug was applied for the treatment of new or old- world hantaviruses infections. The size of square indicates the study size, and pooled estimated is denoted by a diamond at the bottom

bias, but due to the limited number of selected studies were chosen for its non-use.

The results of this systematic, quantitative meta-analysis are based on data from seven studies. Four studies described ribavirin treatment in vivo for etiologic agents of HPS [2, 11, 14, 15], and the remainder [3, 5, 6] evaluated the effects of this drug for etiologic agents of HFRS. A total of 506 participants were recruited, and of these 177 died, regardless of whether ribavirin was used.

Previous studies have aimed at understanding the pathology of HPS, with the goal of seeking a suitable animal model that would clinically resemble the pathology caused by this disease in humans. The effectiveness of ribavirin in treating hantavirus infections is an important question, and animal studies are important it can contribute to understanding of pathogenesis and point toward potential treatments. In recent studies, the Syrian hamster has been used for this purpose, allowing studies of the pathogenesis, treatment and search for a vaccine [10]. Additionally, this meta-analysis included two studies using this animal model, to verify the effects of ribavirin as a single treatment, considering that in the treatment groups, survival was 100 % compared to untreated cases, showing

clear evidence of the beneficial effects of the drug in this animal model [14, 15].

In HPS, the most aggravating factor in the pathogenesis of the disease is the formation and progression of fulminant pulmonary edema, which consequently results in cardiogenic shock; this clinical condition is usually fatal for the patient [7, 9]. Previous studies report that the great “villain” of hantavirus pathogenesis in the cardiopulmonary phase is the immune system itself, which is associated with the hyperresponsiveness, resulting in the disordered activation of immune cells that attack the tissues of the infected individual, especially lung endothelial cells and platelets, which possess receptor β_3 integrin to which hantavirus have tropism [7, 9, 19]. Recent findings have begun to elucidate the pathogenesis of HPS cause by hantavirus infection at the molecular in cellular level. Gupta et al. [4] found that endothelial cells infected by the virus promote cell protection by blocking caspase 3 and granzyme B, which are induced by the cellular cytotoxicity mediated by lymphocytes. However, there are still various molecular mechanisms involved in the pathogenesis of HPS, and these molecules and pathways need to be investigated [7, 8].

In our quantitative meta-analysis of studies involving ribavirin treatment in humans with HPS, the results were contrary to the studies using animals, i.e., there was no significant difference between the treated and untreated groups. Therefore, the effects of this drug in humans with HPS remain unclear for the following reasons [2, 11]: (1) the small number of existing studies, which involved relatively small recruited populations with only 1/3 of the total patients with HFRS; (2) an inability to follow the effects of antiviral therapy due to the severity of disease; and (3) the difficulty in recruiting participants. Significantly, in severe cases of HFRS, mortality usually occurs one week after admission to the hospital, while in HPS, this outcome is observed on average on the third day after hospitalization. Thus, the drug typically has a longer time to take effect in HFRS.

The clinical manifestations of HPS can be divided into the prodromal stage (fever, myalgia, nausea, diarrhea, headache, vomiting), followed by the progression of the patient to the cardiopulmonary phase, resulting in rapid pulmonary edema and cardiovascular compromise, generally progressing to shock and death (approximately 9 days after onset of symptoms) [19]. In view of this, the studies included in the meta-analysis had more patients in the cardiopulmonary phase [2, 11]. Given the rapid progression of the disease to death at this stage, there seems a relatively short amount of time to evaluate the benefits of ribavirin.

In this meta-analysis, we included both HPS and HFRS because these two clinical conditions constitute an imminent hazard to the public health. Moreover, in the era of globalization, there has been ever-increasing communication between various countries through trade or tourism. Although globalization has allowed for financial and technological advances for humanity, it has caused concern for public health systems due to the intense flow of people and goods that can promote the dissemination of hantavirus to other countries. In this context, imported cases of HPS were reported in Europe [13]. This situation reinforces the need to improve and maintain constant international surveillance for hantavirus. In particular, it is important to prevent the global spread of the reservoir hosts of the new-world hantaviruses.

HFRS has been reported in several Asian and European countries. The country with the highest number of the cases of HFRS is China, with approximately 1.5 million cases and at least 46,000 deaths [20]. Studies of hantavirus infections in India are in early stages, but already show cases of hantaviruses, since that levels of 5.2 % for anti-hantavirus IgM antibodies have been found in febrile patients [1]. Therefore, this evidence pointed to the possibility of hantaviruses constitute an emerging public health problem in this region.

Currently, the treatment of HFRS is better documented than that of HPS, moreover due to gravity is lower in

HFRS, allowing for greater clinical monitoring of the effects of drugs used to treat this disease. Furthermore, promising results from *in vitro* studies regarding the high sensitivity of ribavirin for hantavirus have been followed up in *in vivo* study. An *in vivo* study in patients with HFRS providing more significant results on the effects of ribavirin was conducted by Huggins [6]. In this study, the survival of the participants who used the drug was three times higher than in the control group. In addition, there is an ongoing a safety study in Germany on the application of ribavirin in patients with HFRS, and this study is scheduled for completion in the year 2014 (<http://clinicaltrials.gov/show/NCT00868946>).

During the selection of studies for this review, it was not possible to extract the temporal variables related to how many days after the onset of symptoms ribavirin was applied. This information is important because the period with the highest probability of high virus titers in the organism is short, occurring three to 6 days after the onset of symptoms [7, 9, 12, 19]. Thus, unfortunately, we not could to compare the effects of the drug when it was applied immediately after the onset of symptoms versus when it was applied later. Another limiting factor is the fact that the only randomized placebo-controlled experimental study on the HPS [11] was completed earlier than expected, which may have reduced the capacity and affected the summary effect estimates in the meta-analysis. Moreover, the majority of patients in this study, although there were few, had already been treated by the time they reached the lung stage, i.e., in the more severe cases of disease, the drug might have had less time to have an effect.

However, despite the limiting factors in this meta-analysis, this study provides promising results. This study is the first of its kind to address the effects of the drug ribavirin for the treatment of hantaviruses infections in humans and animals. We found that there is a lack of studies documenting the clinical outcomes in patients treated with this drug. Due to difficulties in recruiting patients for randomized placebo-controlled studies on HPS, increased publication of case reports is needed to provide better documentation of patients with regard to the start time of the treatment after the development of symptoms and the clinical outcome. Additionally, benefits associated with glucocorticoid drugs, vasoactive amines and ribavirin applied together in the treatment of HFRS and HPS should be further understood because the treatment often consists of several drugs. With the increasing availability of this type of information, more variables will be available for analysis. Additionally, we believe that the use of real time RT-PCR will provide better characterization of the benefits of antiviral therapies applied at the onset of symptoms.

In short, the small number of available studies evaluated in this meta-analysis limits our ability to examine the

effects of therapy with ribavirin in humans with HPS. On the other hand, there was a significant reduction in mortality in animals, although only four studies evaluated the effects of drugs in animals.

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