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Investigational use of ribavirin in the treatment of severe acute respiratory syndrome, Singapore, 2003

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Summary

OBJECTIVE Ribavirin is a broad spectrum nucleoside analogue efficacious in the treatment of several viral infections. In the recent severe acute respiratory syndrome (SARS) outbreak, ribavirin was used in various countries against this novel coronavirus. We conducted a retrospective analysis to assess the efficacy of ribavirin in the treatment of SARS in Singapore.

METHODS A total of 229 cases were analysed. Ninety-seven (42.4%) patients received ribavirin at a mean of 6.4 days of illness. Clinical and laboratory parameters taken at the start of ribavirin treatment were compared with day 5, 6 or 7 parameters of those patients not on treatment. The two groups were compared using Fisher's exact test and Student's *t*-test. Multivariate analysis was performed using Cox regression model with death as the outcome of interest.

RESULTS The treatment group had younger women with more symptoms of myalgia ($P < 0.001$). The crude death rate was 12.9% in the control and 10.3% ($P = 0.679$) in the treatment group. After correction for age, male sex, lactate dehydrogenase levels and steroid use, the hazard ratio was 1.03 (95% CI: 0.44–2.41, $P = 0.939$).

CONCLUSION In this retrospective, uncontrolled cohort analysis, use of ribavirin did not appear to confer any benefit for patients with SARS.

keywords mortality, ribavirin, severe acute respiratory syndrome, Singapore

Introduction

Ribavirin is a very broad spectrum synthetic nucleoside analogue. It is efficacious in the treatment of respiratory syncytial virus infection among vulnerable children, Lassa fever, haemorrhagic fever with renal syndrome, Crimean-Congo haemorrhagic fever, severe adenovirus infection in immunocompromised patients, and in combination with interferon-2b for hepatitis C infection. Because of its broad spectrum and low relative toxicity it is often tested on a compassionate basis for new and re-emerging viral infections. Thus, it was used in several countries after the dramatic appearance of severe acute respiratory syndrome (SARS) in East Asia, including Singapore and Canada. We performed a retrospective cohort study assessing the effectiveness of ribavirin use in our patients with SARS.

Patients and method

Patients were identified from the existing hospital registry on probable SARS admitted between 1 March and 31 May 2003. Diagnosis of SARS was based on World Health Organization (2003) criteria at the time of the acute illness. The use of ribavirin was doctor-dependent. Oral ribavirin was dosed at 1.2 g three times a day; intravenous ribavirin at 400 mg every 8 h for sicker patients and those who could not take it *per os*. Routes of administration varied depending on whether the patient could tolerate feeding. We collected data on demographics, presenting symptoms, signs and laboratory results on the day of admission.

In the univariate analysis, we used Fisher's exact test and the Student *t*-test to compare these data between patients on ribavirin and those who were not. In the multivariate analysis, we used the Cox regression model to provide both crude and adjusted hazard ratios for use of ribavirin. The aim was to adjust for covariates distributed unevenly between the two groups, which may be related to outcome. Death was the primary outcome of interest. Cox regression analysis was used to compare the survival time between those on ribavirin and those who were not. Survival time is defined as the interval between date of onset and date of death. With the exception of three patients, we used 6 August 2003, when the last SARS patient was discharged from the hospital, as the date of last follow-up for all patients who survived SARS. Three patients who were discharged alive after SARS subsequently died from other

unrelated causes. Their survival time was thus defined as the interval between the date of onset and the date of the last known follow-up at our hospital. Adjustments for known and possible confounders were done in stages, to highlight the incremental effect of these confounders on use of ribavirin. Data was analysed using STATA version 6.0 software (Stata Corp., College Station, TX, USA), and all tests were conducted at the 5% level of significance.

We also attempted to identify complications of drug treatment in the treatment group, namely myocardial injury and anaemia. Myocardial injury was defined as serially elevated creatinine kinase (creatinine kinase that was progressively rising) with electrocardiogram changes (development of elevated ST segment or Q waves). Anaemia was a fall of more than 2 g/dl of haemoglobin by day 7 of treatment.

Results

In Singapore, 238 cases of probable SARS were identified; 231 cases were admitted to our hospital. Of these, 199 were diagnosed as probable SARS during the acute period of illness; 32 cases were identified retrospectively with a positive serology test result done at follow-up. A total of 229 cases were available for analysis, of whom 97 (42.4%) patients received ribavirin. The use of ribavirin throughout the epidemic is represented in Figure 1. The mean starting point of ribavirin treatment was at 6.4 days after onset of illness. Treatment duration was for a mean of 5.6 days. We compared the results of clinical and laboratory investigations at the start of ribavirin treatment with correspondent parameters of patients who did not receive ribavirin on day 6 of illness. When clinical and laboratory results for the day were not available, results from the day before or after were used instead. Both groups shared similar characteristics on admission. The treatment group had fewer males, younger patients, with more symptoms of myalgia. The patients in this group had better oxygen saturation as measured by pulse oximetry on admission compared with controls. Clinical and laboratory results taken at the start of ribavirin in the treatment group *vs.* day 5, 6 or 7 of illness of the control group were similar (Table 1). All but three patients had a positive serology result for SARS-CoV. These three were diagnosed as probable SARS during hospitalization; none of them received ribavirin.

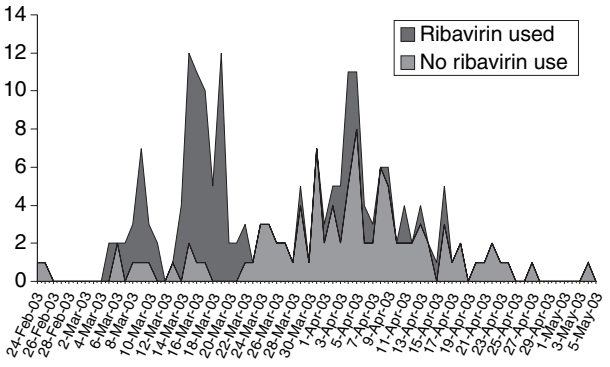

Figure 1 Plot of patients with and without use of ribavirin against date of onset of illness.

At the time of data analysis, all patients in the study had been discharged or had died from the illness. Crude death rates were comparable between the two groups; 10.3% in the treatment group and 12.9% in the controls ($P = 0.679$). There was no significant difference in the proportion of patients admitted to intensive care unit (ICU) between the two groups (Table 1). The hazard ratio of death among those on ribavirin was 0.78 (95% CI: 0.36–1.70, $P = 0.533$) compared with controls. Both groups had an uneven distribution of male sex and older men, known markers of poorer prognosis (Booth *et al.* 2003; Lee *et al.* 2003; Wong *et al.* 2003; Karlberg *et al.* 2004). The enzyme lactate dehydrogenase (LDH) has also been identified as a predictor of poorer prognosis in several studies (Booth *et al.* 2003; Lee *et al.* 2003; Wong *et al.* 2003). After adjustment for these factors and steroid use, the hazard ratio of death was 1.03 (95% CI: 0.44–2.41, $P = 0.939$) for those on ribavirin (Table 2). There was no statistically significant difference between the two groups in acute myocardial infarctions or anaemia.

Discussion

In Hong Kong and Canada, ribavirin was used to treat SARS for want of a specific form of treatment (Tsang *et al.* 2003). Justification of its use came from previous experience with the mouse hepatitis coronavirus (Ning *et al.* 1998). Together with the use of steroids, the potential antiviral and immunomodulatory effects of ribavirin would appear appropriate for the proposed immunopathological damage in the second week of SARS (Peiris *et al.* 2003). However, its use is now challenged. No controlled studies are available, and *in vitro* evidence suggests that the drug has no activity against SARS-CoV at the concentrations for ribavirin-sensitive viruses (MMWR 2003).

In Toronto, ribavirin was used (without steroids routinely) in 126 of 144 (88%) of patients (Booth *et al.* 2003), of whom 40% received steroids concurrently. The majority (91%) received ribavirin within the first 48 h of hospitalization. Patients were given a loading dose followed by a decreasing maintenance dose. Treatment duration was for a mean of 6 days. The authors reported poorer outcomes (death or ICU admission with or without mechanical ventilation) for patients on ribavirin although this was not

Table 1 Comparison of demographics, symptoms, laboratory values and outcome by prescription of ribavirin

Variable	Non-ribavirin (%, $n = 132$)	Ribavirin (%, $n = 97$)	P-value§
Sex (male)	51 (38.6)	22 (22.7)	0.014¶
Race			
Chinese	85 (64.4)	63 (65.0)	>0.999¶
Others	47 (35.6)	34 (35.0)	
Mean age in years (SD)	42.6 (17.7)	34.4 (14.3)	<0.001
Symptoms			
Fever	118 (89.4)	92 (94.9)	0.155¶
Myalgia	34 (25.8)	52 (53.6)	<0.001¶
Cough	45 (34.1)	44 (45.4)	0.100¶
Shortness of breath	19 (14.4)	11 (11.3)	0.556¶
Mean day of illness on admission (SD)	3.99 (3.73)	3.53 (2.39)	0.284¶
Number (%) with positive serology at follow-up	129 (97.8)	97 (100)	0.264¶
Signs and laboratory values (mean and SD)			
On admission			
Temperature	38.4 (0.9)	38.6 (0.8)	0.082
Lowest SaO_2 (%)	97.1 (2.5)	97.5 (1.7)	0.110
Highest FiO_2	0.28 (0.20)	0.25 (0.16)	0.309
*On start of ribavirin**			
TWC ($\times 10^9$/l)	5.1 (2.8)	5.3 (2.6)	0.661
Neutrophil (%)	67.3 (14.4)	69.0 (13.2)	0.420
Lymphocyte (%)	24.1 (12.2)	21.1 (11.0)	0.066
LDH (U/l)	621.2 (362.8)	597.8 (345.0)	0.635
No (%) with $FiO_2 \geq 30\%$	8/105 (7.6)	3/78 (3.9)	0.358¶
SaO_2 (%)	96.0 (9.7)	96.7 (2.1)	0.480
Mean days from illness to X-ray change (SD)	5.4 (3.8)	4.8 (4.4)	0.302
Concurrent use of steroids	17/98 (17.4)	21/97 (21.7)	0.475¶
Concurrent use of antibiotics	94/98 (95.9)	84/97 (86.6)	0.024¶
Complications			
Myocardial injury†	4 (3.0)	3 (3.1)	>0.999¶
Anaemia‡	27 (20.5)	24 (24.7)	0.521¶
Outcome			
Number of dead (%)	17 (12.9)	10 (10.3)	0.679¶
Number of ever admitted to ICU (%)	27 (20.5)	19 (19.6)	>0.999¶
Mean day of illness to start of ribavirin (SD)	NA	6.4 (2.8)	NA
Mean cumulative dose in mg (SD)	NA	13592 (8147)	NA
Mean days on ribavirin (SD)	NA	5.6 (2.5)	NA

TW, total white; PLT, platelets; LDH, lactate dehydrogenase; FiO_2, oxygen expressed as a fraction of the inspired air; SaO_2, saturation of oxygen; ICU, intensive care unit; NA, not available.

* Parameters taken on start of treatment for those on ribavirin, and day 6 for the control group. When results were not available, day 5 or 7 parameters were used instead.

† Myocardial injury was defined as serially elevated creatinine kinase (creatinine kinase that was progressively rising) with electrocardiogram changes (development of elevated ST segment or Q waves) from admission till day 14 of illness.

‡ Anaemia was defined as a fall in more than 2 g/dl of haemoglobin from admission till day 14 of illness.

§ All P-values from Student's t-test unless otherwise indicated.

¶ P-values from Fisher's exact test.

statistically significant (RR 1.9; 95% CI: 0.45–8.0; $P = 0.36$). Adverse events reported included a significant fall in haemoglobin in 71 patients, with 54 patients (37.5%) having had evidence of haemolysis (1.5-fold increase in bilirubin or decrease haptoglobin level). A separate report also from Canada documents complications of haemolytic anaemia in 61%, hypocalcaemia in 58% and hypomagnesium in 48% of patients (Knowles *et al.* 2003).

In Hong Kong, combination treatment of ribavirin and steroids was given to patients with fever, radiographic abnormality and a history of contact with an index SARS patient (So *et al.* 2003). The dosage regimen was similar to ours except that prednisolone was given in addition to

Table 2 Crude and adjusted hazard ratios of the effect of ribavirin

Covariate	HR	95% CI	*P*-value
Ribavirin only	0.78	0.36–1.70	0.533
Ribavirin adjusted for sex and age	1.55	0.67–3.57	0.303
Ribavirin adjusted for sex, age and LDH	1.28	0.54–3.02	0.574
Ribavirin adjusted for sex, age, LDH and steroid use	1.03	0.44–2.41	0.939
Ribavirin adjusted for sex, age, LDH, steroid and antibiotic use	1.03	0.44–2.42	0.939

HR, hazard ratio; LDH, lactate dehydrogenase; CI, confidence interval.

ribavirin at a dose of 1 mg/kg of body weight. Most patients received this combination therapy but no comparative efficacy data from treatment and control groups were available.

In Singapore, the dosing regimen paralleled the Hong Kong Study, but steroid was not routinely administered together with ribavirin: only 21 patients (21.7%) received ribavirin and steroid concurrently. Ribavirin was offered at each managing doctor's discretion, within 48 h of confirming the diagnosis of probable SARS. Unlike the Canadians, our patients were started on ribavirin at a mean of 6.4 days of illness. There was no treatment protocol requiring all patients to be started on ribavirin. This resulted in two groups of patients in our hospital.

The two groups of study subjects were dissimilar. The control group comprised older males ($P < 0.001$), both risk factors of poorer prognosis (Lee *et al.* 2003; Karlberg *et al.* 2004). This was not unexpected with the study design. LDH has been well-described as a predictor of severity of illness; hence, we used its levels to correct for the severity of illness at the start of ribavirin use. LDH levels at the start of ribavirin in the treatment group were compared against levels at day 5, 6 or 7 of illness of the control group, where the mean start date of ribavirin was on day 6.4 of illness. The hazard ratio of 1.03 (95% CI: 0.44–2.41, $P = 0.939$) associated with ribavirin after correcting for predictors of mortality, is in line with the Canadian study. It was, thus, not surprising that Health Canada does not recommend its use routinely in patients with probable SARS (Health Canada 2003).

The use of steroids and antibacterial antibiotics differed between the two groups. Steroids use was defined as having ever been treated with hydrocortisone, prednisolone or methylprednisolone, singly or in combination. Antibiotic use refers to the administration of an antibacterial agent (e.g. ceftriaxone, erythromycin, clarithromycin, amoxicillin-clavulanate, levofloxacin) during the treatment of pneumonia. There were more patients with concurrent use of steroids in the treatment group, and fewer with concurrent use of antibiotics. Steroids were frequently used in combination with ribavirin in the earlier part of the epidemic. In the control group, a steroid was used together with intravenous γ-globulin as a new treatment regimen in the later part of the epidemic. After correcting for steroid use, the hazard ratio of death for patients treated with ribavirin was 1.03 (95% CI: 0.44–2.41, $P = 0.939$).

In the earlier part of the epidemic, some patients were identified as SARS based on existing guidelines and epidemiological links. They were given ribavirin without concurrent antibacterial agents. This accounted for the fewer patients who received antibacterial agents in the treatment group. This group of 13 patients had mild disease and recovered quickly subsequently. There were no deaths, and nobody required ICU admission.

Adverse effects related to the use of ribavirin have been well-described. Complications include haemolytic anaemia, bone marrow suppression, elevated lactate and pyruvate levels, hypocalcaemia, hypomagnesaemia, hyperammonaemia and pancreatitis. Booth *et al.* (2003) reported 49% of patients on ribavirin experienced a decrease in haemoglobin, and that 76% of these patients had evidence of haemolysis. Premature discontinuation of ribavirin was necessary in 18% of their patients.

In our study complications of myocardial injury and anaemia did not differ between the two groups. Three patients (3.1%) had a myocardial event, while 24 patients (24.7%) had a decrease in haemoglobin of 2 g/dl in the treatment group. This was comparable with four (3.0%) and 27 (20.5%) respectively in the non-treatment group. The cause of anaemia was not established in all cases, and we were unable to ascertain the number of patients who had haemolytic anaemia secondary to the use of ribavirin.

Limitations

Our study was a retrospective analysis with two groups of patients being treated at different times of the epidemic. Most of the patients who received ribavirin were admitted early during the epidemic. The apparent lack of clinical efficacy, supported by a subsequent *in vitro* report, led to the decline in use of this drug. Different management practices in these two temporal groups would affect our

interpretation of the results. Use of steroid (dosage and route) was different throughout the epidemic. There was also a change in treatment protocol. In our own hospital's unpublished analysis, we observed that overall survival outcomes improved in the later part of the epidemic, with fewer admitted to the ICU with lower APACHE scores. These may have skewed the results to favour those not receiving ribavirin.

Our study used only in-hospital mortality as the only outcome indicator. Other effects of ribavirin on patients (e.g. lung function, time to defervescence of fever and infectivity) were not assessed. This limits further interpretation of our study.

Conclusion

In this retrospective, uncontrolled cohort analysis, use of ribavirin alone does not appear to confer any benefit for patients with SARS and is consistent with *in vitro* data. New therapeutic options should be tried including immune therapy.

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