

## Original Article

# Influence of glucagon-like peptide-2 on intestinal barrier function and immune function and changes in TGF- $\beta$ 1 levels in obstructive jaundice model rats

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Received December 30, 2019; Accepted March 3, 2020; Epub May 15, 2020; Published May 30, 2020

**Abstract:** Objective: The aim of the current study was to analyze the influence of glucagon-like peptide-2 (GLP-2) on intestinal barrier function (IBF) and immune function (IF), as well as changes in TGF- $\beta$ 1 levels, in obstructive jaundice (OJ) model rats. Methods: A total of 60 healthy SD rats were selected and divided into the observation group (OG), including 20 intraperitoneally injected with GLP-2 as OJ models, the surgical control group (SCG), including 20 intraperitoneally injected with phosphate buffered solution (PBS) as OJ models, and the control group (CG), including 20 intraperitoneally injected with PBS and treated with laparotomy but not with common bile duct ligation (CBDL). These rats were killed in batches at 1 day, 3 days, 7 days, and 14 days after surgery, evaluating IBF and IF and measuring TGF- $\beta$ 1 levels. Results: (1) Proliferating cell nuclear antigen (PCNA) levels of CG were much higher than those of OG and SCG at 1 day, 3 days, and 7 days after surgery ( $P < 0.05$ ); (2) Caspase-3 levels of SCG were much higher than those of OG and CG at 3 days and 7 days after surgery ( $P < 0.05$ ); (3) Intestinal villus heights (IVH) of SCG levels were much lower than those of OG and CG at 1 day, 3 days, and 7 days after surgery ( $P < 0.05$ ); (4) CD4<sup>+</sup> and CD4<sup>+</sup>/CD8<sup>+</sup> of OG and CG were higher than those of SCG and CD8<sup>+</sup> of OG and CG was lower than that of SCG at 1 day and 7 days after surgery ( $P < 0.05$ ); and (5) TGF- $\beta$ 1 levels of SCG were much higher than those of OG and CG at 7 days after surgery ( $P < 0.05$ ). Conclusion: GLP-2 can improve IBF and IF, enhancing TGF- $\beta$ 1 levels of OJ model rats.

**Keywords:** Obstructive jaundice, rats, glucagon-like peptide-2, intestinal barrier function, immune function, TGF- $\beta$ 1 level

## Introduction

With higher incidence rates found in the Department of Hepatobiliary Surgery, OJ has been mainly treated through surgery [1]. It has been found, clinically, that OJ patients are easily complicated with endotoxemia (ETM), infections, and even multiple organ dysfunction syndrome. They tend to produce many cell factors, seriously affecting their IF levels. This leads to severe and complicated pathological and physiological changes [2, 3].

After OJ, the bile salt can neither inhibit the intestinal bacteria normally nor degrade the endotoxin effectively. Moreover, ETM and intestinal bacterial translocation will be caused by the gradual decline of intestinal mucosal barrier function (IMBF). This may lead to exacerbation and even death [4, 5]. ETM has an impor-

tant impact on OJ patient prognosis, with preoperative endotoxin levels directly related to postoperative mortality [6]. Surgery will cause severe trauma to OJ patients, with obvious changes in levels of cell factors, seriously influence IF [7]. JunChen et al. found that IF was improved, to a certain extent, through drug treatment. They also found that GLP-2, as a polypeptide, could accelerate the growth of intestinal mucosa and improve IMBF [8].

At present, there are relatively few studies concerning the effects of GLP-2 on intestinal barrier function, immune function, and TGF- $\beta$ 1 after treatment of obstructive jaundice at home and abroad. Therefore, the current study is somewhat innovative. To this end, 60 rats were selected for animal experimentation after grouping and modeling. The aim of the study was to discuss the influence of GLP-2 on IBF and IF,

as well as changes in TGF- $\beta$ 1 levels, in the treatment of OJ rats, endeavoring to provide more clinical methods.

## Material and methods

### Materials

A total of 60 healthy SD rats, with body masses of 200 g-250 g, were selected and divided into OG, SCG, and CG, with each group including 20 rats. They were raised under normal light at a temperature of 18-25°C. They were provided ample food and water. Humidity was controlled at 60%-80%. They were raised and managed in strict accordance with Animal Protection Association guidelines. Every procedure was approved by the Animal Care and Use Committee of the Third Affiliated Hospital of Nanchang University.

### Methods

(1) Preparation of 20 model rats for OG: The rats were intraperitoneally injected with 3 mL/kg 10% chloral hydrate after preoperative fasting for 12 hours. After successful anesthesia, disinfection, and draping in sequence, an incision was made in the center of upper abdomen to expose ligamentum hepatoduodenal. Dissociation of the common bile duct was performed in the first porta hepatis, followed by double ligation. Next, the abdominal wall was sutured in two layers. Upon the completion of surgery, the rats were intraperitoneally injected with GLP-2 phosphate buffered solution (PBS), with a purity of over 95%, twice per day. The volume dose of GLP-2 was controlled at 250  $\mu$ g/(kg·d); (2) Preparation of 20 model rats for SCG: After the same surgery applied with OG, the rats were intraperitoneally injected with 0.01 mmol/L PBS every day; and (3) Preparation of 20 model rats for CG: The rats were treated with abbreviated laparotomy procedures, but not with CBDL. They were then intraperitoneally injected with 0.01 mmol/L PBS daily.

The rats ate freely after surgery and were killed in batches at 1 day, 3 days, 7 days, and 14 days after surgery for evaluation of therapeutic effects.

### Observation targets

IBF: The three groups were measured with respect to PCNA, Caspase-3, and IVH at 1 day,

3 days, and 7 days after surgery. ① PCNA detection: The laparotomy procedure was performed immediately after the rats were killed. A 2-cm-long jejunum was excised in the position 1 cm away from the distal end of Treitz ligament. Contents in the enteric cavity were washed thoroughly through isotonic saline and fixed in 40 g/L formaldehyde solution for preparation of 3- $\mu$ m-thick slices. The ready-to-use anti-PCNA mouse monoclonal antibody was used as a primary antibody in immunohistochemical (IHC) staining, while the biotin-labeled rabbit anti-rat serum was used as a secondary antibody. The IHC specimen was measured through software. Mean optical density (MOD) levels of positive cells were regarded as results; ② Caspase-3 detection: Slicing and measurement were carried out according to the above methods. The ready-to-use anti-caspase-3 mouse monoclonal antibody was used as a primary antibody and the secondary antibody was the same as above; and ③ IVH measurement: Paraffin-embedded intestinal tissues were sliced and H&E staining was performed routinely. IVH was measured through the CAST system by measuring 10 villi for each slice, recording results based on the mean value.

IF: The laparotomy procedure was performed immediately after the rats were killed. A 2-cm-long ileum was excised in the position 1.5 cm away from the cecum. The enteric cavity was washed using the above methods, with the mucosal layer reserved. The ileum mucosa was ground through a glass grinder and filtered through a 200-mesh filter. The single-cell suspension was adjusted to the cell number of  $1 \times 10^6$ /mL. Flow cytometry was used to calculate the T lymphocyte percentage of CD4<sup>+</sup> and CD8<sup>+</sup> in intestinal mucosa, calculating the ratio of CD4<sup>+</sup>/CD8<sup>+</sup>.

TGF- $\beta$ 1 levels: The rats were intraperitoneally injected with 3 mL/kg 10% chloral hydrate at 7 days and 14 days after surgery. After anesthesia, the abdominal cavity was opened in a sterile environment for decollement of portal vein. A total of 1.5 mL of blood was drawn from portal veins through sterile injector and placed into the test tube. Centrifugation was then performed at a speed of 3,000 rpm for 10 minutes, aiming to extract the serum. The serum was then stored at a temperature of -70°C for later measurement. DAS-ELISA was used to measure TGF- $\beta$ 1 levels in strict accordance with instructions.

**Table 1.** Comparison of IBF between OG and CG at different time points after surgery ( $\bar{x} \pm s$ )

Group	Time	PCNA	Caspase-3 ( $\mu\text{g/ml}$ )	IVH ( $\mu\text{m}$ )
OG (n=20)	1 d after surgery	127.58 $\pm$ 10.13	157.89 $\pm$ 9.63	435.26 $\pm$ 58.31
	3 d after surgery	125.66 $\pm$ 15.24	159.83 $\pm$ 8.64	432.56 $\pm$ 55.27
	7 d after surgery	119.89 $\pm$ 12.34	160.27 $\pm$ 10.21	420.16 $\pm$ 40.28
SCG (n=20)	1 d after surgery	121.66 $\pm$ 17.79	168.34 $\pm$ 12.42	400.33 $\pm$ 86.92
	3 d after surgery	109.87 $\pm$ 13.53	191.25 $\pm$ 10.42	352.42 $\pm$ 58.79
	7 d after surgery	85.24 $\pm$ 13.29	211.78 $\pm$ 18.24	313.26 $\pm$ 62.27
CG (n=20)	1 d after surgery	134.42 $\pm$ 7.58	150.78 $\pm$ 12.81	434.42 $\pm$ 75.66
	3 d after surgery	132.63 $\pm$ 12.86	153.66 $\pm$ 9.69	452.27 $\pm$ 75.31
	7 d after surgery	130.26 $\pm$ 19.35	155.57 $\pm$ 9.31	457.89 $\pm$ 70.86
P1		0.012	<0.001	0.001
P2		0.001	0.008	0.003
P3		0.015	0.003	0.005

Note: P1 represents the *P* value obtained by comparison of 1 d after surgery of the three groups; P2 represents the *P* value obtained by comparison of 3 d after surgery of the three groups; and P3 represents the *P* value obtained by comparison of 7 d after surgery of the three groups.

### Statistical methods

SPSS22.0 was used for statistical analysis. Measurement data are represented by mean  $\pm$  standard deviation. Independent-samples *t*-tests were used for data in conformity with normal distribution. Mann-Whitney U-tests were used for data not in conformity with normal distribution. Comparisons within groups were conducted through paired-samples *t*-tests. Enumeration data are represented by [n (%)] and was compared through  $\chi^2$  tests between groups.  $P < 0.05$  indicates statistical significance.

### Results

#### Comparison of IBF between OG and CG

PCNA levels of CG were much higher those that of OG and SCG at 1 day, 3 days, and 7 days after surgery ( $P < 0.05$ ). Levels of OG were much higher than those of SCG at 1 day, 3 days, and 7 days after surgery ( $P < 0.05$ ). There was little difference in PCNA levels of CG at 1 day, 3 days, and 7 days after surgery ( $P > 0.05$ ). PCNA levels at 3 days and 7 days after surgery were much lower than those at 1 day after surgery, respectively, in OG and SCG ( $P < 0.05$ ). There was little difference in Caspase-3 levels at 1 day, 3 days, and 7 days after surgery, respectively, in OG and CG ( $P > 0.05$ ). There were obvious differences in Caspase-3 levels at 1 day, 3 days, and 7 days after surgery in SCG ( $P < 0.05$ ). Caspase-3 levels of SCG were much higher than those of

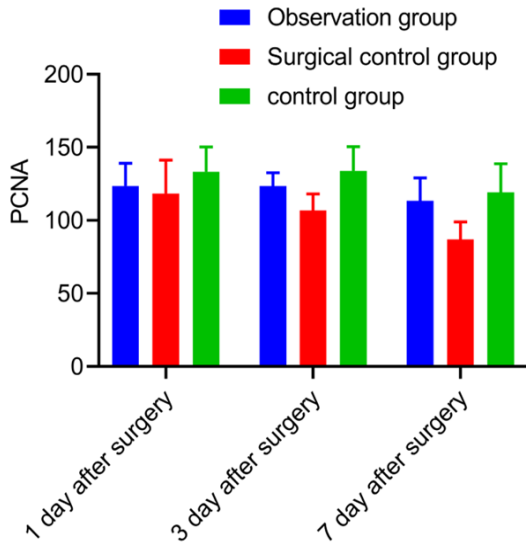
OG and CG at 1 day after surgery and levels of OG were higher than those of CG ( $P < 0.05$ ). Caspase-3 levels of SCG were much higher than those of OG and CG at 3 days and 7 days after surgery ( $P < 0.05$ ). IVH at 7 days after surgery was much lower than that at 1 day and 3 days after surgery in OG ( $P < 0.05$ ). There were obvious changes in IVH at 1 day, 3 days, and 7 days after surgery in SCG ( $P < 0.05$ ). IVH at 1 day after surgery was much lower than that at 3 days and 7 days after surgery in CG ( $P < 0.05$ ). IVH of SCG was much lower than that of OG and CG at 1 day, 3 days, and 7 days after surgery ( $P < 0.05$ ) (**Table 1; Figures 1-3**).

#### Comparison of IF between OG and CG

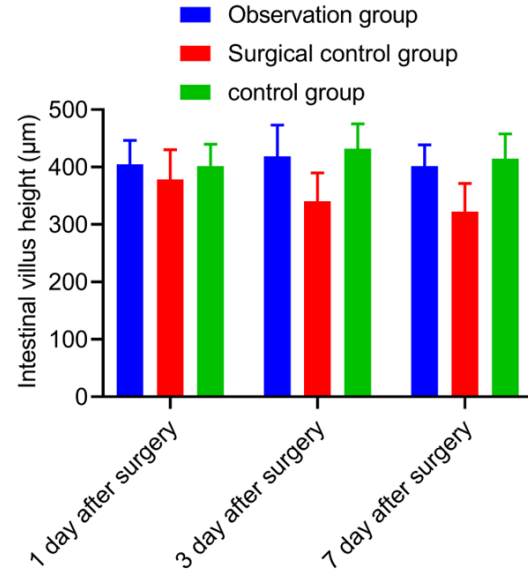
CD4<sup>+</sup> and CD4<sup>+</sup>/CD8<sup>+</sup> of OG and CG were higher than those of SCG and CD8<sup>+</sup> of OG and CG was lower than that of SCG at 1 day and 7 days after surgery ( $P < 0.05$ ). There were no statistical differences in CD4<sup>+</sup>, CD8<sup>+</sup> and CD4<sup>+</sup>/CD8<sup>+</sup> at 1 day and 7 days after surgery, respectively, in OG, CG, and SCG ( $P > 0.05$ ) (**Table 2**).

#### Comparison of changes in TGF- $\beta$ 1 levels between OG and CG

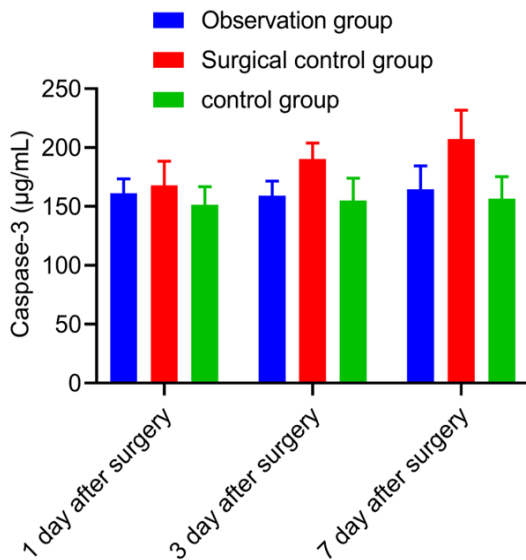
There were no obvious differences in TGF- $\beta$ 1 levels between OG and CG at 7 days and 14 days after surgery ( $P > 0.05$ ). TGF- $\beta$ 1 levels of SCG were much higher than those of OG and CG at 7 days after surgery ( $P < 0.05$ ). There was little difference in TGF- $\beta$ 1 levels at 7 days and



**Figure 1.** PCNA levels of the three groups at different time points after surgery. PCNA levels of CG were much lower than those of OG and SCG at 1 day, 3 days, and 7 days after surgery ( $P<0.05$ ). PCNA levels of OG were much higher than those of SCG at 1 day, 3 days, and 7 days after surgery ( $P<0.05$ ). There was little difference in PCNA levels of CG at 1 day, 3 days, and 7 days after surgery ( $P>0.05$ ). PCNA levels at 3 days and 7 days after surgery were much lower than those at 1 day after surgery, respectively, in OG and SCG ( $P<0.05$ ).



**Figure 3.** IVH of the three groups at different time points after surgery. IVH at 7 days after surgery was much lower than that 1 day and 3 days after surgery in OG ( $P<0.05$ ). There were obvious changes in IVH at 1 day, 3 days, and 7 days after surgery in SCG ( $P<0.05$ ). IVH at 1 day after surgery was much higher than that 3 days and 7 days after surgery in CG ( $P<0.05$ ). IVH of SCG was much lower than that of OG and CG at 1 day, 3 days, and 7 days after surgery ( $P<0.05$ ).



**Figure 2.** Caspase-3 levels of the three groups at different time points after surgery. There were unobvious changes in Caspase-3 levels at 1 day, 3 days, and 7 days after surgery, respectively, in OG and CG ( $P>0.05$ ) and obvious changes in SCG ( $P<0.05$ ). Caspase-3 levels of SCG were much higher than those of OG and CG at 1 day, 3 days, and 7 days after surgery ( $P<0.05$ ).

14 days after surgery, respectively, in OG and CG ( $P>0.05$ ). TGF-β1 levels at 14 days after surgery were much higher than those at 7 days after surgery in SCG ( $P<0.05$ ) (Table 3 and Figure 4).

**Discussion**

OJ occurs when the bile in intestinal tract cannot enter the biliary system due to partial or complete obstruction. This may be caused by different reasons [9]. Biliary calculus is an important cause for OJ. It is also affected by inflammation, congenital biliary obstruction, biliary stricture, iatrogenic bile duct injury, biliary tumors, ampullary carcinoma, and parasites [10, 11]. Surgery can achieve good effects on OJ. However, there is a higher risk of postoperative complications and case fatality rate. This is closely related to intestinal endotoxemia (IETM), mainly caused by microecological disorders of intestinal flora, cell dysfunction, lack of bile salt, and intestinal mucosal damage [12, 13]. Thus, it is of great significance to improve IMBF and IF levels for OJ patients through effective treatment methods.

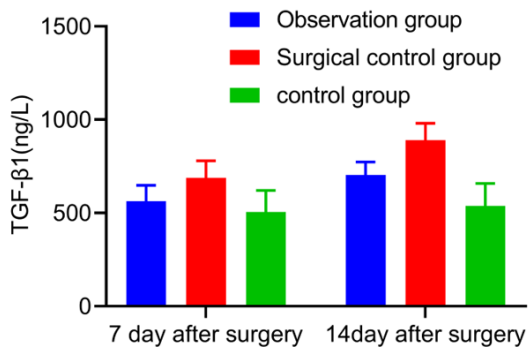
**Table 2.** Comparison of IF between OG and CG at different time points after surgery ( $\bar{x} \pm s$ )

Group	Time	CD4 <sup>+</sup> (%)	CD8 <sup>+</sup> (%)	CD4 <sup>+</sup> /CD8 <sup>+</sup>
OG (n=20)	1 d after surgery	49.06±15.27	7.52±2.38	5.89±1.37
	7 d after surgery	51.23±16.37	7.21±1.96	6.32±1.42
SCG (n=20)	1 d after surgery	33.62±13.28	10.85±3.27	2.68±1.06
	7 d after surgery	35.69±14.22	9.98±4.23	2.74±1.23
CG (n=20)	1 d after surgery	48.27±13.65	7.55±2.78	5.79±2.30
	7 d after surgery	45.36±15.37	7.20±1.96	5.82±1.63
P1	0.001	0.028	0.007	0.001
P2	<0.001	0.015	0.001	<0.001

Note: P1 represents the *P* value obtained by comparison of 1 d after surgery of the three groups; P2 represents the *P* value obtained by comparison of 7 d after surgery of the three groups.

**Table 3.** Comparison of changes in TGF-β1 levels at different time points after surgery ( $\bar{x} \pm s$ , ng/L)

Group	7 d after surgery	14 d after surgery
OG (n=20)	535.62±150.28	579.52±138.72
SCG (n=20)	775.62±168.49	923.57±118.27
CG (n=20)	546.39±138.52	559.62±105.41
P	0.001	0.013



**Figure 4.** TGF-β1 levels of the three groups at different time points after surgery. TGF-β1 levels of SCG were much higher than those of OG and CG at 7 days after surgery ( $P < 0.05$ ). There was little difference in TGF-β1 levels between OG and CG at 7 days and 14 days after surgery ( $P > 0.05$ ). TGF-β1 levels at 7 days after surgery were much lower than those at 14 days after surgery in SCG ( $P < 0.05$ ).

A single-chain polypeptide, GLP-2 is composed of 33 amino acids and is the product of proglucagon (PG) gene expression [14]. Some scholars found the promoting effects of GLP-2 on growth of specific intestinal mucosa in the late 20th century [15]. With a high conservation in mammals, GLP-2 is mainly secreted by intestinal L-cells. It is further decomposed by dipeptidyl peptidase IV (DPP-IV), finally excreted th-

rough the kidneys [16]. GLP-2 receptor can bring the biological effects into play. This receptor has a partial agonistic action on GLP-2, but without any response to other proglucagon-derived peptides [17]. GLP-2 receptor has a lot of expression in the gastrointestinal tract, which are distributed in jejunum, duodenum, ileum, the colon, and stomach, in sequence [18]. Matthew D J et al. verified that GLP-2 was combined with GLP-2 receptors, respectively, in intestinal mucosa, under mucosa, and on myenteric neuron. They also found that the neurons had a specific effect on progenitor cells of columnar epithelium through signal transduction. This could promote the proliferation of columnar epithelial cells, absorption of nutrients, and the growth, differentiation, and repair of cell population in intestinal crypt. These factors would maintain the permeability of intestinal mucosa [19].

PCNA levels are highly correlative with cell proliferation. These levels can reflect cell proliferation activity during cell division cycle and accurately judge cell proliferation [20]. With the effect of an apoptotic actuator, Caspase-3 protein is a crucial enzyme protein in inducing cell apoptosis in mammals [21]. OG was treated with GLP-2 in the present study. PCNA levels, Caspase-3 levels, and IVH of OG were better than those of SCG and in accord with those of CG. Results suggest that GLP-2 could protect the intestinal villi, delay the damage process, inhibit cell apoptosis in intestinal mucosa, and effectively improve IMBF. As the core of cell immunoregulation, such T lymphocytes as CD4<sup>+</sup> and CD8<sup>+</sup> can accurately reflect intestinal immune function by measuring the number and ratio of T lymphocyte subsets [22]. Mosawi SH et al. found the reduction of CD4<sup>+</sup> and increase

of CD8<sup>+</sup> after OJ and also a reduced ratio of CD4<sup>+</sup>/CD8<sup>+</sup> [23]. CD4<sup>+</sup>, CD8<sup>+</sup> and CD4<sup>+</sup>/CD8<sup>+</sup> of OG were better than those of SCG and in accord with those of CG at 7 days after surgery. Results suggest that GLP-2 effectively improved the IF of OJ rats.

TGF- $\beta$  is mainly secreted by blood platelets and macrophages as a multifunctional cytokine for cell growth and differentiation. It accounts for the largest proportion in somatic cells with the highest activity. It also has a variety of biological functions, such as IF, inflammation, cell growth and differentiation, tissue repair, and regulation of embryonic development [24]. TGF- $\beta$  levels of OG were much lower than those of SCG and in accord with those of CG at 7 days and 14 days after surgery. Results indicated that GLP-2 could accelerate epithelial healing after mucosal damage, quicken the neovascularization of impaired wounds, enhance the blood supply, and provide essential nutrients for histocytes [25].

In conclusion, GLP-2 obviously improves IBF and IF and reduces TGF- $\beta$ 1 levels in the treatment of OJ model rats. Thus, it has good application value. However, this was an animal experiment study with fewer indexes. Results may not be representative. More extensive and intensive studies should be conducted based on humans, aiming to provide more references for clinical treatment of OJ.

#### Acknowledgements

The present work was supported by the Natural Science Foundation of Jiangxi Province [grant number 20161BAB205255].

#### Disclosure of conflict of interest

None.

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