The Effects of Liver Cirrhosis on Electrical Autorhythmicity of Isolated Duodenum in Rats

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Background
The gut-liver axis describes the reciprocal interaction between the gut and liver under physiologic and pathologic conditions such as cirrhosis. One phenomenon that occurs in liver cirrhosis is a reduction in small bowel motility and transit time, with uncertain mechanisms. Intestine motility depends first on the electrical activity and autonomic nervous system of the interstitial cells of Cajal (ICC). If cirrhosis directly affects the ICC network such as inflammation caused by microbiome amplification as a result of cirrhosis, which can be altered through liver cirrhosis, the previous study showed the impairment of the gut-liver-brain axis in cirrhotic patients. Furthermore, the reduction of small bowel motility in liver cirrhosis is associated with gastrointestinal microbiome multiplication, which triggers inflammation and alters epithelium and vascular intestinal barriers.

Therefore, the roles of intrinsic or extrinsic intestinal mechanisms that affect cirrhotic bowel motility are not clear. If liver cirrhosis directly affects the intrinsic mechanism of intestinal motility, the isolated bowel of cirrhotic cases will also exhibit an electrical abnormality compared with normal cases.

Materials and Methods
Six weeks after bile duct ligation (BDL) surgery, animals were anesthetized, and blood samples were obtained from the heart for the evaluation of plasma cirrhosis indices, including weight, plasma albumin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), bilirubin, and direct bilirubin. Then, the duodenum of rats was isolated and compared with the control group for the rate of electrical slow waves and the maximum amplitude of slow waves.

Results:
Significant differences were observed between the control and BDL group in terms of weight, plasma albumin, SGOT, SGPT, bilirubin, and direct bilirubin. Moreover, there was a significant decrease in the maximum amplitude of slow waves in the BDL group compared with the control.

Conclusion:
It seems that different factors directly harm the ICC network such as inflammation caused by microbiome amplification as a result of cirrhosis, which in turn reduces small bowel motility.

Keywords:
Liver cirrhosis, Interstitial cells of Cajal, Gastrointestinal microbiome
All of the animals were then weighed at the start as well as at the end of the experiment. Five rats underwent bile duct ligation (BDL) surgery as described in the literature. During the following weeks, ascitic fluid was observed in the peritoneum cavity with signs of jaundice. Six weeks after BDL surgery, the animals were anesthetized with ketamine and xylazine (60 mg/kg and 6 mg/kg, respectively). The other five rats that went through the whole process without the BDL surgery were considered the control group.

For the evaluation of liver cirrhosis establishment through BDL surgery, at the end of the experiment and after anesthetizing, blood samples were directly obtained from the heart, and albumin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), bilirubin, and direct bilirubin of plasma were measured through spectrophotometry technique by Pars Azmoon kits.

After blood sampling, the anesthetized rats were sacrificed and their duodenums were isolated. The electrical activity of approximately 1 cm of isolated duodenum which was 2 cm after the pylorus sphincter was compared with the control group. The isolated duodenum was cut along the mesenteric border, pinned flat on a base, and super-fused using carbonized Krebs–Henseleit solution (pH = 7.35–7.45, temperature = 37°C) in the tissue isolator.

Three blunt voltmeter electrodes were used for luminal surface electrical recording. The positive and negative electrodes were stated on the proximal and distal surfaces of the isolated tissue, respectively. Then, a third electrode, which was the reference electrode, was fixed on the solution content.

The electrical signals were digitized at the sampling rate of 1 kHz and displayed on LabChart 7 by PowerLab system (model 4/35, AD Instruments, Sydney, Australia). The raw sample electrical pattern recorded from the isolated duodenum in the control and BDL groups is illustrated in Figures 1A and 1B, respectively. Any cycle with an amplitude higher than 0.5 mV was considered a slow wave. After 10 minutes of stabilization, the rate of slow waves was measured for one minute, and the maximum amplitude of slow waves was measured for 15 minutes.

**Statistical Analysis**
The data were analyzed by unpaired t-test using GraphPad Prism 9 software (GraphPad Software, San Diego, CA) and were presented as mean ± standard error of the mean. Moreover, P values less than 0.05 were considered statistically significant.

**Results**

**Verification of Liver Cirrhosis**

Table 1 presents the results of the comparison between the control and BDL group regarding weight at the start of the experiment and after 6 weeks of the experiment, albumin of plasma, SGOT, SGPT, bilirubin, and direct bilirubin. As seen in Table 1, the weigh-in start of the experiment does not show a significant difference. However, other measured variables indicate differences for BDL animals compared to the control group. These alterations of variables confirm the cirrhosis induction under the BDL situation.

**Rate and Amplitude of Duodenum Slow Waves**
The results are presented in Figures 2A and 2B. As demonstrated in Figure 2A, there was no significant difference between the rate of slow waves in control vs. BDL groups (31.8 ± 0.9165 cycles per minute vs. 31.8 ± 1.02 cycles per minute, respectively, P > 0.9999).

On the other hand, a significant difference was observed in the maximum amplitude of slow waves between control and BDL groups.

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**Table 1. The Comparison of Different Variables between the Control and BDL Group**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control Group</th>
<th>BDL Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight in start (g)</td>
<td>216.3 ± 4.9</td>
<td>224.2 ± 5.3</td>
<td>0.7033</td>
</tr>
<tr>
<td>Weight after 6 weeks (g)</td>
<td>309.2 ± 8.1</td>
<td>261.7 ± 12.4</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Albumin of plasma (g/dL)</td>
<td>4 ± 1.6</td>
<td>2.94 ± 0.08</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>SGOT (U/L)</td>
<td>154 ± 5.5</td>
<td>624.2 ± 25.9</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>SGPT (U/L)</td>
<td>97.7 ± 4.6</td>
<td>195 ± 6.9</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>0.46 ± 0.04</td>
<td>8.1 ± 0.55</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dL)</td>
<td>0.14 ± 0.02</td>
<td>6.54 ± 0.89</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Note: BDL: Bile duct ligation; SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase.
and BDL groups (24.53 ± 1.367 mV vs. 4.458 ± 0.7644 mV, respectively, *P* < 0.0001), as depicted in Figure 2B.

**Discussion**

The bowel motility is subordinate to ICC electrical autorhythmicity. The alteration of bowel electrical activity in health and disease can change enteric motility. Further, the reduction of small bowel motility and transient time in liver cirrhosis emerged with unclear mechanisms. However, the effect of liver cirrhosis on the number and maximum amplitude of slow waves has not been investigated yet. Therefore, the present in-vitro research was designed to clarify the direct role of liver cirrhosis in the electrical autorhythmicity of rat duodenum out of the body.

At first, plasma factors and weight of animals verified the BDL technic for induction of cirrhosis after six weeks. The loss of weight, reduction of serum albumin, increase of SGPT as well as SGOT, and elevation of bilirubin content displayed that the cirrhosis is established after six weeks.

The results of the recorded electrical activity of the isolated duodenum indicated that the pacing function of ICC in the duodenum is robust, even when isolated, to the extent that it is not altered even after six weeks of BDL and gastrointestinal microbiome elevation. On the other hand, a decline in the maximum amplitude of slow waves showed changes in electrical irritability and conduction in the gut syncytium.

According to prior research, it is clear that autonomic dysfunction correlates positively with motility disorders and gastroparesis in cirrhotic patients, but hepatic branch vagotomy modulates the gut-liver-brain axis in murine cirrhosis. A study in mice suggested that ICC plays a major role in inflammation-induced motor disturbances in the small intestine. Moreover, Liu et al explained that neuroinflammation in murine cirrhosis is dependent on the gut microbiome. In addition, the gastrointestinal microbiome amplification caused by cirrhosis can decrease small bowel motility with uncertain mechanisms.

It seems that extrinsic factors such as the autonomic nervous system did not play a role in decreasing the propagation of electricity in liver cirrhotic conditions. However, the rise of gastrointestinal microbiome and inflammation has an effect greater than the alteration of epithelium and vascular intestinal barriers in liver cirrhosis.

**Conclusion**

In conclusion, the present study indicates that intrinsic factors such as gastrointestinal microbiome and/or other agents may refer to the interaction between liver cirrhosis factors and the ICC network, which in turn reduces small bowel electrical activity as well as motility.

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**Authors’ Contribution**

Conceptualization: Gholamreza Bayat, Roham Mazloom.
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**Competing Interests**

The authors declare that they have no conflict of interest.

**Ethical Approval**

Animal ethics committee of Alborz University of Medical Sciences (ethical code: IR.ABZUMS.REC.1398.139)

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