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The Monitoring of Cytokines as an Evaluation of Ribavirin as a Treatment for SARS

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Abstract:

The administration of ribavirin has been proposed as a potential treatment for SARS. It was the objective of this study to monitor the immune response in murine lungs in response to SARS infection and subsequent ribavirin treatment.

To evaluate the significance of the modest inhibitory activity of ribavirin to SARS coronavirus (SARS-CoV) in vitro, ribavirin was evaluated for efficacy in a SARS-CoV replication mouse model. Four sets of mice were used, including two control and two experimental groups. Lung samples were taken three and seven days post infection. The lung samples were homogenized and cytokine levels measured with the Quansys Q-Plex™ Mouse Cytokine array. This platform was chosen for its ability to simultaneously measure 16 different mouse cytokine levels with a small sample volume (30µl). Specifically, levels were quantified for IL-1α, IL-1ß, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12, MCP-1, IFNγ, TNFα, MIP-1α, GM-CSF, and RANTES.

The day three and day seven virus-infected mice showed elevated cytokine levels over the control groups for all tested cytokines except IL-3 and IL-9. However, the day three viral infected, ribavirin-treated mice showed only slightly elevated cytokine levels, whereas there were significant increases in the IL-1α, IL-1ß, IL-2, IL-4, IL-5, IL-6, IL12, MCP-1, and TNFα levels by day seven.

The SARS infection resulted in higher cytokine levels measured in the murine lung samples. Though this response was muted in the day three ribavirin treated mice, it was elevated by day seven. Whether the virus was able to overcome the effects of the ribavirin by day seven or the ribavirin had an initial suppressive effect on the immune system is still to be determined.

Introduction:

With the outbreak of Severe Acute Respiratory Syndrome (SARS) in early 2003 there has been a rush to fully understand this virus and find potential treatments. Severe acute respiratory syndrome is a viral respiratory illness caused by a coronavirus, called SARS-associated coronavirus (SARS-CoV). In the SARS outbreak of 2003, 8,098 people worldwide became infected with the SARS-CoV, 774 of these dying. In an attempt to combat an infection with the SARS-CoV, Ribavirin has become the most widely prescribed treatment.

Ribavirin has been chosen as a therapy for SARS because of its broad-spectrum antiviral activity against many DNA and RNA viruses. Ribavirin is a purine nucleoside analogue. It prevents replication of a large number of RNA and DNA viruses by inhibiting the enzyme inosine monophosphate dehydrogenase, which is required for the synthesis of guanosine triphosphate. The use of ribavirin as a treatment for SARS is somewhat controversial. It has not shown any activity against SARS-CoV in vitro. There is also a lack of evidence to suggest that this drug has any in vivo efficacy. Additionally, many patients who are treated with high doses of ribavirin have adverse side effects such as hemolytic anemia, elevated transaminase levels and bradycardia. Despite it's ineffectively against SARS-CoV, it is still the most widely used drug to directly combat a SARS-CoV infection.

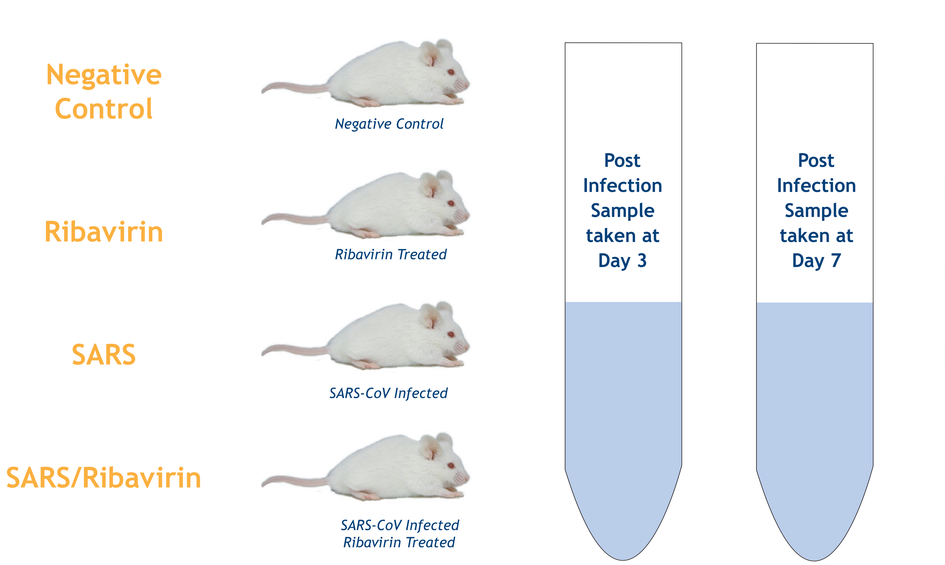
The goal of this study was to better understand the effects of ribavirin in an SARS-CoV infected host. This was accomplished by monitoring the cytokine levels during a SARS-CoV infection and subsequent treatment. We expect to see comparable cytokine levels in both the ribavirin treated and untreated mice. This finding would confirm that ribavirin is not an effective treatment for SARS.

Results:

Both sets of mice that were infected with SARS-CoV experienced increased cytokine levels. At day three the infected untreated mice had elevated IL-1α, IL-1ß, IL-5, IL-6, IL-10, IL-12, MCP-1, IFNγ, TNFα, MIP-1α, GMCSP, and RANTES. The infected ribavirin treated mice at day three did not show the same cytokine elevation as the untreated with the exception of IL-1α, IL-6, IFNγ, and MIP-1α. The measured levels for IL-1ß, IL-5, IL-6, IL-9, and IL-10 had dropped at day seven for the infected untreated mice when compared to the day three values for these mice. In contrast at day seven the infected ribavirin treated mice developed much higher IL-1α, IL-1ß, IL-2, IL-4, IL-5, IL-12, MCP1, IFNγ, TNFα, GMCSF, and RANTES levels when compared to the day 3 values for these mice. In addition the infected treated mice at day seven had 3 logs higher viral titers than did the infected mice (data not shown).

Conclusion:

We demonstrated that ribavirin lacks efficacy against SARS-CoV, possibly exacerbating the infection. The cytokine profiles of the ribavirin treated mice would indicate that the immune response of the mice was delayed when infected with SARS-CoV. The untreated mice had mounted a full inflammatory response by day three where as it took the infected and treated mice 7 days to mount this response. Moreover, this suppressed immune response was further demonstrated in the fact that the viral titers in the ribavirin treated mice were 3 log levels higher than found in the untreated mice.



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