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Abstract:

Hepatic encephalopathy is a complex life-threatening neuropsychiatric syndrome, which can be associated with acute inflammation. It can be found in cases of acute liver failure caused by a viral infection. Reports of patients infected with SARS-CoV-2 have described hepatic encephalopathy. Therapy with immunomodulators can be an effective choice for this clinical condition. CIGB-258 is an immunomodulatory peptide with anti-inflammatory properties derived from cellular stress protein 60 (HSP60).

We report a case of a 55-years-old woman diagnosed with COVID-19 and hepatic encephalopathy characterized by episodes of anxiety, delirium, confusion and seizure, according to her clinical history, laboratory and radiological data. Levels of aspartate aminotransferase, alanine aminotransferase, plasma ammonia and alkaline phosphatase were increased and inflammatory biomarkers such as interleukin 6 and 10 were over the normal range. The patient received an intravenous administration of 1 mg of CIGB-258, every 12 hours during
four days, followed by 1 mg daily for another three days without adverse reactions. Neurological symptoms disappeared completely at the fourth day after starting therapy, and inflammatory biomarkers noticeably decreased, but not all of them reach the normal values.

This case highlights the outcomes of a severe COVID-19 patient with hepatic encephalopathy, treated with CIGB-258. The patient recovered successfully and the liver enzymes, plasma ammonia and biomarkers associated with hyperinflammation were reduced. These results support clinical investigations of CIGB-258 as a therapeutic agent in COVID-19.

**TRIAL REGISTRATION:** RPCEC00000313

**Keywords:** COVID-19, SARS-CoV-2, hepatic encephalopathy, CIGB-258

**Introduction:**

SARS-CoV-2 leads, in many cases, to a life-threatening pneumonia and acute respiratory distress syndrome (ARDS) (1). Though the most common and significant presentation is respiratory disease, reports of neurological features are growing (2,3). These neurological manifestations are complications of the systemic effects of COVID-19 considered to be direct effects of the virus on the nervous system, or associated to the immune-mediated disease (4). Some authors have reported hepatic encephalopathy (HE) in patients infected with SARS-CoV-2 (5,6). HE is a pathological process in the brain associated with liver dysfunction that frequently develops over hours to days and can show signs as changes in personality, behavior, cognition, or consciousness. Plasma ammonia levels are considered an important factor in the pathogenesis of HE and ammonia concentrations are high in the systemic circulation of patients with HE (<76 μmol/L) (7).

The treatment of COVID-19 patients with HE is complex, it comprises ammonia blood level reduction measures according to the International Society for Hepatic Encephalopathy and Nitrogen Metabolism guidelines, to down regulate dietetic and intestinal flora nitrogen input, including probiotic supplements (8). However, the use of immunomodulators to control the inflammatory processes mediated by SARS-CoV-2 add an essential missing component.
CIGB-258 is an immunomodulatory peptide derived from the human heat shock protein (HSP) 60. It was able to induce regulatory effects associated to the inhibition of inflammation in several experimental inflammatory models and in patients with rheumatoid arthritis (9–12).

Recently, we studied the effect of CIGB-258 on peripheral blood mononuclear cells (PBMC) isolated from rheumatoid arthritis patients. The peptide reduced the levels of calprotectin (paper submitted for publication), a biomarker for inflammatory diseases released by monocytes and neutrophils (13).

The peptide showed a favorable clinical safety profile and received an Emergency Use Authorization by the Cuban Regulatory Authority (CECMED, http://www.cecmed.cu) for the treatment of seriously or critically ill COVID-19 patients. Therapy outcomes of COVID-19 patients treated with CIGB-258, were associated to a decrease in lung inflammation and in circulating levels of C-reactive protein (CRP), ferritin, lactate dehydrogenase (LDH), creatinine and molecules linked to the cytokine storm (14). These results suggested that CIGB-258 may exert a wide spectrum of actions to limit the inflammatory events of COVID-19 cases.

Here, we report the outcomes of a severe COVID-19 patient with HE, treated with CIGB-258.

Case presentation:

On May 4th 2020, a 55-year-old woman with a 5-year history of controlled hypertension (enalapril 20 mg/day), and without a psychiatric history, tested positive to SARS-Cov-2 by RT PCR. The patient received the standard treatment for COVID-19 at the “Luis Diaz Soto Hospital in Havana, Cuba”, according to the protocol of the Cuban Ministry of Public Health (http://infomed.sld.cu/anuncio/2020/05/11/ministerio-de-salud-publica-protocolo-de-actuacion-nacional-para-la-COVID-19). At admission, the patient was asymptomatic with normal results of her laboratory tests (Table 1). Starting on day eight of hospitalization, the patient developed episodes of anxiety, sleep disorders, delirium, and high blood pressure (160/90 mmHg). No alteration was found in the physical examination. The patient was treated with diazepam (5 mg/8 hours) and diphenhydramine (150 mg/day). Blood pressure was stabilized (120/80 mmHg). Psychiatric symptoms worsened at day 10 after...
hospitalization, showing hypomnesia and disorientation in time and space, as well as seizures and vomiting (Figure 1).

Levels of aspartate aminotransferase (AST; 1214 U/L), alanine aminotransferase (ALT; 460 U/L) and white blood cell count increased, and inflammatory markers such as ferritin, CRP and LDH were above their normal range. Plasma ammonia was twice the normal level (170 μmol/L; normal value is 25-76 μmol/L) (Table 1). Serum calprotectin concentration was 33919 ng/mL (normal range: 481-6540 ng/mL)(Figure 2). Chest and abdominal roentgenograms and brain computed tomography were normal.

The patient was diagnosed with HE, classified as being seriously ill and transferred to an intensive care unit (ICU). She was treated with 1mg of CIGB-258 every 12 hours by the intravenous route (RPCEC00000313 at the Cuban Clinical Trial Registry/ www.registroclinico.sld.cu). The diet was restricted to energy intake of 35–40 kcal / kg body weight, including 1.2–1.5 g protein / kg body weight (8), with probiotic supplements.

White blood cell count, liver enzymes, plasma ammonia, inflammations biomarkers and cytokines decreased during the treatment with CIGB-258 (Table 1). Calprotectin levels normalized after 96 hours of treatment, reaching a value of 6374 ng/mL (Figure 2). The patient showed marked improvement in her neurological condition, she was conscious, communicative, speaking clearly and fluently and well oriented (Figure 1). The therapy with CIGB-258 continued for another 72 hours (1 mg of peptide/ day). She was discharged from the hospital when she tested negative for COVID-19 by RT PCR. Liver transaminases and LDH remained above the normal range at the time of her medical discharge. After leaving the hospital, the patient was checked through the primary health system and specialized services, as established in the treatment protocol in Cuba for all COVID-19 patients.
Table 1. Clinical Laboratory tests

<table>
<thead>
<tr>
<th>Blood cell count</th>
<th>Ref Range and Units</th>
<th>Day of Admission (Day 1)</th>
<th>Day of ICU admission (Day 10)</th>
<th>CIGB-258 therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cell (10^9/L)</td>
<td>4 - 11</td>
<td>11,24</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>Platelet count (10^9/L)</td>
<td>150 - 450</td>
<td>-</td>
<td>380</td>
<td>214</td>
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<tr>
<td>Prognostic Markers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>230-460</td>
<td>459</td>
<td>3846</td>
<td>4730</td>
</tr>
<tr>
<td>Ferritine (µg/L)</td>
<td>30-300</td>
<td>-</td>
<td>383</td>
<td>536</td>
</tr>
<tr>
<td>C-Reactive Protein (mg/L)</td>
<td>≤ 5</td>
<td>3,32</td>
<td>37,17</td>
<td>91,99</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>≤ 6</td>
<td>-</td>
<td>46,49</td>
<td>26,67</td>
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<tr>
<td>IL-10 (pg/mL)</td>
<td>≤7,8</td>
<td>-</td>
<td>349,35</td>
<td>68,05</td>
</tr>
<tr>
<td>Metabolic Panel</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>47-125</td>
<td>105</td>
<td>119</td>
<td>180</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>32-39</td>
<td>26</td>
<td>1214</td>
<td>1004</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>32-40</td>
<td>39</td>
<td>460</td>
<td>480</td>
</tr>
<tr>
<td>Ammonia (µmol/L)</td>
<td>25-76</td>
<td>-</td>
<td>170</td>
<td>165</td>
</tr>
<tr>
<td>Alkaline Phosphatase (U/L)</td>
<td>35-126</td>
<td>184</td>
<td>178</td>
<td>143</td>
</tr>
</tbody>
</table>

LDH: Lactate dehydrogenase , AST: Aspartate aminotransferase , ALT: Alanine aminotransferase , ICU: Intensive care unit

Results and Discussion

The progression of the COVID-19 pandemic has increased the cases with neurological manifestations (4).

Although case reports of HE associated with SARS-CoV-2 are infrequent. The hyperinflammatory response induced by SARS-CoV-2 is a major cause of disease severity (15) and the exaggerated synthesis of cytokines and other inflammatory molecules can lead to an acute severe systemic inflammatory response. Neurological complications -such as HE- could be a consequence of systemic inflammatory effects induced by SARS-CoV-2 (16). In these cases, drugs that modulate immune response may be an attractive therapeutic choice.

Here, we report the first case of HE associated with COVID-19- treated with CIGB-258, an immunomodulatory peptide. To our knowledge, after 5 months of the epidemic, this is the only report of HE associated to COVID-19 in Cuba. The patient started with the CIGB-258 treatment, immediately after being diagnosed with HE. Neurologic symptoms improved during the treatment, which were associated with a reduction of liver enzymes, inflammatory biomarkers, IL-6 and IL-10. These cytokines are linked to hyperinflammation in COVID-19 patients (15).
These results agree with our recent report that included sixteen patients with COVID-19 in serious (31%) or critical (69%) conditions. All critically ill patients recovered from respiratory distress, while all seriously ill patients considerably improved. Levels of biomarkers associated with hyperinflammation and interleukin (IL)-6, IL-10 and tumor necrosis factor (TNFα) significantly decreased during the treatment (14). These results correspond to the mechanism of action of CIGB-258. This peptide shows a wide biodistribution, targeting multiple organs including the liver (17). Its molecular mechanism is associated to an increase in regulatory T cells and a decrease of proinflammatory cytokines (9,11,12).

In this case we introduced the evaluation of calprotectin levels taking into account previous evidence of the peptide effects on the production of this molecule by monocytes and macrophages (submitted for publication). Calprotectin significantly increases in the serum of patients with inflammatory diseases (18), stimulating macrophages and neutrophils recruitment, and inducing cytokine secretion (13). In particular, in HE patients, high calprotectin levels are related to gastrointestinal inflammation. In the present case, CIGB-258 reduced calprotectin to normal levels after four days of treatment, which concurs with the resolution of hepatic encephalopathy symptoms. The treatment with CIGB-258 was well tolerated, and the patient’s clinical condition markedly improved after 96 hours.

These results indicate that CIGB-258 reduced the systemic inflammation component induced by SARS-CoV-2 and resolved HE diagnosed in the patient.

Conclusions

In the present case, HE, which was, a consequence of systemic inflammatory effects induced by SARS-CoV-2, was successfully treated by the intravenous administration of the CIGB 258 peptide, using 1mg every 12 hours during 96 hours, without any adverse effects. The patient markedly improved her neuropsychiatric condition and liver transaminases, plasma ammonia, alkaline phosphatase, inflammatory biomarkers, IL-6 and IL-10 were all reduced. This result supports the ongoing controlled clinical trials of CIGB-258 as a therapeutic drug in COVID-19.
Acknowledgments
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Conflict of interest
The authors have no conflicts of interest

References


Figure 1. Development of hepatic encephalopathy symptoms throughout the course of COVID-19. Schedule of the CIGB-258 therapy
Figure 2. Therapy with CIGB-258 caused the reduction of Calprotectin in the patient. Concentrations of Calprotectin in the serum were measured by a specific ELISA (Quantikine, R&D Systems), according to the manufacturer's instructions. Serum samples were obtained before and during the treatment. Normal range: 481-6540 (ng/mL)