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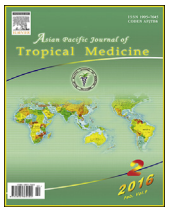

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Influence of lactulose on interventional therapy for HCC patients with hepatocirrhosis and hypersplenism

Deng-Wei Zong, Chen-Yang Guo, Hong-Tao Cheng, Hong-Tao Hu, Jin-Cheng Xiao, Hai-Liang Li*

Department of Radiology Intervention, Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou 450008, China

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ABSTRACT

Objective: To investigate the influence of lactulose on immunity of hepatocellular carcinoma (HCC) patients with hepatocirrhosis and hypersplenism after double-interventional therapies.

Methods: A total of 40 HCC patients with hepatocirrhosis and hypersplenism, hospitalized during January 2013 to June 2014, were enrolled and randomized into control group and observation group. Both groups received partial splenic embolization combined with transcatheter arterial chemoembolization. Besides, observation group orally took lactulose 30 mL/d. Four days before interventional therapies and at days 1, 3, 7 and 14 after therapies, fasting venous blood was collected to detect white blood cell count, red blood cell count (RBC), and platelet count (PLT). Four days before therapies and at days 7 and 14 after therapies, the levels of alanine aminotransferase, aspartate transaminase, total bilirubin, malondialdehyde, super-oxide dismutase (SOD), IFN-γ, and IL-4 as well as the distribution of T cell subsets in peripheral blood were tested. Complications were observed after interventional therapies.

Results: Before interventional therapies the levels of white blood cell count, PLT and RBC in both groups showed no difference, while after interventional therapies the levels of PLT and RBC in both groups showed an increasing tendency ($P < 0.05$). At day 14 after interventional therapies, the level of blood cell as well as that of SOD, IFN-γ and IL-4 in serum were significantly higher than that before therapies; meanwhile, the levels of alanine aminotransferase and total bilirubin of observation group after therapies were significantly lower than before and control group ($P < 0.05$), the levels of $CD4^+/CD8^+$, SOD and IFN-γ were all higher than before and control group ($P < 0.05$).

Conclusions: Oral administration of lactulose could adjust the imbalance of oxidation system/antioxidant system in HCC patients with hepatocirrhosis and hypersplenism after interventional therapies, and improve the antitumor immunity and prognosis.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common types of malignant liver cancer with high morbidity and mortality. In the past decades, the global morbidity of HCC has been on a sharp upward trend; the morbidity of HCC in United States is even twice as much as the average level of the world [1]. As most patients are already at the advanced stage when HCC is diagnosed, the tumors are often inoperable. Therefore, palliative therapy is the principle therapeutic method of HCC. Among all the palliative therapies, transcatheter arterial chemoembolization (TACE) is most frequently adopted. It can significantly increase survival of HCC patients with unresectable tumors [2–4]. It's reported that about 80% of HCC patients have hepatocirrhosis, because HCC mostly develops from hepatocirrhosis or liver fibrosis. In addition, hepatocirrhosis usually also causes portal hypertension and hypersplenism. Recent studies have shown that partial splenic embolization (PSE) can permanently improve thrombocytopenia and hypersplenism caused by portal hypertension [5].

*Corresponding author: Hai-Liang Li, Department of Radiology Intervention, Affiliated Cancer Hospital of Zhengzhou University, No. 127 Dongming Road, Zhengzhou, China.
Tel: +86 13903861969
E-mail: cjr.lihailiang@vip.163.com

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As an oral disaccharide, lactulose can promote nitrogen balance and excretion; therefore, it is widely used in the treatment of hepatic encephalopathy and constipation [6,7]. The pharmacokinetic characteristics of lactulose make it an effective and safe option for the treatment of such indications. However, many of its potential effects are still unknown. In this study, HCC patients with hepatocirrhosis and hypersplenism received lactulose orally, to investigate the influence of lactulose on the efficacy of interventional therapy and antioxidant system, thus providing theoretical basis for the comprehensive treatment of the disease.

2. Materials and methods

2.1. General data

A total of 40 HCC patients with hepatocirrhosis and hypersplenism, hospitalized during January 2013 to June 2014, were chosen as subjects. The diagnosis of HCC fits in with relevant guidelines [8], and the diagnosis of hypersplenism should fit in with the following criteria: type-B ultrasound or CT scan shows hepatocirrhosis, spleen pachydiameter >4.1 cm, class I splenomegaly, white blood cell count (WBC) $<3 \times 10^9$/L, and platelet count (PLT) $<100 \times 10^9$/L [9]. The liver condition of all patients was either Child–Pugh A or Child–Pugh B. No patient had taken immunomodulator within 30 d before the treatment.

The patients were randomly divided into control group and observation group, with 20 patients in each group. Double intervention with TACE and PSE began after no patient was found contraindications.

2.2. Therapeutic methods

In case there might be ascites, sufficient support therapy was given to all patients before the intervention. During the procedure, all patients fitting in with the inclusion criteria received PSE first, and then TACE. PSE procedures: The catheter was inserted into the femoral artery via Seldinger method, following with abdominal angiogram and selective splenic arteriography to observe the distribution of splenic artery and routes of collateral circulation. Then, the tip of the catheter was placed at the splenic hilum and the gelatin sponge was suspended in the contrast media to form embolization; the scope of splenic embolization was assessed by angiogram. When a 40%–60% ablation of the splenic parenchyma was obtained, the embolization was terminated. After the PSE procedure, TACE was performed under aseptic condition: the catheter was inserted into the femoral artery as in the PSE procedure, following with abdominal angiogram and selective splenic arteriography to observe tumor blood-supply, the distribution of hepatic artery and routes of collateral circulation. The tip of the catheter was placed at the feeding artery of the tumor and embolization was performed using an emulsion mixture of lipoidal ultra-fluid, perarubicin and DDP (the dose of embolization depends on tumor size, blood-supply and hepatic function of the patient). When the tumor was filled well with emulsifier, the embolization was terminated.

In addition, from day 3 before the interventional therapies to day 14 after therapies, the observation group took lactulose orally; the control group took placebo. For the observation group, lactulose syrup was administered as 30 mL/d in divided doses for a stool frequency of two to three soft defecations per day.

2.3. Observation items

Clinical symptoms and complications were observed after the interventional therapies. Four days before interventional therapies and at days 1, 3, 7 and 14 after them, fasting venous blood was collected to detect WBC, red blood cell count (RBC), and PLT. Four days before interventional therapies and at days 7 and 14 after them, levels of alanine aminotransferase (ALT), aspartate transaminase (AST) and total bilirubin (TBil) were tested; meanwhile, peripheral venous blood was collected for measuring levels of $CD4^+$ T cell and $CD8^+$ T cells by flow cytometry, in order to obtain the ratio of $CD4^+/CD8^+$.

Four days before interventional therapies and at days 7 and 14 after them, fasting blood sample was collected; thiobarbituric acid chromatometry was used to assay the content of malondialdehyde (MDA) in blood, xanthin oxidase method was used to assay the content of super-oxide dismutase (SOD), and ELISA was used to detect the levels of IFN-γ and IL-4 in serum. All reagent kits were bought from Shijiazhuang Huarui Biotech R&D Center.

2.4. Data statistics

SPSS 16.0 software was used for statistical analysis and all the data was represented by mean ± SD. The paired *t*-test was used to compare the indexes of the same group before and after the procedure; the independent *t*-test was used to compare the indexes of two groups; enumeration data was compared by χ^2 test. When $P < 0.05$, the differences were considered statistically significant.

3. Results

3.1. Levels of peripheral blood cell and transaminase after interventional therapies

After interventional therapies, WBC content and PLT content of both groups showed significant increase compared with the preoperative content ($P < 0.05$); at day 14 after interventional therapies, levels of ALT and TBil of the observation group patients were higher than preoperative levels and those of control group ($P < 0.05$); concerning the RBC and AST level, the post-treatment level in both groups showed no significant difference compared with the preoperative level (Table 1).

After the therapies, both groups showed a slowly rising trend on the percentage of $CD4^+$ T cell, but a slowly decreasing trend on the percentage of $CD8^+$ T cell; at day 14 after the therapies, the ratio of $CD4^+/CD8^+$ in the observation group was significantly higher than that before therapies ($P < 0.05$).

3.2. Levels of SOD, MDA, IFN-γ and IL-4 before and after interventional therapies

According to Table 2, at day 14 after interventional therapies, levels of SOD, IFN-γ and IL-4 in both groups increased significantly, while the observation group showed higher levels of SOD and IFN-γ than the control group ($P < 0.05$). At day 14 after therapies, MDA content of the observation group was significantly lower than that before therapies ($P < 0.05$); however, there was no significant change in MDA content of the control group ($P > 0.05$).

Table 1
Blood cells and aminotransferase contents at different time points.

Groups		WBC level ($\times10^9$/L)	PLT level ($\times10^9$/L)	RBC level ($\times10^{12}$/L)	ALT (U/L)	AST (U/L)	TBil (μmol/L)
Control group	Pre Op	2.57 ± 0.61	52.81 ± 7.44	3.22 ± 0.51	54.43 ± 17.62	52.06 ± 17.08	26.61 ± 9.55
	POD 1	4.32 ± 1.47*	63.04 ± 9.20	3.26 ± 0.48	–	–	–
	POD 3	7.72 ± 1.32*	76.54 ± 10.85*	3.31 ± 0.67	–	–	–
	POD 7	6.85 ± 0.92*	117.07 ± 14.35*	3.42 ± 0.71	54.75 ± 14.63	51.52 ± 15.93	26.48 ± 10.11
	POD 14	6.04 ± 0.82*	148.73 ± 17.50*	3.39 ± 0.55	55.02 ± 17.21	51.22 ± 15.04	25.74 ± 8.96
Observation group	Pre Op	2.59 ± 0.52	54.02 ± 9.21	3.19 ± 0.47	54.78 ± 15.40	51.43 ± 16.45	25.94 ± 8.54
	POD 1	4.97 ± 0.80*	71.25 ± 11.62	3.37 ± 0.62	–	–	–
	POD 3	8.54 ± 1.26*	89.20 ± 7.26*	3.62 ± 0.42	–	–	–
	POD 7	7.69 ± 1.03*	137.51 ± 6.57*	3.88 ± 0.56	50.11 ± 16.55	45.30 ± 13.82	22.14 ± 7.23
	POD 14	6.95 ± 1.22*	155.94 ± 18.93*	3.71 ± 0.61	41.33 ± 15.74*,#	40.66 ± 10.95	18.22 ± 6.90*,#

Note: Pre Op: pre-operation; POD: postoperative day. * *vs.* Pre Op, $P < 0.05$; # *vs.* control group, $P < 0.05$.

Table 2
Levels of serum antioxidants and cytokines at different time points before and after interventional therapies.

Groups		SOD (U/mL)	MDA (nmol/mL)	IFN-γ (pg/mL)	IL-4 (pg/mL)
Control group (n = 20)	Pre Op	168.57 ± 14.98	6.72 ± 2.01	4.37 ± 0.94	5.92 ± 1.94
	POD 7	207.35 ± 18.71*	5.89 ± 1.74	5.22 ± 1.20*	6.81 ± 2.15
	POD 14	218.40 ± 20.34*	5.78 ± 1.65	5.65 ± 1.54*	7.32 ± 2.21*
Observation group (n = 20)	Pre Op	169.34 ± 16.30	6.68 ± 1.69	4.32 ± 0.97	5.87 ± 1.82
	POD 7	219.64 ± 16.94*	5.75 ± 1.33	5.67 ± 1.31*	6.99 ± 2.30
	POD 14	269.04 ± 22.17*,#	5.47 ± 1.82*	6.78 ± 1.72*,#	7.67 ± 2.46*

Pre Op: pre-operation; POD: postoperative day. * *vs.* Pre-operation, $P < 0.05$; # *vs.* control group, $P < 0.05$.

3.3. Incidence of complications after interventional therapies

After interventional therapies, patients of both groups suffered pain, fever, nausea and vomiting, and left subdiaphragmatic effusion. However, in generally, two groups showed no difference in complications incidence ($P > 0.05$).

4. Discussion

In clinic, lactulose is mainly used in the treatment of constipation and hepatic encephalopathy. In fact, its effects are far more than that. It can also reduce endotoxin, affect trace element metabolism, regulate intestinal flora balance, affect the circulation of bile acid, resist oxidation, and strengthen immunity and so on. In the study, all patients received double-interventional therapies. Moreover, lactulose was given to patients of observation group to observe the influence of lactulose on therapeutic effect. Our results showed that the observation group and the control group showed no difference in the levels of blood cell and transaminase after the therapies and also in the postoperative complications incidence. However, levels of antioxidant enzyme and cytokines were more significantly improved in patients having taken lactulose than in those having not ($P < 0.05$).

Hepatocirrhosis is the advanced stage of chronic liver disease, which is a result of various repeated or long-term damages to the liver. In China, hepatocirrhosis is mainly caused by viral hepatitis and develops from liver fibrosis [10]. Although hepatocirrhosis is the result of many factors, the role of oxidative stress should not be ignored, which influences the development of hepatocirrhosis from various aspects [11,12]. It has been verified that the reactive oxygen species (ROS) can induce lipid peroxidation leading to inflammation and fibrogenesis through the activation of stellate cells [13]. In addition, ROS is the critical mediator of liver fibrosis, and it also contributes to hepatic fibrosis from various kinds of liver injuries [12,14]. SOD is an antioxidant enzyme that constitutes the first line of defense against ROS. It reflects the body's ability and degree of reducing free radical. MDA represents the change of ROS content and the degree of lipid peroxidation and indirectly reflects the degree of ROS's damage to tissues. Joint detection of MDA and SOD can indicate the body's condition of oxidation and peroxidation.

This study found that lactulose can significantly improve patients' postoperative level of SOD and meanwhile inhibit MDA content, which indicated that lactulose can improve the curative effect of interventional therapy by reducing oxidative stress. Yu *et al.* [15] investigated the potential influence of lactulose administration on liver regeneration, and they concluded that lactulose administration accelerates posthepatectomized liver regeneration in rats by inducing hydrogen, which may result from attenuation of the oxidative stress response and excessive inflammatory response. In the mouse model of human ulcerative colitis, Chen *et al.* [16] found that lactulose can prevent the development of DSS-induced colitis and alleviate oxidative stress in the colon, probably by increasing endogenous H_2 production. In a word, lactulose can eliminate free radicals, adjust the imbalance between oxidation system and antioxidant system, and alleviate liver injury.

Cellular immunity often plays an important role in tumor immunity. When the body has strong antitumor immunity, there is mainly the secretion of Th1 cytokines; while there is mainly the secretion of Th2 cytokines, the body's antitumor immunity is inhibited. IFN-γ and IL-4 are representative cytokines respectively produced by Th1 cells and Th2 cells. The study showed that at day 14 after interventional therapies, the level of IFN-γ in observation group was significantly higher than that in control group, while the two groups showed no difference in the level of IL-4, which suggested that Th1/Th2 cell polarization evidently

shifted to Th1 cells, and patients' antitumor immunity was significantly enhanced after the administration of lactulose. Vendemiale *et al.* [17] also found the effect of activation on cell-mediated immune system depressed during liver cirrhosis, produced by lactulose. There are many other studies having confirmed the positive role of lactulose on the immune defense and immune regulation [18,19]. However, further studies are still needed to confirm and clarify the possible mechanisms involved.

In summary, oral administration of lactulose could adjust the imbalance between the oxidation system and the antioxidant system of HCC patients with hepatocirrhosis and hypersplenism after interventional therapies, alleviate liver injury, improve the antitumor immunity and prognosis.

Conflict of interest statement

We declare that we have no conflict of interest.

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