Tropical Journal of Pharmaceutical Research August 2021; 20 (8): 1705-1710
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v20i8.22

Original Research Article

Xuebijing injection alleviates liver injury in patients with hepatocellular carcinoma by inhibiting inflammatory response after transarterial chemoembolization

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Sent for review: 20 April 2021 *Revised accepted: 27 July 2021*

Abstract

Purpose: *To study the influence of Xuebijing (XBJ) injection on patients with hepatocellular carcinoma (HCC) after transarterial chemoembolization (TACE), and to elucidate the underlying mechanism of action.*
Methods: *Thirty-nine HCC patients were retrospectively analyzed. They were assigned to 3 groups: negative group which received no XBJ injection, low-dose XBJ group given XBJ injection (50 ml), and high-dose XBJ group given 100 mL of XBJ injection. Peripheral blood was separately collected and analyzed on the first day after admission and TACE.*
Results: *TACE led to liver damage in HCC patients, with increased serum activities of ALT and AST, and total bilirubin (tBLB). Moreover, TACE elevated white blood cells, neutrophil count, C-reactive protein (CRP) and expression levels of inflammation cytokines. In the groups treated with XBJ injection, there were dose-dependent mitigation of liver dysfunction, and reduced levels of inflammatory cytokines, when compared with the negative group. However, XBJ injection did not affect myelosuppression or regulatory T cells.*
Conclusion: *XBJ dose-dependently decreases liver injury in HCC patients after TACE by suppressing inflammatory response. Thus, XBJ may exert hepatoprotective effect on HCC after TACE in humans in clinical practice.*

Keywords: *Xuebijing injection, Transarterial chemoembolization (TACE), Hepatocellular carcinoma, Inflammation*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common malignant cancer in the world, and it has a poor prognosis. The main curative strategies for HCC include liver resection, transplantation, radiofrequency ablation and transarterial chemoembolization (TACE) [1]. The TACE procedure is recommended as an efficient therapy for HCC so as to alleviate local tumor growth, prolong survival, inhibit tumor recurrence, palliate symptoms and bridge the time to liver transplantation [2-4]. The TACE procedure relies on the transport of anticancer

agents to the targeted area, followed by blocking of hepatic blood vessels by embolic particles, resulting in cancer cell ischemia and necrosis [5]. In addition, cancer cell injury can trigger inflammation. Furthermore, TACE may exacerbate the already vulnerable liver function of HCC patients, leading to accentuation of liver damage. The incidence of acute hepatic failure after TACE is 5 - 20 % [6-8]. However, the associated mortality may reach 60 - 80 %. In this study, some serological parameters were measured to assess liver function and the levels of pro-inflammatory cytokines. These parameters were serum transaminases, bilirubin, TNF-α and IL-6.

Xuebijing (XBJ) injection is a traditional Chinese herbal preparation from five Chinese herbs: safflower, red peony root, Chinese angelica, *Radix Salviae* Miltiorrhizae and *Szechuan Lovage* Rhizome. The XBJ injection improves microcirculation, alleviates oxidative stress, inhibits inflammation, and modulates immune response [9,10]. The injection was approved in 2004 by the National Medical Product Administration, and it is widely used for treating sepsis [9,11]. However, the effect of XBJ injection on HCC patients with TACE has not been investigated.

METHODS

Patients and methods

Sixty HCC cases were retrospectively analysed between September, 2019 and November, 2019 at the Hepatobiliary Centre of the First Affiliated Hospital of Nanjing Medical University (Table 1). The standard of diagnosis for HCC was in line with the guidelines of the American Association for the Study of the Liver. The study followed the ethical guidelines of the 1975 Declaration of Helsinki [12], and was approved by the First Affiliated Hospital of Nanjing Medical University (approval no. NJMU201902). The inclusion criteria in this research were as follows: Child-Pugh stage A or B, TACE as initial therapy and monotherapy, and unresectable HCC.

The TACE protocol was implemented by selectively placing a catheter through the femoral artery into the hepatic artery, and then injecting a mixture of iodized oil (5-10ml) (Yantai Luyin Pharmaceutical Co. Ltd, China) and lobaplatin (30 mg/m^2; Hainan Changan International Pharmaceutical Co. Ltd, China). The HCC patients in the intervention groups received XBJ injection (Tianjin Chase Sun Pharmaceutical Co. Ltd, China) at a dose of 50 ml or 100 ml for two days (Figure 1).

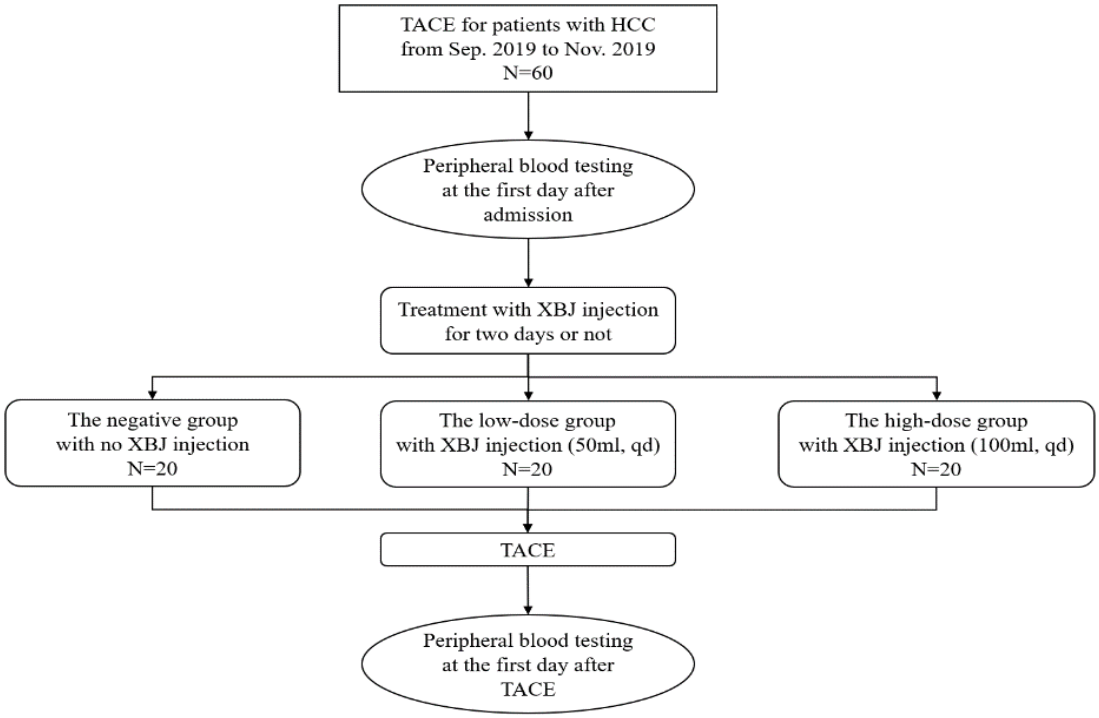

Figure 1: Scheme showing research protocol used. HCC = hepatocellular carcinoma; TACE = transarterial chemoembolization; XBJ = *Xuebijing*

Table 1: Clinical characteristics of the HCC patients

Characteristic	Negative control group	Drug group		*P*-value
		Low-dose	*High-dose*	
Case	20	20	20	-
XBJ injection	-	5-0 ml	100 ml	-
Age (years)	60.6 (42-74)	62.9 (42-78)	60.4 (47-74)	0.384
Sex (male/female)	18/2	17/3	14/6	0.235
Child-Pugh A vs. B	10/10	12/8	9/11	0.627

Data for gender and Child-Pugh stage were analyzed by Chi-square test; data for mean age were analyzed by ANOVA

Technical information

Peripheral blood was collected on the first day after admission and TACE, and the blood samples were subjected to analysis at the clinical laboratory of the First Affiliated Hospital of Nanjing Medical University. Serum alanine aminotransferase (sALT) and sAST activities, and levels of tBIL, white blood cells (WBCs), neutrophil count (NE), red blood cells (RBCs), platelets (PLTs) and C-reactive protein (CRP) were measured using an automated chemical analyzer (Olympus Automated Chemistry Analyzer AU5400, Tokyo, Japan). Pro-inflammatory cytokine levels were assayed with enzyme-linked immunosorbent assay (ELISA) kits (Beyotime, China), while the level of Tregs was measured using a flow cytometer (BD Bioscience, USA).

Statistical analysis

Results are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was employed for comparison of the different groups using SPSS software. Values of $p < 0.05$ were taken as indicative of significant differences.

RESULTS

Hepatocellular carcinoma (HCC) affected liver function, as reflected in changes in the levels of sALT, sAST and tBIL. Moreover, liver function was worsened by TACE. However, XBJ injection attenuated liver injury, as was evident in results from the different groups. It was also found that XBJ injection relieved liver damage, and its protective effect was dose-dependent. These results are shown in Figure 2.

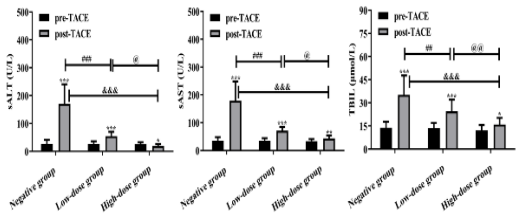

Figure 2: Effect of XBJ injection on liver. *The levels of sALT, sAST and tBIL were assayed pre-TACE and post-TACE, as indices of hepatic function. *P<0.05, **p < 0.01, ***p<0.001, vs. pre-TACE in different groups; ##p < 0.01, ###p < 0.001, &&&p<0.001, vs. post-TACE in negative group; @p<0.05, @@p < 0.01, vs. post-TACE inc low-dose group*

The results also showed that XBJ injection alleviated liver inflammation. It was found that WBC, NE, CRP, TNF-α and IL-6 were markedly up-regulated after TACE. The TACE procedure led to liver injury by blocking the hepatic artery, delivering antineoplastic drugs, and then amplifying inflammation during treatment. However, XBJ injection inhibited inflammation in a dose-dependent manner. In the high-dose group, the levels WBC, NE, CRP, TNF-α and IL-6 were lowest amongst all the groups (Figure 3). It is known that chemotherapy and contrast medium may lead to myeloproliferative disorders [13,14]. However, the XBJ injection did not ameliorate myeloproliferative disorders. The populations of RBCs and platelets were markedly decreased by TACE treatment, except for WBC. These results are presented in Figure 4. As shown in Figure 5, XBJ injection had no effect on level of Tregs. Studies have shown that Tregs play an important role in HCC because they protect the viability of cancer cells [15,16]. However, in this study, there were no obvious differences in levels of Tregs between the negative control and drug groups. On the other hand, TACE decreased the level of Tregs (Figure 5).

DISCUSSION

The present research has demonstrated that XBJ injection dose-dependently protected the liver from TACE by inhibiting inflammation. However, XBJ injection did not affect the level of Tregs. In addition, the antineoplastic drugs and contrast medium given via TACE disturbed myeloproliferative functions, but XBJ injection did not mitigate this condition.

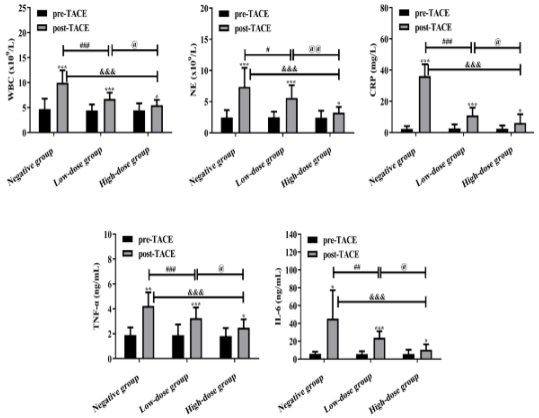

Figure 3: Effect of XBJ on inflammation. *The levels of white blood cells (WBCs), neutrophil count (NE), C-reactive protein (CRP), TNF-α and IL-6 were determined in the different groups. *P < 0.05, **p < 0.01, ***p < 0.001, vs. pre-TACE in different groups; ##p<0.01, ###p<0.001, &&&p < 0.001, vs. post-TACE in negative group; @p<0.05, @@p<0.01, vs. post-TACE in low-dose group*

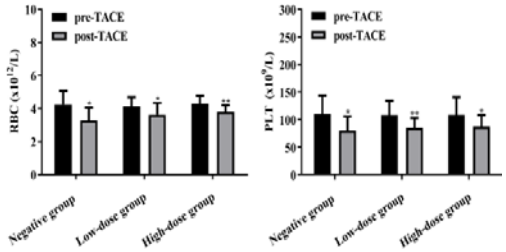

Figure 4: Effect of XBJ injection on myeloproliferative disorders. *The effect of XBJ on the number of red blood cells (RBCs) and platelets (PLT) was determined in the study. *P < 0.05, **p < 0.01, vs. pre-TACE in different groups*

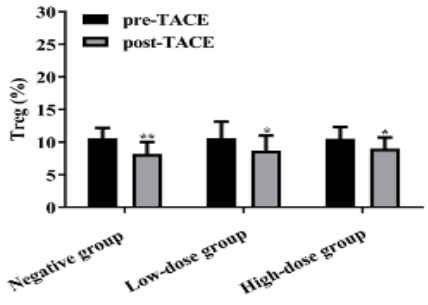

Figure 5: Effect of XBJ injection on levels of Tregs. *The level of Tregs was decreased in all groups after TACE treatment. *P < 0.05, **p < 0.01, vs. pre-TACE in different groups*

There is a high incidence of HCC in China, which accounts for half of the number of HCC cases worldwide. The high-risk population is within the age range of 55 to 65 years. However, there is an increasing trend in the incidence of HCC with young people [17]. It is unfortunate that most HCC patients are diagnosed at advanced stage, thereby losing the opportunity for surgery [18]. However, for these patients, other treatments e.g., radiofrequency ablation, chemotherapy and TACE, are used. The TACE procedure is recommended by the Barcelona Clinic Liver Cancer (BCLC) staging system for patients with intermediate-stage HCC. The procedure has been shown to lengthen median survival of patients [19,20]. The effect of TACE is dependent on chemotherapeutic and embolic agents delivered to the tumor through its abundant blood supply. In conventional TACE, a chemotherapeutic agent and lipiodol are transported to the tumor via a catheter placed in the artery. Ultimately, TACE leads to an ischemic/hypoxic microenvironment, focal angiogenesis, partial tumor necrosis and regional inflammatory reactions [21,22]. The chemotherapeutic agent used in our department is lobaplatin. Compared with systemic chemotherapy, TACE decreases the side effects of cytotoxic chemotherapy [21]. The TACE treatment is beneficial for HCC patients. However, it also compromises liver function. In this study, it was revealed that TACE had effect on the levels of sALT, sAST and TBIL, due to deterioration of hepatic function. Besides, TACE resulted in myelosuppression [14]. These results are related to the chemotherapy drug, embolism of blood vessels, and inflammation caused by the death of the tumor cells and liver ischemia/reperfusion injury.

It has been revealed that the bioactive constituents of XBJ injection are ferulic acid, ligustrazine, paeoniflorin, carthamin yellow A, tanshinol and protocatechualdehyde [23]. It has been reported that XBJ injection is used clinically for treating sepsis and multiple organ dysfunction syndrome [24]. The protective effect of XBJ injection on blood circulation is due to attenuation of *blood stasis* and elimination of toxins. Moreover, XBJ injection downregulates proinflammatory cytokines and mitigates oxidative stress. It alleviates liver injury by suppressing hyperactive inflammation and reducing serum levels of ALT, AST and TBIL following liver surgery [10]. In this study, it was found that XBJ injection ameliorated hepatic function and inhibited proinflammatory factors, which are consistent with previous findings. Some studies have demonstrated that TACE affects the level of Tregs [16,25]. In this study, there was also a relationship between Tregs and TACE. However, XBJ injection had no effect on the levels of Tregs.

CONCLUSION

The findings of this study show that although TACE is beneficial to HCC patients, it also inevitably compromises liver function, primarily as a result of inflammation. The results obtained suggest that XBJ injection may alleviate hepatic lesion through suppression of inflammatory response. However, the injection did not affect myelosuppression or Treg levels. Thus, XBJ injection should be applied with caution in HCC patients, mostly as a last resort.

DECLARATIONS

Acknowledgement

This study was supported by the Natural Science Foundation for Young Scientists of Jiangsu Province, China (Grant no. BK20171084).

Conflict of interest

No conflict of interest is associated with this work.

Authors' contribution

We declare that this work was performed by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Chen Zhong and Feng Cheng designed the study, supervised the data collection, and analyzed the data. Chen Zhong interpreted the data and prepared the manuscript for publication. Feng Cheng supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

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