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Original Article

The roles of the glucagon-like peptide-2 and the serum TGF-β1 levels in the intestinal barrier and immune functions in rats with obstructive jaundice

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Abstract: Objective: To determine the mechanisms by which glucagon-like peptide-2 (GLP-2) impacts the intestinal barrier function, the immune function, and the serum transforming growth factor-β1 (TGF-β1) levels in rats with obstructive jaundice. Methods: Overall, 72 SPF-grade healthy Wistar rats were randomly divided into 4 groups containing 18 rats each: the observation group (ligation of common bile duct, intraperitoneal GLP-2 injection), the control group (ligation of common bile duct, normal saline), the sham-operated group (common bile duct exposed without ligation, normal saline), and the blank group. The serum immune function and the TGF-β1 levels were measured on days 3, 7, and 14 after the intervention. Results: The body mass was determined to be significantly less in the control group than in the other three groups on day 14 after the intervention ($P < 0.05$). The TGF-β1, endotoxin, alanine aminotransferase (ALT), and bilirubin were expressed at significantly higher levels in the control group compared with the blank and sham-operated groups and were the highest at each time point, but the levels in the observation group were significantly decreased after the intervention ($P < 0.05$). Conclusions: We found that GLP-2 can decrease the serum TGF-β1 levels, regulate the immune function, reduce the endotoxin and bilirubin, and protect the intestinal barrier function in rats with obstructive jaundice.

Keywords: Glucagon-like peptide-2, obstructive jaundice model, intestinal barrier function, immune function, TGF-β1

Introduction

Obstructive jaundice is a common disease of the biliary and liver tracts that is associated with higher morbidity and mortality and leads to various pathophysiological disorders, reduced immune function, malnutrition, coagulation dysfunction, and even death [1-3]. The intestinal barrier function is composed of multiple protective mechanisms and is the key pathophysiological function of obstructive jaundice [4-7], which can prevent the spread of bacteria and toxins to other organs and tissues [8]. Obstructive jaundice damages the tight junction of the intestinal mucosa through cytokine release, reduced Kupffer cell functioning in the liver, bile deficiency, and oxidative stress, thereby reducing the defense function of the intestinal tract; thus, intestinal bacteria may migrate or induce endotoxemia [9]. Therefore, effective treatment and prevention against intestinal barrier damage must be investigated.

Glucagon-like peptide-2 (GLP-2) can enhance the biological function of the intestines [10]. GLP-2 can upregulate the tight junction protein content in rats with obstructive jaundice to restore and maintain the integrity of the intestinal mucosal epithelial barrier [11]. Transforming growth factor-β1 (TGF-β1) is a cytokine with multiple biological functions that is primarily involved in fibrosis promotion and inflammation management [12]. Fang et al. showed that simvastatin can enhance liver regeneration and activation by reducing TGF-β1-induced apoptosis in rats with obstructive jaundice [13]. Reportedly, GLP-2-induced TGF-β1 expression and the vascular endothelial growth factor are involved in mucosal epithelial cell migration associated with mucosal

regeneration [14]. In addition, GLP2 plays a role in osteoclast formation through TGF-β1 signaling [15]. IgA, IgG, and IgM are immunoglobulins and are important effector molecules of humoral immunity. IgA can protect the intestinal mucosa, and IgM and IgG can enhance and promote the phagocytosis of other cells in the body [16]. CD3+, CD4+, and CD8+ are subsets of T lymphocytes that coordinate with each other to maintain normal immune function in the body' normal state. Changes in the number of each subset can easily cause immune disorders [17, 18]. Currently, the effects of GLP-2 and TGF-β1 on the immune function of rats with obstructive jaundice remains to be elucidated further.

Therefore, the present study was designed to determine the effects of the GLP-2 and TGF-β1 levels on the intestinal barrier function and the immune function in rats with obstructive jaundice.

Materials and methods

Animal materials

Overall, 72 SPF-grade, healthy Wistar rats (aged 6-8 weeks) were obtained from the Beijing Vital River Laboratory Animal Technology Co., Ltd., with license number: SYXK (Beijing) 2017-0022. All the animals were housed in the Experimental Animal Center of Beijing Anzhen Hospital for 1 week. During the experiment, the animals were housed in ventilated cages, with ad libitum access to food and water, and the indoor temperature was maintained at 18°C-25°C. The approval of the Committee on the Ethics of Animal Experiments of Guizhou Provincial People's Hospital was obtained prior to the initiation of the study.

Grouping and model preparation

The rats were randomized and placed in the observation, control, sham-operated, and blank groups, with 18 rats in each group. The rat model for obstructive jaundice was established: All the rats were fasted for 12 h and anesthetized after being weighed, and 10% chloral hydrate (Qingdao Yulong Algae Co., Ltd., with SFDA approval number: H370226-73) was intraperitoneally injected at 350 mg/kg into the mice in which no peritonitis or severe pain was observed. After a routine disinfection, an upper abdominal median incision was made to expose the hepatoduodenal ligament, and the bile duct was carefully dissociated. The bile duct was double ligated along the hepatic portal of the bile duct using 5-0 surgical silk, and finally, the abdominal cavity was closed and sutured layer by layer. Aseptic processing was required during the surgery to prevent postoperative infections. The rats in the observation group had obstructive jaundice and were intraperitoneally injected with GLP-2 250 μg/kg (Shanghai Hengfei Biotechnology Co., LTD., with item number: bs-0208R-2) on the day of their surgeries.

The rats in the control group had obstructive jaundice, and the same volume of normal saline was injected daily postoperatively. In the sham-operated group, the common bile duct was exposed without ligation, and the same volume of normal saline at 5 mL/kg was injected daily postoperatively. The blank group received no treatment and was provided normal feeding.

Detection indicators

After the establishment of the model, the rats were sacrificed in batches on days 3, 7, and 14 after the intervention, and venous blood was collected into the corresponding test tubes. The blood was then centrifuged at 4000 rpm for 5 min. The serum TGF-β1 and endotoxin levels were measured using an HR-801 enzyme-labeled analyzer (Shenzhen Highcreation Technology Company Limited, China), and the procedure was performed in strict accordance with the instructions of the enzyme-linked immunosorbent assay kit (Wuhan FineTest Biotechnology Co., Ltd., with item number: ER1378, CD-108426-EA). Measurements of the alanine aminotransferase (ALT), total bilirubin (TBIL), and direct bilirubin (DBIL) levels expressed in the blood were conducted using an automatic biochemical analyzer PUZS-300 (Perlong Medical). The peripheral blood T lymphocyte subsets (CD3+, CD4+, and CD8+) were measured using an FACSCalibur automatic flow cytometry (Becton Dickinson, Franklin Lakes, NJ, USA), and the immunoglobulin levels (IgG, IgM, and IgA) were measured using immunoscattering turbidimetry.

Observation indicators

The changes in the rats' body masses in the four groups was observed on day 14 after the

Table 1. Comparison of the general conditions (x ± SD)

Categories	Observation group (n = 18)	Control group (n = 18)	Sham-operated group (n = 18)	Blank group (n = 18)	F/χ^2	P
Sex					2.24	0.52
Male	12 (66.67)	10 (55.56)	13 (72.22)	14 (77.78)		
Female	6 (33.33)	8 (44.44)	5 (27.78)	4 (22.22)		
Age (weeks)	7.67 ± 0.25	7.53 ± 0.31	7.72 ± 0.37	7.59 ± 0.28	1.37	0.26
Length (cm)	17.67 ± 1.34	18.35 ± 1.01	18.14 ± 1.23	18.07 ± 1.12	0.96	0.42
Body mass before modeling (g)	217.36 ± 28.37	221.51 ± 29.39	214.23 ± 25.76	219.34 ± 30.11	0.21	0.89
Indoor temperature (°C)	23.89 ± 1.18	23.68 ± 1.35	24.03 ± 1.02	23.37 ± 1.23	1.02	0.39
Indoor humidity (%)	50.34 ± 2.61	51.76 ± 1.96	50.97 ± 2.07	51.07 ± 2.12	1.25	0.30

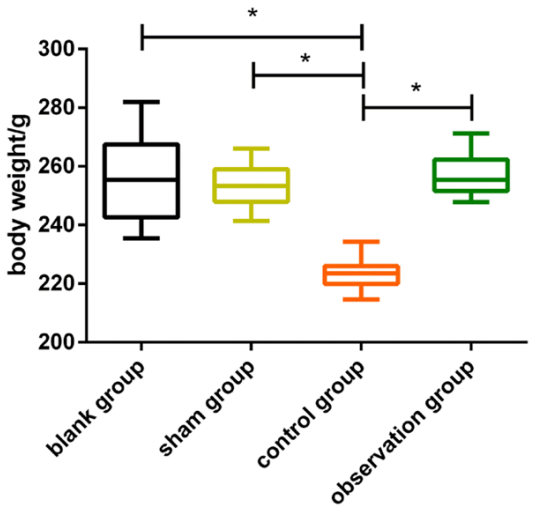

Figure 1. Comparison of the body masses on day 14 after the intervention. On day 14 after the intervention, the body masses in the control group were the lowest among the four groups, which was significant (P < 0.05), but no significant differences were observed among the blank, sham-operated, or observation groups (P > 0.05). Note: * represents a comparison between the two groups, where P < 0.05.

intervention. The immune function and the TGF-β1 expressions were measured on days 3, 7, and 14 after the intervention to compare the serum endotoxin, ALT, TBIL, and DBIL levels in the four groups.

Statistical analysis

The statistical analysis was performed using SPSS 18.0 (IBM Corp, Armonk, NY, USA), and the data were plotted using GraphPad Prism 7. The count data in the groups were analyzed using chi-squared tests and expressed as the number of cases/percentage [n (%)]. The measurement data were the mean values ± SDs. One-way analyses of variance were used to analyze the intra-group comparisons of the mean data, and Bonferroni tests were used for the post hoc measurements. A *P* value of > 0.05 indicated statistical significance.

Results

Comparison of the general information

Table 1 shows a statistical comparison of the general conditions between the randomly-grouped rats. The four groups did not differ significantly in terms of their general conditions such as sex, age (weeks), length, body mass before modeling, indoor temperature, or indoor humidity (P > 0.05).

Comparison of the body masses on day 14 after the intervention

The rats' body masses in each group sacrificed on day 14 were determined, and the body masses in the control group were significantly lower than the body masses in the other three groups after the intervention (**Figure 1**; P < 0.05). The body masses of the rats in the blank, sham-operated, and observation groups showed no significant differences (P > 0.05).

Immune-related indicators

Comparison of the cellular immunity indexes of the rats in the four groups after the intervention: The rats in the blank and control groups showed slight differences in their cellular immune indexes at all time points after the intervention (P > 0.05). The control group had a lower CD3+/% and CD4+/% and a higher CD8+/% than the other three groups. The CD4+/% of the observation group on days 3 and 7 after the intervention was lower than it was in the blank and sham-operated groups, but the CD8+/% was higher than it was in these groups (P < 0.05). The comparison on

Table 2. Comparison of cellular immunity indexes of rats in the four groups after the intervention (x ± SD)

	Cases (n)	Day 3 after intervention	Day 7 after intervention	Day 14 after intervention
CD3+/%				
Blank group	18	63.89 ± 8.86	62.23 ± 9.01	62.34 ± 8.93
Sham-operated group	18	62.38 ± 8.37	61.31 ± 8.25	60.14 ± 8.09
Control group	18	51.34 ± 6.12[a,b]	50.11 ± 5.88[a,b]	46.32 ± 6.11[a,b]
Observation group	18	56.89 ± 5.76[a,b,c]	57.45 ± 6.34[c]	58.96 ± 6.34[c]
F		10.72	9.77	16.84
P		0.023	0.034	0.015
CD4+/%				
Blank group	18	34.43 ± 3.76	33.82 ± 4.02	34.11 ± 3.73
Sham-operated group	18	35.77 ± 4.09	32.67 ± 3.27	33.09 ± 3.17
Control group	18	24.12 ± 3.23[a,b]	20.57 ± 2.87[a,b]	18.27 ± 1.43[a,b]
Observation group	18	27.32 ± 3.01[a,b,c]	29.56 ± 2.77[a,b,c]	32.71 ± 2.85[c]
F		44.80	60.60	119.90
P		0.0001	0.0001	= 0.0001
CD8+/%				
Blank group	18	29.34 ± 4.87	31.07 ± 4.98	30.76 ± 4.67
Sham-operated group	18	30.12 ± 5.01	30.76 ± 4.89	31.13 ± 5.03
Control group	18	40.98 ± 5.11[a,b]	42.45 ± 5.12[a,b]	46.37 ± 5.23[a,b]
Observation group	18	36.34 ± 4.33[a,b,c]	35.31 ± 5.08[a,b,c]	32.08 ± 4.38[c]
F		23.27	21.20	43.75
P		0.0001	= 0.0001	= 0.0001

Note: [a]represents a comparison with the blank group, where $P < 0.05$; [b]represents a comparison with the sham-operated group, where $P < 0.05$; [c]represents a comparison with the observation group, where $P < 0.05$.

day 14 after the intervention among the three groups showed no significant differences. The CD3+/% of the observation group was lower than it was in the blank and sham-operated groups on day 3 after the intervention, but was not significantly different on days 7 and 14 (**Table 2**).

Comparison of the rats' humoral immunity in the four groups after the intervention: No significant differences were observed in the humoral immunity levels between the blank and the sham-operated groups at each time point after the intervention. The humoral immunity index of the control group showed a gradual decrease. The IgG and IgM levels were lower in the observation group than they were in the blank and sham-operated groups on day 3 after the intervention, but days 7 and 14 after the intervention did not show any significant variations among the three groups. The observation group had lower IgA levels than the blank and sham-operated groups on days 3 and 7 after the intervention. The observation group showed higher humoral immunity indexes than the control group at each time point after the intervention (**Table 3**; $P < 0.05$).

The rats' serum TGF-β1 levels in the four groups at the different time points after the intervention: **Figure 3** shows the rats' serum TGF-β1 levels in the four groups on days 3, 7, and 14 after the intervention. The TGF-β1 level in the control group was the highest at each time point and presented an increasing trend. The TGF-β1 level in the observation group at each time point was significantly lower than it was in the control group. However, the TGF-β1 level in the observation group was higher than it was in the blank and sham-operated groups on days 3 and 7 after the intervention ($P < 0.05$). Moreover, the TGF-β1 level in the observation group was significantly lower than it was in the control group at each time point after the intervention ($P < 0.05$).

Comparison of the rats' endotoxin and ALT values in the four groups at the different time points after the intervention: **Figures 2** and **3**

Table 3. Comparison of the humoral immunity of the rats in the four groups after the intervention (x ± SD, g/L)

	Cases (n)	Day 3 after intervention	Day 7 after intervention	Day 14 after intervention
IgG				
Blank group	18	13.87 ± 3.27	14.33 ± 3.76	13.98 ± 4.01
Sham-operated group	18	14.08 ± 3.17	13.33 ± 2.99	13.89 ± 3.31
Control group	18	9.34 ± 2.34[a,b]	8.31 ± 2.63[a,b]	6.68 ± 1.86[a,b]
Observation group	18	11.47 ± 2.21[a,b,c]	12.87 ± 2.32[c]	13.07 ± 2.77[c]
F		11.60	14.51	23.20
P		0.027	< 0.014	< 0.003
IgM				
Blank group	18	1.85 ± 0.37	1.82 ± 0.40	1.90 ± 0.42
Sham-operated group	18	1.89 ± 0.33	1.77 ± 0.31	1.81 ± 0.34
Control group	18	1.34 ± 0.33[a,b]	1.29 ± 0.32[a,b]	1.16 ± 0.28[a,b]
Observation group	18	1.57 ± 0.34[a,b,c]	1.74 ± 0.44[c]	1.87 ± 0.41[c]
F		10.18	7.86	16.57
P		= 0.024	0.038	0.012
IgA				
Blank group	18	2.77 ± 0.44	2.71 ± 0.48	2.82 ± 0.50
Sham-operated group	18	2.83 ± 0.51	2.76 ± 0.47	2.79 ± 0.43
Control group	18	1.89 ± 0.31[a,b]	1.76 ± 0.28[a,b]	1.61 ± 0.21[a,b]
Observation group	18	2.19 ± 0.25[a,b,c]	2.31 ± 0.33[a,b,c]	2.59 ± 0.34[c]
F		24.47	24.15	39.46
P		0.002	0.0017	< 0.0035

Note: [a]represents comparison with the blank group, where P < 0.05; [b]represents a comparison with the sham-operated group, where P < 0.05; [c]represents a comparison with the observation group, where P < 0.05.

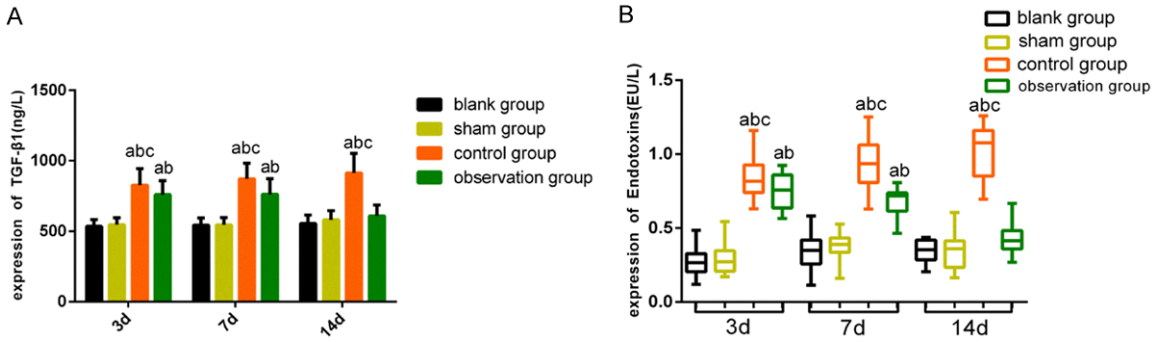

Figure 2. The serum TGF-β1 and endotoxin I levels at the three time points after the intervention. A: No significant differences were observed in the TGF-β1 level between the blank and sham-operated groups at each time point (P > 0.05). The TGF-β1 level was the highest and presented an increasing trend in the control group at each time point. It was higher in the observation group than it was in the blank and sham-operated groups on days 3 and 7 after the intervention (P < 0.05). However, the TGF-β1 level in the observation group was significantly lower than the TGF-β1 level in the control group at each time point after the intervention (P < 0.05). B: No significant differences were observed in -β1 serum endotoxin levels between the blank and sham-operated groups at all the time points (P > 0.05). The serum endotoxin levels in the control group showed an increasing trend at each time period, with higher expressions than those in the other three groups (P < 0.05). After the intervention, the serum endotoxin levels of the observation group gradually decreased on days 3, 7, and 14 after the intervention (P < 0.05). No significant differences were observed in the serum endotoxin levels between the blank and sham-operated groups on day 14 after the intervention (P > 0.05). Note: [a]represents a comparison with the blank group at P < 0.05; [b]represents the comparison with the sham-operated group at P < 0.05; [c]represents a comparison with the observation group at P < 0.05.

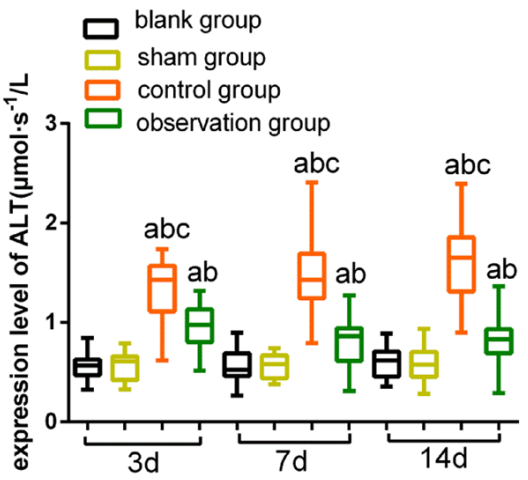

Figure 3. Comparison of the ALT values at the three time points after the intervention. No variations of significance were observed in the serum ALT levels between the blank and sham-operated groups at the three time points ($P > 0.05$). The serum ALT levels were increased in the control group, with the control group showing the highest expressions among the four groups ($P < 0.05$). The observation group had significantly higher ALT levels than the blank and sham-operated groups on days 3, 7, and 14 after the intervention ($P < 0.05$). Note: [a]represents a comparison with the blank group at $P < 0.05$; [b]represents a comparison with the sham-operated group at $P < 0.05$; [c]represents a comparison with the observation group at $P < 0.05$.

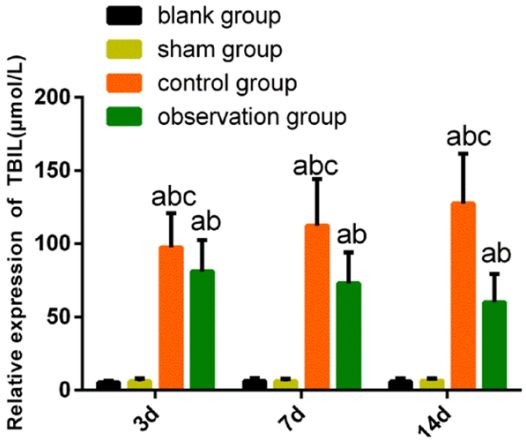

Figure 4. Comparison of the TBIL levels at the three time points after the intervention. No significant differences were observed in the TBIL levels between the blank and sham-operated groups at each time point, but this level showed a significant decrease in comparison to the levels in the observation and control groups ($P < 0.05$). The control group showed an increased TBIL level after the intervention. The observation group had significantly lower TBIL levels compared with the control group at each time point after the intervention ($P < 0.05$). Note: [a]represents a comparison with the blank group, where $P < 0.05$; [b]represents a comparison with the sham-operated group, where $P < 0.05$; [c]represents a comparison with the observation group, where $P < 0.05$.

show that no significant differences were observed in the serum endotoxin and ALT levels between the blank and sham-operated groups at each time point ($P > 0.05$). The serum endotoxin and ALT levels were increasingly higher in the control group at the three time points, and the expressions were the highest among all groups ($P < 0.05$). Compared with the blank and sham-operated groups, the observation group had significantly higher ALT levels on days 3, 7, and 14 after the intervention ($P < 0.05$). The serum endotoxin levels in the observation group gradually decreased after the intervention, but they were higher than the serum endotoxin levels in the blank and sham-operated groups on days 3 and 7 after the intervention ($P < 0.05$). No significant differences were observed in serum endotoxin levels between the blank and sham-operated groups on day 14 after the intervention ($P > 0.05$).

Comparison of the rats' bilirubin levels in the four groups at different time points after the intervention: The changes in the TBIL and DBIL levels at the three measurement points after the intervention are shown in **Figures 4** and **5**. No significant differences were observed in the TBIL or DBIL levels between the blank and sham-operated groups at each time point, and both levels were significantly higher in the observation and control groups at the corresponding time point ($P < 0.05$). These levels in the control group showed an increasing trend after the intervention. The observation group expressed significantly lower TBIL and DBIL levels at each time point after the intervention compared with the control group ($P < 0.05$).

Discussion

Obstructive jaundice is due to intrahepatic cholestasis or an obstruction of the bile excretions caused by an obstruction of the extrahepatic biliary tract system. It has a low survival rate and causes a series of homeostasis disorders [19, 20]. The intestinal epithelium is mainly composed of intestinal, discoid, and goblet cells and is the core component of the intestinal barrier [21]. Studies have shown

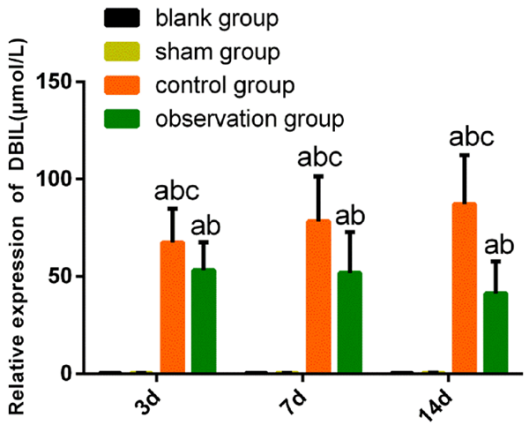

Figure 5. Comparison of the DBIL levels at the three time points after the intervention. No differences were observed in the DBIL levels between the blank and sham-operated groups at each time point, but both groups showed a significant reduction compared with the observation and control groups ($P < 0.05$). The DBIL level showed an upward trend in the control group after the intervention and a significant decrease in the observation group at each time point, demonstrating a sharp contrast ($P < 0.05$). Note: [a]represents a comparison with the blank group, where $P < 0.05$; [b]represents a comparison with the sham-operated group, where $P < 0.05$; [c]represents a comparison with the observation group, where $P < 0.05$.

that the reduction of goblet cells in the small intestine leads to reduced MUC2 secretions, which plays an important role in intestinal mucosal injuries [21]. Therefore, the intestinal barrier must be protected from obstructive jaundice. GLP-2R is the main receptor for the function of GLP-2 and becomes an important regulatory factor of intestinal mucosa by stimulating epithelial growth [22, 23]. GLP-2 can enhance intestinal mucosal proliferation by binding to GLP-2R in intestinal epithelial cells and indirectly protect the intestinal barrier function by binding to GLP-2R receptors distributed in other areas [24]. In addition, GLP-2 has been proven to increase villus growth and intestinal weight by inducing a higher proliferation rate in crypt cells [25].

TGF-β1 is an important anti-inflammatory cytokine that can inhibit the occurrence and development of inflammation and accelerate the repair of epithelial injury by reducing immune overreaction [26]. In addition, previous studies have shown that TGF-β1 can be used as an immune modulator in human diseases, and its process includes the enhancement of the antitumor immune response mediated by the CD8+ and T cells, thereby increasing the infiltration of natural killer cells and T cells at the metastatic site [27, 28]. Studies have shown that TGF-β1 has a correlation with immune cytokines in the body [29, 30]. The CD3+ cells mainly exist on the cell surface and are common markers of T lymphocytes [31]. CD4+ cells are effector cells with auxiliary humoral and cellular immune functions, and CD8+ cells are cytotoxic T cells that can recognize and kill infected cells [32]. IgG, IgM, and IgA are three crucial players that regulate the body's immune function [33]. Studies have shown that GLP-2 can significantly improve the intestinal mucosal immune barrier in mice with severe acute pancreatitis, regulating the expressions of the intestinal mucosal CD3+, CD4+, CD8+ lymphocytes and IgA lambda chain, thereby showing a protective effect [34]. However, studies on the effect of GLP-2 on obstructive jaundice are scarce.

The rats' body masses in the control group were significantly lower than the body masses of the rats in the other three groups on day 14 after the intervention ($P < 0.05$), and no significant differences were observed in the other three groups, which is consistent with an earlier study [24]. These results suggest that GLP-2 can protect the intestinal mucosa from injury and improve the weight loss caused by obstructive jaundice. The proportion of the IgA and CD4+ and CD8+ T lymphocytes reportedly decreases after the establishment of the obstructive jaundice model in rats, suggesting that obstructive jaundice weakens the immune function of the intestinal mucosa [35]. Studies have shown that obstructive jaundice can significantly reduce patients' cellular immune function, leading to severe liver damage. Postoperative treatment with thymopeptide α1, an immune-enhancing agent, can significantly improve the cellular liver function and the immune function of such patients [36]. Lei et al. [37] reported that GLP-2 treatment can improve the innate and acquired intestinal immunity in mice. This study shows that the CD3+, CD4+, IgG, IgM, and IgA levels are significantly reduced, with increased CD8+ levels, after the establishment of the obstructive jaundice model. The immune function of the body significantly decreased with the aggravation of the obstruction in the control group, but

the immune function in the observation group gradually recovered with time, suggesting that inflammation subsides and the natural repair of injuries requires time. It indicated that GLP-2 injections can significantly control the development of the disease and improve the immune damage caused by obstructive jaundice. Piao et al. [26] studied the effect of the recombinant growth hormone on the intestinal mucosal barrier in rats with obstructive jaundice and showed that the serum TGF-β1 level was lowered to a greater extent in the rats injected with recombinant growth hormone than it was in the obstructive jaundice group. In the present study, the TGF-β1 levels were observed and compared on days 3, 7, and 14 after the intervention in the four groups of rats. The TGF-β1 levels in the control group were the highest at each time point and showed an increasing trend. The TGF-β1 expression was higher in the observation group than it was in the blank and sham-operated groups on days 3 and 7 after the intervention ($P < 0.05$). The TGF-β1 level was significantly lower in the observation group than it was in the control group at each time point after the intervention ($P < 0.05$). The increase in the serum TGF-β1 levels may be due to the compensatory mechanism produced by the body when the intestinal mucosa is severely damaged, and obstructive jaundice can lead to a significant increase in the serum TGF-β1 level in rats based on the previous studies. The injection of GLP-2 leads to a faster repair of the local mucosal damage and to faster intestinal epithelial regeneration and significantly reduces the serum TGF-β1 level. Obstructive jaundice may present with an excessive pro-inflammatory response to endotoxemia [38]. Studies have shown that N-acetylcysteine injections administered intravenously in patients with obstructive jaundice can significantly improve their serum ALT and bilirubin levels and protect their liver function [39]. In the present study, the serum endotoxin and ALT levels in the control group increased with the passage of the modeling time, and higher expression levels compared with those in the other groups of rats were observed ($P < 0.05$). The serum endotoxin and ALT levels in the observation group decreased with the passage of the modeling time but remained higher than those in the blank and sham-operated groups at each time point after the intervention. This finding suggests that obstructive jaundice can lead to a significant increase in the endotoxin and ALT levels, and GLP-2 can inhibit the upregulation of the serum endotoxin and ALT levels and protect the intestinal barrier function in the rats from obstructive jaundice. The serum bilirubin level gradually increases with the ligation time after establishing a rat model of obstructive jaundice. Fei et al. [40] showed that Astragalus injections can protect rats with obstructive jaundice against multiple organ injuries and significantly improve the TBIL and DBIL levels. According to Kong et al. [41], spironolactone can significantly inhibit the TBIL and DBIL levels in the kidney tissues of rats with obstructive jaundice. In the present study, the bilirubin levels in the rats with obstructive jaundice were observed. Compared with the gradual increase of the TBIL and DBIL levels in the control group after the intervention, the observation group had significantly lower levels ($P < 0.05$), indicating that GLP-2 can significantly downregulate the bilirubin elevation caused by obstructive jaundice.

In this study, the role of GLP-2 in improving the intestinal barrier function in rats with obstructive jaundice by measuring the immune function, the TGF-β1 level, and the serum endotoxin level was studied. Currently, the application of GLP-2 in obstructive jaundice is limited to animal studies, and GLP-2 has shown a good effect in intestinal diseases [42]. However, a large number of studies are required to prove the role of GLP-2 in obstructive jaundice and subsequently conduct clinical trials. In addition, the long-term monitoring of the changes in the patients' indicators at multiple time points is required for such trials, and the influence of GLP-2 on the prognosis requires careful monitoring and further elucidation. However, the integration mechanism between the main signaling pathways of GLP-2 as well as the key regulatory points and the feedback inhibition of integration require further investigation. Moreover, owing to certain issues at the early stage of our study design, a sham GLP-2 treatment group was not designed, which is one of the limitations of the study. Furthermore, there is a need for the role of GLP-2 in humans to be substantiated by adequate research data.

In conclusion, the innovations of our study are that we found that GLP-2 can decrease the serum TGF-β1 level, regulate immune function indexes, reduce the endotoxin and bilirubin levels, and play a protective role in intestinal barrier function in rats with obstructive jaundice.

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Disclosure of conflict of interest

None.

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