Ruxolitinib in Alleviating the Cytokine Storm of Hemophagocytic Lymphohistiocytosis

Li Jianguo, MD, Zhou Zhixuan, BS, Med, Liu Rong, BS, Med, Shi Xiaodong, MD

Hemophagocytic lymphohistiocytosis (HLH) is a rare and life-threatening syndrome classified into primary HLH and secondary HLH. Secondary HLH is always caused by autoimmune disease, infections, or cancer. The first-line therapy for secondary HLH is the HLH 2004 protocol, including dexamethasone, etoposide, and supportive therapy. However, up to 30% of patients, especially pediatric patients, remain unresponsive to first-line treatment, and the mortality rate reaches 50% in children with HLH. Furthermore, some children who have special conditions, such as an active virus infection, are not suitable for immunosuppressants treatment. Recently, several HLH-promoting cytokines have been identified, including interferon-γ, interleukin-2, and interleukin-6. Janus kinase 1 and 2 control the signaling of many cytokines, notably interferon-γ, interleukin-2, and interleukin-6. Janus kinase 1 and 2 inhibitors, such as ruxolitinib, have been successfully used to treat HLH in mice. Here, we report that a boy, diagnosed with HLH and high titer of hepatitis B virus–DNA copies, improved quickly, and the cytokine storm of HLH was alleviated after receiving ruxolitinib. Five days after ruxolitinib treatment, entecavir was introduced and serum titer results of hepatitis B virus–DNA returned negative. With 3 months of ruxolitinib treatment and following-up 1 year, the boy’s situation maintained sustained remission. In this study, it is suggested that ruxolitinib might be a first-line drug, which could alleviate the cytokine storm of HLH. This treatment may be ushering in the age of glucocorticosteroid-free HLH treatment, which is particularly meaningful for children because it avoids the side effects of glucocorticosteroid.
by the above cytokines, and blockade of this pathway will decrease cytokine signaling and inflammation of HLH. JAK1/2 inhibitors, such as ruxolitinib, have been successfully used to treat HLH in mice.\textsuperscript{5–7} Recently, 2 adults and 1 child with HLH were treated by using a combination strategy of first-line drugs and ruxolitinib.\textsuperscript{8–10} Here, we report that a boy with HLH and active hepatitis B infection received ruxolitinib as a treatment of HLH. Amazingly, his temperature returned to normal and the cytokine storm of HLH was alleviated. Five days after ruxolitinib treatment, entecavir was introduced to control active hepatitis B infection. Serum titer of hepatitis B virus (HBV)-DNA decreased after 1 month and the results returned negative after 3 months of treatment. Fortunately, this patient remained stable at 1 year follow-up.

**CASE REPORT**

A 6-year-old boy was hospitalized because of high fever lasting for >1 month. Because of prolonged fever (>38.5°C), anemia, leukopenia, enlarged spleen, hypertriglyceridemia (3.75 mmol/L), hypofibrinogenemia (1.61g/L), high level of serum ferritin (SF) (9736 ng/mL), high level of interleukin-2 receptor (IL-2R) (4898 U/mL), high level of soluble CD25\(^+\) (41561 pg/mL), and hemophagocytosis observed on the bone marrow puncture specimen, the patient was diagnosed with HLH according to the HLH 2004 protocol. The functional involvement of the central nervous system and respiratory system was not found in this patient. Infectious disease workup results, including bacteria, fungi, tuberculosis, viruses, and parasite, were negative except for HBV. The serum titer of HBV-DNA was 6.7 \(\times 10^6\) copies per mL. The patient carried HBV from birth through vertical transmission. Before coming to our hospital, the patient had been treated with pegylated interferon-\(\alpha\)-2a (peg-IFN \(\alpha\)-2a) for 2 months before developing HLH because the HBV-DNA copy number was 3.9 \(\times 10^4\) copies per mL and abnormal liver function was detected. The local doctors considered that the fever might be caused by peg-IFN \(\alpha\)-2a, so they stopped using peg-IFN \(\alpha\)-2a. No other triggers, such as autoimmune disease and/or malignancy of HLH, had been found. Whole-exon sequencing was performed and no HLH-related genes mutations were found. All laboratory results are listed in Tables 1 and 2.

Considering that the patient had chronic HBV infection, high titer of HBV-DNA copies, and the cytokine storm of HLH, conventional glucocorticoid and cytotoxic chemotherapy were likely to aggravate the HBV infection. As an alternative, on day 2 of hospitalization after communication with parents, ruxolitinib was prescribed at 2.5 mg twice daily on the basis of previous experience in children with refractory cancer.\textsuperscript{11} The patient’s body temperature rapidly returned to normal after 3 days of treatment (Fig 1 shows the temperature peak change on observed days). Because entecavir recommended by infectious expert needed to be ordered and delivered to our hospital, it was delayed until 5 days after ruxolitinib treatment. After 6 days, the level of cytokines, such as IL-2R, decreased from 4898 to 3790 U/mL. After 11 days, the patient’s white blood cell (WBC) count and hemoglobin (Hb) levels were significantly elevated, C-reactive protein (CRP) level was back to normal, SF and IL-2R levels were significantly reduced, and meanwhile, splenomegaly

### TABLE 1 Clinical Indexes From Admission to Subsequent Follow-up

<table>
<thead>
<tr>
<th>Day</th>
<th>WBC</th>
<th>Hb</th>
<th>PLT</th>
<th>NEUT#</th>
<th>ESR</th>
<th>CRP</th>
<th>SF</th>
<th>ALT</th>
<th>AST</th>
<th>Triglyceride</th>
<th>LDH</th>
<th>Fibrinogen</th>
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<td>57</td>
<td>9736</td>
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<td>146</td>
<td>1.61</td>
<td>38.818</td>
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<tr>
<td>2</td>
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<td>77</td>
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<td>ND</td>
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<td>122.2</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>1.76</td>
<td>79</td>
<td>221</td>
<td>0.32</td>
<td>36</td>
<td>55</td>
<td>&gt;2000</td>
<td>19.1</td>
<td>118.1</td>
<td>2.32</td>
<td>1541</td>
<td>1.55</td>
<td>39.132</td>
<td>19.6</td>
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<tr>
<td>5</td>
<td>2.56</td>
<td>82</td>
<td>365</td>
<td>0.34</td>
<td>ND</td>
<td>31</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
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<td>110.3</td>
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<td>ND</td>
<td>ND</td>
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<td>ND</td>
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<tr>
<td>80</td>
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<td>21</td>
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<td>ND</td>
<td>ND</td>
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</tr>
<tr>
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<td>307</td>
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<td>ND</td>
<td>40.2</td>
<td>46.3</td>
<td>1.2</td>
<td>224</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
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<td>128</td>
<td>2.65</td>
<td>111.4</td>
<td>125</td>
<td>18.5</td>
</tr>
</tbody>
</table>

\(\text{Day}\) describes the day after the initial hospital day. ALT, alanine aminotransferase; AST, aspartate aminotransferase; D-dimer, metabolite of secondary fibrinolysis; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; ND, no data; NEUT#, neutrophil granulocyte count; PLT, platelet count; TT, thrombin time.
improved. One month later, almost all clinical indexes returned to normal and the titer of HBV-DNA was decreased to $4.7 \times 10^3$ copies per mL. Table 1 reveals related laboratory examination results from the first hospitalization to the last follow-up time, and Table 2 indicates HBV-DNA copy numbers, decreased cytokine levels, such as of IL-2R, IL-6, interleukin-8 (IL-8), interleukin-10 (IL-10), and TNF-α, and corresponding treatment, further revealing the therapeutic effect. Ruxolitinib was administered for a 3-month treatment regimen and entecavir was used for 6 months. At 1-year follow-up, the patient’s condition remained normal, and no suspected unexpected serious adverse reaction was found. Abbreviations and the reference range of laboratory indexes are shown in Supplemental Table 3.

### DISCUSSION

HLH, a rare and life-threatening syndrome, can take the form of primary HLH or secondary HLH. Primary HLH results from genetic mutations, and secondary HLH is caused by autoimmune disease, infections, or cancer. The patient in this study had a high titer of HBV-DNA who had no related genetic mutation and did not show any malignancy or autoimmune disease. He had been treated with peg-IFN α-2a for 2 months before being diagnosed with HLH, but whether these factors were related to the pathogenesis of HLH was not clear. The administration of interferon α-2a has been reported to cause HLH in the course of treating chronic hepatitis C virus infection, as well as interferon β-1a used in multiple sclerosis. No other factors were found, such as other infection, connective tissue diseases and tumor, and we deduced that immune-related drugs, such as interferon, may cause HLH.

Although glucocorticoids and immunosuppressants are conventional therapies, some patients remain unresponsive and may require alternative therapies. Janus kinase (JAK) 1 and JAK 2 recruit signal transducers and activators of transcription to cytokine receptors, leading to modulation of gene expression. Ruxolitinib, as a JAK inhibitor with selectivity for subtypes JAK 1 and JAK 2, inhibits dysregulated JAK signaling and is thus a good treatment of cytokine storm disease. It has been reported that 2 adults and 1 child with refractory HLH benefited from a combination of ruxolitinib and first-line therapy.
In this study, ruxolitinib was used to control the cytokine storm of secondary HLH, and the patient's body temperature rapidly returned to normal after 3 days of treatment. Entecavir was introduced to treat the HBV infection after 5 days of ruxolitinib treatment. The reason that entecavir was delayed was because it was initially unavailable in our hospital. The patient's hemogram, spleen size, and inflammation status rapidly improved thereafter and returned to normal 1 month later. In addition, ruxolitinib treatment lasted for 3 months with no side effects. No other HLH recommended medication was used in the whole treatment period. With this case study, we confirmed the therapeutic effect of ruxolitinib in HLH, which highlights that ruxolitinib is a promising drug as a first-line treatment of the cytokine storm of HLH. Of note, during the course of treatment, the patient had also received antiviral therapy, so the clinical improvement and sustained remission in the patient stemmed from the combination therapy of ruxolitinib and entecavir. In addition, this report may be indicated as an alternative glucocorticosteroid-free HLH treatment, which is particularly meaningful for children because it avoids the side effects of glucocorticosteroid. Further investigation with clinical trials is needed to identify the optimal dose, duration, and adverse effects of ruxolitinib in HLH.

ACKNOWLEDGMENTS

We thank the patient and his family who participated in this study for their support.

ABBREVIATIONS

CRP: C-reactive protein
Hb: hemoglobin
HBV: hepatitis B virus
HLH: hemophagocytic lymphohistiocytosis
IL-2R: interleukin-2 receptor
IL-6: interleukin-6
IL-8: interleukin-8
IL-10: interleukin-10
JAK: Janus kinase
JAK1/2: Janus kinase 1 and 2
peg-IFN α-2a: pegylated interferon α-2a
SF: serum ferritin
TNF-α: tumor necrosis factor α
WBC: white blood cell

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