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Original Research Article

Effect of a combination of Xiaochaihu decoction and teprenone on peripheral blood T lymphocytes in chronic atrophic gastritis, and on expression of COX-2 in gastric mucosa

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Abstract

Purpose: *To study the effects of the combined use of Xiaochaihu decoction and teprenone on peripheral blood T lymphocytes in chronic atrophic gastritis (CAG) and gastric mucosal expression of cyclooxygenase-2 (COX-2).*
Methods: *Patients with CAG who were treated at Traditional Chinese Medicine Hospital of Jiaxing from January 2017 to January 2018 were used as subjects. They consisted of observation and control groups (99 patients per group). Both groups were treated with teprenone (50 mg, thrice daily), but patients in the observation group received 200 mL of Xiaochaihu decoction, in addition to teprenone. The treatments were given orally, and lasted for 3 weeks. Comparisons were made between the two groups with respect to the effects of the treatments on peripheral blood T lymphocytes, CAG, and quality of life.*
Results: *Peripheral T lymphocytes, chronic atrophic gastritis and quality of life in patients in the observation group were significantly improved, relative to control group patients. The combined treatment led to a significant decrease in the expression of COX-2. After treatment, there was upregulation in $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ levels in both groups, relative to the corresponding levels prior to drug exposure ($p < 0.05$). However, the observation group levels of $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ were higher than corresponding control values ($t = -14.45$, $p < 0.001$; $t = -12.47$, $p < 0.001$; $t = -3.49$, $p < 0.001$, respectively). Moreover, results from histopathological studies showed marked improvement in the observation group.*
Conclusion: *A combination treatment of Xiaochaihu decoction and teprenone improves the condition of CAG patients via changes in peripheral blood lymphocytes and COX-2 expression in gastric mucosa.*

Keywords: *Xiaochaihu decoction, Teprenone, Chronic atrophic gastritis, T lymphocytes, Cyclooxygenase-2*

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INTRODUCTION

Chronic atrophic gastritis (CAG) is caused by various factors. The typical clinical manifestations are dull pain in abdomen and a feeling of fullness or belching. It is a common digestive system disease characterized by atrophy of gastric epithelial tissue and glands,

attenuated mucosa, and thickening of mucosal muscle layer [1]. It is often accompanied by intestinal metaplasia, erosion, and abnormal hyperplasia. Studies have shown that CAG is positively correlated with the incidence of gastric cancer [2].

Gastric cancer usually develops in stages, from superficial gastritis to atrophic gastritis, and thereafter to gastric mucosal epithelial hyperplasia, then to gastric mucosal intraepithelial neoplasia. This last stage gives rise to gastric cancer. Currently, the methods used for treating CAG in the clinics are chemotherapy and Chinese Materia Medica [3]. Traditional Chinese medicine (TCM) has produced good clinical results in the treatment of CAG [4, 5]. Although the combination of *Xiaochaihu* decoction and teprenone in the treatment of CAG has yielded certain beneficial effects, the mechanism involved in this treatment is still unclear [6].

It is known that in CAG, T lymphocyte sub-group in peripheral blood, and COX-2 in gastric mucosa in CAG patients are abnormally expressed. In addition, COX-2 is implicated in the etiology of gastric cancer. Indeed, the interaction between *H. pylori* infection, COX-2 and positive expression of p53 may enhance gastric cancer incidence [7]. It has also been reported that CD3+, CD4+ and CD4+/CD8+ levels are decreased, whereas the levels of pro-inflammatory cytokines are increased in gastric cancer patients.

The immune function of cancer patients is compromised, and this is aggravated by deepening of the degree of mucosal lesion [8]. It has been found that CD3+ cells are useful for assessing the degree of lesion in gastritis and the effectiveness of treatment, while providing new therapeutic ideas [9]. No studies have been carried out so far on the effect of combination of *Xiaochaihu* decoction and teprenone on T lymphocyte subgroup and COX-2 levels in CAG patients. The aim of this study was to investigate the influence of combination of *Xiaochaihu* decoction and teprenone on peripheral blood lymphocytes, as well as its effect on the expression of COX-2 in CAG patients.

METHODS

Study subjects

One hundred and ninety-eight CAG patients treated in Traditional Chinese Medicine Hospital of Jiaxing from January 2017 to January 2018 were used as subjects. They were divided into two groups: control group and observation (99 cases per group). The general conditions of patients in the two were comparable. This research was approved by the Ethical Committee of Traditional Chinese Medicine Hospital of Jiaxing (no. 20175619), and conducted according to the guidelines of Declaration of Helsinki promulgated in 1964 as amended in 1996 [10].

Inclusion criteria

Patients who were diagnosed with CAG through gastroscopy and pathological biopsy, with manifest clinical symptoms such as distended upper abdomen, belching, acid regurgitation and nausea were included in the study. Their ages ranged from 21 to 67 years.

Exclusion criteria

The excluded patients were those who received antibody or anti-Hp four weeks before hospital admission, and patients with other digestive system diseases or kidney dysfunction and severe heart dysfunction, as well as patients who took other medicines for treating gastritis.

Treatment methods

Each patient in the control group was given 50 mg teprenone (Eisai China Inc., SFDA approval number = H20093656) medication after meals, three times daily. One treatment course was of 7-day duration, and the treatment lasted for 3 weeks. Patients in the observation group were each given the same teprenone treatment, in addition to 200 mL of *Xiaochaihu* decoction twice daily. The water decoction was prepared from *Radix bupleuri* (12 g) *Secutellaria baicalensis* (12 g), ginseng (9 g), *Pinellia ternate* (9 g), *Radix glycyrrhizae preparata* (6 g), ginger (6 g) and *Jujube* (4 g).

Peripheral blood T lymphocyte subgroup

After an overnight fast, T lymphocytes in peripheral blood samples were assayed using flow cytometric techniques.

Gastroscopic assessment of treatment effectiveness

Gastroscopic observation was conducted before and after treatment. Endoscope scores were recorded according to observed conditions such as gastric mucosa congestion, edema, erythema, erosion and inflammatory cell infiltration. The scores were categorized as follows: 0 point for normal gastric mucosa; 1 point for localized and

scattered edema and/or congestion, 2 points for plaque, congestion or edema only in antrum of stomach or body or fundus; and 3 points for ubiquitous congestion and edema over antrum of stomach or body.

Pathological grading was made according to degree of infiltration of inflammatory cells thus: grade I (mild) for inflammatory cell infiltration in one third of gastric mucosa; grade II (moderate) for inflammatory cell infiltration in one-to-two thirds of gastric mucosa, and grade III (severe) for inflammation infiltration in two-thirds of gastric mucosa. Treatment outcome was classified as *effective* for endoscopy score ≥1 point, or *invalid* if no change was seen or if endoscope scores increased to ≥1 point. In addition, the effect of treatment on CAG pathology was classified as *effective* i.e. there was improvement in inflammatory grade, or *invalid* if no improvement in pathology occurred or if the condition became more severe.

COX-2 determination in gastric mucosa

Antrum stomach tissue was cut into slices. Immuno-histochemical staining was conducted using two-step methods. The appearance of yellow or brown stain in the cells was evidence of COX-positive cells. Optical density value was measured in an IBAS 2000 image analyzer. Values from 10 slices were averaged as mean value.

Judgement of effectiveness

Effectiveness was graded as *excellent* if symptoms disappeared or basically disappeared, and endoscopy score was 0; *effective* if there was some improvements/relief in the symptoms, with endoscopy scores decreased to ≥ ½; or *invalid* if symptoms did not improve and/or if they even got worse, with endoscopy scores decreased to <½. The *total effectiveness* was calculated as shown in Eq 1.

$$T\ (\%) = \{(e_1+e_2)/t\}100 \ldots\ldots\ldots\ldots (1)$$

where T is total effectiveness, e_1 is the excellent cases, e_2 is the effective cases and t is the total cases.

Evaluation of quality of life

The life quality of patients before and after treatment was evaluated using SF-36. The parameters assessed included general well-being, vitality, sociability, emotional status, as well as physical functionality, pain**,** and psychological health. There were a total of 36 items in different dimensions. The higher the scores, the higher the life quality.

Criteria for evaluation of effectiveness

Scores were given according to the main symptoms of CAG such as discomfort after meals, early feeling of fullness, and pain in the upper abdomen. The symptoms and scores were: no symptoms = 0 point, mild symptoms = 2 points, moderate symptoms = 4 points, and severe symptoms = 6 points. The treatment index was calculated as in Eq 2.

$$T\ (\%) = \{(t_1 - t_2)/t_3\}100 \ldots\ldots\ldots\ldots (2)$$

where T is treatment index, t_1 is score before treatment, t_2 is total score after treatment and t_3 is the total score before treatment. *Recovery* occurred when the main symptoms disappeared or were basically disappeared, and effectiveness was ≥ 95 %. *Excellent* was inferred if the main symptoms were significantly improved, and effectiveness was 70 to 94 %; treatment was *effective* when the main symptoms were significantly improved, and effectiveness ranged from 30 to 69 %, and *invalid* referred to a situation where there were no significant improvements in the main symptoms, or where the symptoms even got worse, with effectiveness below 30 %.

Statistical analysis

Data input and treatment were conducted using SPSS 17.0 software. Numerical data are presented as mean ± standard deviation (SD) while attribute data are presented as %. Comparison before and after treatment was carried out with paired *t*-test, which was also for two-group comparisons. Effectiveness was compared using Chi squared (χ^2) test. Values of $p < 0.05$ were deemed significant.

RESULTS

T lymphocytes of peripheral blood of patients

The values of T lymphocytes of peripheral blood in both groups were comparable prior to drug exposure with respect to $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ (t = -1.45, p = 0 .149; t = -1.17, p = 0.243; t = -0.58, p = 0.565, respectively, Table 1). However, these parameters were significantly upregulated in the observation group after exposure to combination therapy, relative to control (t = -14.45, $p < 0.001$; t = -12.47, $p < 0.001$; t = -3.49, $p < 0.001$, respectively).

Table 1: T lymph cells of peripheral blood of patients

Group	Time of evaluation	$CD3^+$ (%)	$CD4^+$ (%)	$CD4^+/CD8^+$
Control	Before treatment	60.32 ± 5.28	43.25± 2.14	1.86 ± 0.64
	After treatment	68.27±4.16	51.27 ± 3.74*	2.31 ± 0.71
Observation	Before treatment	61.41 ± 5.33	43.68 ± 2.96	1.91 ± 0.58
	After treatment	80.26 ± 7.13*#	58.14 ± 4.01#	2.69 ± 0.82#

*Values are expressed as mean ± SD (n = 99. *p < 0. 05, relative to value prior to drug exposure; #p < 0. 05, relative to control post-exposure to drug*

COX-2 expression in gastric mucosa

The results in Table 2 show that COX-2 expression in patients were comparable in the combined treatment group and control patients prior to treatment (t = -0.56, p = 0.580). However, after treatment, there were significant downregulations of COX-2 expression in both groups, relative to values before treatment (p < 0.05), with patients on combination treatment having lower COX-2 than control patients (t = 8.20, p < 0.001).

Table 2: Gastric mucosal COX-2 expression in the two groups

Group	Time of evaluation	COX-2
Control	Before treatment	17.24 ± 3.61
	After treatment	7.82 ± 1.53
Observation	Before treatment	17.53 ± 3.74
	After treatment	6.15 ± 1.33*#

*Values are expressed as mean ± SD (n = 99); *p < 0. 05, relative to value prior to drug exposure; #p < 0. 05, relative to control post-drug exposure*

Quality of life

The patients in the two groups were comparable with respect to different aspects of SF-36 before treatment (Table 3). However, after treatment, the SF-36 scores were significantly elevated, when compared with values prior to drug exposure (p < 0.05). The SF-36 scores for physical functionality, physical functionality, physical pain, general well-being, life vitality, sociability, emotional stability, and psychological health of patients in the observation group were significantly higher than those in the control group (t = -2.02, p = 0.045; t = -4.22, p < 0.001; t = -2.59, p = 0.010; t = -3.59, p < 0.001; t = -3.50, p < 0.001; t = -2.25, p = 0.026; t = -3.22, p = 0.002; and t = -3.52, p < 0.001, respectively).

Treatment effectiveness in patients

As shown in Table 4, total effectiveness in the combined treatment group was higher than that in the control group (χ^2 =7.479, p = 0.006).

Gastroscopic and histopathological observations in patients

The effectiveness seen in gastroscopy and histopathological observations in the combined treatment group was superior to that in the group given teprenone only (χ^2 = 9.175, p = 0.003; and χ^2 =15.762, p < 0.001, respectively). These findings are presented in Table 5.

Table 3: Quality of life scores of patients (mean ± SD, n = 99)

G	PF	PR	PP	GW	LV	S	ES	PH[I]
BC	88.20±6.13	55.28±10.14	56.18±11.03	68.27±12.49	65.11±11.03	64.15±11.29	51.64±12.36	72.59±11.83
AC	93.66±7.38*	66.39±11.18*	72.36±13.27*	72.17±13.21*	73.94±12.49*	75.13±14.01*	63.61±13.77*	80.31±12.66*
BO	89.02±5.93	56.03±11.32	57.25±11.85	68.59±12.41	65.58±12.39	63.09±10.03	52.06±12.89	72.94±12.10
AO	95.81±7.63*#	73.64±12.93*#	77.05±12.17*#	79.33±12.25*#	80.07±12.18*#	79.66±14.37*#	70.27±15.32*#	87.82±17.03*#

*P < 0. 05, relative to value prior to drug exposure; #p < 0. 05, relative to control group post-treatment; G, group; BC, before treatment in control group; AC, after treatment in control group; BO, before treatment in observation group; AO, after treatment in observation group; PF, physical function; PR, physical role; PP, physical pain; GW, general well-bing; LV, life vitality; S, sociability; ES, emotional stability; PH[I], Psychological health

Table 4: Treatment effectiveness (%) in patients (n = 99)

Group	Excellent	Effective	Invalid	Total effectiveness
Control	48.48	25.25	26.26	73.74
Observation	59.60	29.29	11.11	88.89#

#p < 0.05, relative to teprenone group

Table 6: Incidence of TCM syndrome (%) in patients (n = 99)

Group	Recovered	Excellent	Valid	Invalid	Total effectiveness (%)
Control	35.35	26.26	7.07	31.31	68.69
Observation	40.40	29.29	11.11	19.19	80.81[#]

[#]$p < 0.05$, compared with control group

Table 5: Gastroscopic and histopathological observations in patients (n = 99)

Group	Gastroscopy		Histopathology	
	Valid	*Invalid*	*Valid*	*Invalid*
Control	67.68	32.32	69.70	30.30
Observation	85.86	14.14	91.92[#]	8.08

[#]*p < 0.05, compared with control group*

CAG syndrome

With respect to CAG syndrome, effectiveness in patients given combined treatment was higher than the effectiveness observed in the teprenone-alone group ($\chi^2 = 3.853$, $p = 0.049$).

Adverse reactions in patients

There were no withdrawal cases in the two groups during the treatment. However, there were 7 cases of nausea and 4 cases of vomiting in the patients given teprenone alone, while 6 cases of abdominal pain and 2 cases of nausea were recorded in the combined treatment group. Adverse reactions were comparable in both groups of patients ($\chi^2 = 0.524$, $p = 0.469$).

DISCUSSION

Xiaochaihu decoction is widely used in TCM clinics, especially for digestive system diseases. In TCM, it is believed that chronic gastritis is related to diet and emotional changes, and that the mechanism involves dysfunction in liver, spleen and stomach. Reports have indicated that the application of *Xiaochaihu* decoction for the treatment of chronic gastritis produces good results [11,12].

The total number of T cells produced in differentiation of lymphocytes is generally constant, but $CD4^+/CD8^+$ ratio changes in response to changes in cellular immune function. The $CD4^+/CD8^+$ ratio decreases when there is a disorder in immunity, thereby leading to occurrence of diseases. The results of this study show that although T lymphocytes in the two groups improved significantly after treatment, the improvement in the observation group was more obvious. This indicates that *Xiaochaihu* decoction can regulate body immunity, which is in agreement with previous reports [13-16].

Cyclooxygenase (COX) is a membrane-bound protein and a rate-limiting enzyme in prostaglandin biosynthesis. It maintains various physiological and pathological processes by regulating prostaglandin levels *in vivo*. Cyclooxygenase-2 (COX-2) is a member of the COX family which has been implicated in chronic gastritis. Inflammation can stimulate cells to secrete COX-2 through up-regulation of COX-2 protein expression in gastric mucosa. The results of this study show that COX-2 of gastric mucosa tissue of patients in the two groups were highly expressed. There were significant decreases in COX-2 expression in patients in the two groups after treatment, but the COX-2 expression in the group given combined treatment of teprenone and *Xiaochaihu* was low, relative to COX-2 expression in the group given teprenone alone. This suggests that *Xiaochaihu* inhibits inflammatory reactions.

One limitation in this study is the small sample size used. This might have led to sampling error. Another limitation is the short period of the study.

CONCLUSION

This study has shown that the combination of *Xiaochaihu* decoction with teprenone has enhanced therapeutic effects on CAG via a mechanism related to improvement in T lymphocytes in peripheral blood, and thus resulting in decreased COX-2 expression in gastric mucosa.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

This work was done by the authors named in this article and the authors accept all liabilities resulting from claims which relate to this article and its contents. The study was conceived and designed by Yu Xiaoxiao; Weng Weian and Yu Xiaoxiao collected and analysed the data, and Weng Weian wrote the manuscript. All authors read and approved the manuscript for publication.

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