

Clinical efficacy of weifuchun combined with retinoic acid on treatment of patients with PLGC and effect on expressions of gene *Rb* and *C-erbB-2*.

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Abstract

Objective: To investigate the clinical efficacy of weifuchun combined with retinoic acid on treatment of PLGC (Precancerous Lesion of Gastric Cancer) patients and effect on the expressions of gene *Rb* and *C-erbB-2*.

Methods: A total of 126 patients of gastric mucosa chronic inflammation associated with dysplasia with or without gastric mucosal metaplasia who were confirmed by endoscopic biopsy and pathology were enrolled in this study, randomly divided into the treatment group (63 cases) and control group (63 cases). The patients in the treatment group received the basic treatment of chronic gastritis (omeprazole +sucralfate) and the test drugs (weifuchun+retinoic acid), and patients in the control group received the basic treatment of chronic gastritis. The expressions of *Rb* and *C-erbB-2* before and after treatment were detected by immunohistochemical staining.

Results: The ORR of the treatment group was significantly higher than that of the control group after treatment; in the treatment group, the positive expression rate of *Rb* was significantly higher than that of the control group, while the positive expression rate of *C-erbB-2* in the treatment group was significantly lower than that of the control group.

Conclusion: Weifuchun combined with retinoic acid has a therapeutic effect for Patients with PLGC; it may play its role by up-regulating tumor suppressor gene *Rb* and down-regulating cancer gene *C-erbB-2*.

Keywords: Weifuchun combined with retinoic acid, PLGC, Gene *Rb*, Gene *C-erbB-2*.

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Introduction

Gastric cancer is an insidious disease and it is a genetic disease with abnormal expression of multiple genes caused by a variety of factors [1]. Before carcinogenesis, the carcinogenic factors have serial action or synergistic effect to cause gastric mucosal injury; and during different stages, it may cause abnormal expressions of different genes [2]. During the PLGC, it finally causes the occurrence of gastric cancer. PLGC usually refers to the disease at the early stage of gastric cancer, mainly including intestinal metaplasia or dysplasia. Studies have shown that [3], part of PLGC can be successfully reversed by drug therapy; at present, it has become a hot issue in the studies of gastric cancer prevention.

Weifuchun is the earliest Chinese patent medicine used in PLGC [4], with the effect of eliminating inflammatory reactions and promoting the gastric mucosal repair. Retinoic acid is the active product of vitamin A that is metabolized in human body, which can regulate the proliferation and differentiation and maturation of cells; it can induce differentiation of tumor cells through the retinoic acid receptors, having inhibitory effect on chemical induced cancers of a variety of tissues and organs [5]. However, the clinical

efficacy of Weifuchun combined with retinoic acid in patients with PLGC is still unclear. In this study, we investigated the clinical efficacy of Weifuchun combined with retinoic acid on treatment of patients with PLGC and its effect on the expressions of gene *Rb* and *C-erbB-2*, so as to provide scientific basis for rational drug use in clinical practices.

Data and Methods

General data

A total of 126 patients with gastric mucosa chronic inflammation associated with dysplasia with or without gastric mucosal metaplasia who were confirmed by endoscopic biopsy and pathology from Gastroenterology Clinic from January 2016 to January 2017 in our hospital were selected in this study. They were randomly divided into treatment group (63 cases) and control group (63 cases). There were no statistical differences in the sex, age and clinical symptoms between the two groups, and they were comparable. The experimental study was reviewed and approved by the hospital ethics committee. All enrolled patients signed informed consent.

Inclusion criteria: 1. Subjects who were confirmed by gastroscopy and pathology, with the diagnostic criteria and pathological diagnosis of chronic gastritis conforming to the diagnosis and treatment protocol of combined TCM and western medicine for chronic gastritis and the pathology diagnostic criteria and classification of chronic gastritis in Appendix I; 2. aged from 18 to 65 y; 3. Subjects who did not receive other medications recently.

Exclusion criteria: 1. Subjects with malignant changes confirmed pathologically; 2. subjects with a history of serious mental illness and drug allergy; 3. subject with serious systemic diseases, such as liver, kidney, heart, blood and immune diseases, etc.; 4. women during pregnancy or lactating period; 5. subjects who failed to take medicines as prescribed or the efficacy of drugs could not be determined; 6. subjects allergic to the test drugs in this study.

Treatment

Patients in the treatment group were treated with basic treatment of chronic gastritis (omeprazole+sucralfate) plus test drugs (Weifuchun+retinoic acid); while patients in the control group were treated with the basic treatment of chronic gastritis. In the treatment group, patients took oral weifuchun tablets, 3 tablets/time, tid; retinoic acid tablets, 10 mg/time, tid; omeprazole 20 mg/time, bid; sucralfate 0.75 g/times, tid. In the control group, patients took oral omeprazole 20 mg/time, bid; sucralfate 0.75 g/times, tid. The course of treatment was 12 w. The drugs used were from the same manufacture. During the treatment, patients were prohibited to take other drugs. A questionnaire survey was conducted before and after treatment. The patient history, clinical symptoms and general conditions were recorded, and patients underwent endoscopy and pathological biopsy and tests of blood routine and liver and kidney functions. During the treatment, clinical changes and adverse reactions were followed up periodically.

Specimen collection

The endoscopic biopsy was performed before treatment and within one week after the end of treatment. Four specimen blocks were collected from the sites of 3-clock, 6-clock and 9-clock of the gastric antrum, 2.5 cm from the pylorus and the gastric angle, the specimens were large enough to reach the mucosal muscular layer. Specimens should be collected additionally for the possible or possible lesions, and packaged separately. The sampling sites, endoscopic findings and brief disease history should be provided to the Pathology Department, to describe the lesion and biopsy sites in details. The biopsy sites should be consistent as far as possible before and after treatment.

Efficacy evaluation

The efficacy evaluation was performed with reference to the Regimens for Diagnosis and Treatment of Chronic Gastritis with Integrative Chinese and Western Medicine (Draft) (2003 Edition) [4]. If the intestinal metaplasia or dysplasia was

alleviated by more than two grades (including two grades), it was markedly effective; and if the intestinal metaplasia or dysplasia was alleviated by one grade, it was effective; and if the intestinal metaplasia or dysplasia was not alleviated or was alleviated by less than one grade, it was ineffective.

Outcome measure and evaluation

The expressions of *Rb* and *C-erbB-2* before and after treatment were detected by immunohistochemical staining. The detection was performed in accordance with the instructions of the kits. The standard for *Rb* positive was in accordance with the Mark Kelley laboratory standard [5], evaluation was as follows: 1. staining degree: according to the staining degree, basically no staining: score 0; light staining (yellow): score 1; moderate staining (brown): score 2; strongly positive staining (brown): score 3. 2. according to the percentage of positive cells: the percentage of stained cells $\leq 5\%$, score 0, the percentage of 6%~25%, score 1, the percentage of 26%~75%, score 2; and the percentage $>75\%$, score 3. The scores of the two items were added for making the final evaluation result, score 0: negative (-), scores 1-2, weakly positive (+); scores 3-4, moderately positive (++); scores 5-6, strongly positive (+++). The *C-erbB-2* positive standards referred to the Hercept test evaluation criteria [6]: if the percentage of positive cells $<10\%$ or only staining of cytoplasm, it was negative (-); and if the percentage of positive cells $>10\%$, it was positive; for non-continuous, weak staining (+); for continuous cytomembrane staining, moderate positive (++); and continuous cytomembrane staining, strongly positive (+++).

Statistical processing

SPSS 19.0 software was used for statistical analysis, t test was adopted for measurement data, and $P < 0.05$ was considered statistically significant difference.

Results

Clinical efficacy evaluation

The endoscopy and clinical efficacy evaluation before and after treatment were shown in Table 1. The ORR (69.84) of the treatment group was significantly higher than that of the control group (38.10), with statistically significant difference ($P < 0.05$).

Table 1. Clinical efficacy evaluation of patients before and after treatment in the two groups.

Group	Number of patients (N)	Marked	Effective	Ineffective
Treatment group	63	21 (33.33)	23 (36.51)	19 (30.16)
Control group	63	4 (6.35)	20 (31.75)	39 (61.90)

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Note: Comparison of the treatment group and the control group before treatment, $t=0.159$, $P>0.05$; comparison of the treatment group and the control group after treatment, $t=0.2527$, $P<0.05$.

The expressions of *Rb* before and after treatment

The expressions of gene *Rb* before and after treatment were shown in Table 2. *Rb* expression was located in the nucleus, and the cytoplasmic staining was of no significance. The positive expression rate of *Rb* in the treatment group was

Table 2. Expressions of *Rb* before and after treatment.

Group	N	Before treatment				After treatment				Intra-group comparison		
		+	++	+++	Positive rate	+	++	+++	Positive rate	P		
Treatment group	63	16	15	17	15	74.60%	7	12	15	29	88.89%	0.012
Control group	63	16	14	19	14	74.60%	15	13	16	19	76.19%	0.750

Note: Comparison of the treatment group and the control group before treatment, $t=0.159$, $P>0.05$; comparison of the treatment group and the control group after treatment, $t=2.527$, $P=0.03$

The expressions of *C-erbB-2* before and after treatment

The expressions of gene *C-erbB-2* before and after treatment were shown in Table 3. *C-erbB-2* expression was located in the cell membrane. The positive expression rate of *C-erbB-2* in the treatment group was 34.92% before treatment, which was significantly down-regulated compared with that after treatment (16.67%), with significant difference ($P=0.03$); the

Table 3. Expressions of *C-erbB-2* before and after treatment.

Group	N	Before treatment				After treatment				Intra-group comparison		
		+	++	+++	Positive rate	+	++	+++	Positive rate	P		
Treatment group	63	41	8	9	5	34.92%	55	4	2	2	16.67%	0.003
Control group	63	40	7	8	11	36.51%	44	8	7	4	30.16%	0.532

Note: The treatment group vs. the control group after treatment, $P=0.013$

Discussion

Gastric cancer is a major killer of human health, and it is difficult to find and diagnose for its complex etiology and insidious onset. In recent years, studies have shown that the occurrence of gastric cancer undergoes years of precancerous lesions. To reverse PLGC and block its progressions has become a focus for the prevention of gastric cancer. The development of molecular biology techniques can not only help to reveal the molecular mechanism of gastric cancer process, but also provide the target and basis for the treatment of PLGC. The clinical study in recent years has focused on the on the discovery of occurrence experience of gastric cancer and years of precancerous lesion. To reverse PLGC and block its progressions has become a focus. With the quick development of molecular biology techniques, the molecular mechanism of gastric cancer has been gradually discussed and

74.60% before treatment, which was significantly up-regulated compared with that that after treatment (88.89%), with significant difference ($P=0.012$); the positive expression rate of *Rb* in the control group was not changed significantly before and after treatment, 74.60% and 76.19%, respectively, without significant difference ($P>0.05$). In addition, after treatment, the positive expression rate of *Rb* in the treatment group was significantly higher than that of the control group, with significant difference ($P=0.03$).

positive expression rate of *C-erbB-2* in the control group was not changed significantly before and after treatment, 36.51% and 30.16%, respectively, without significant difference ($P>0.05$). In addition, after treatment, the positive expression rate of *C-erbB-2* in the treatment group was significantly higher than that of the control group, with significant difference ($P=0.013$).

studied and provides the target and basis for the treatment of PLGC.

Weifuchun is a nationally protected TCM for the treatment of PLGC earliest in china, with the efficacy of activating blood and regulating qi-flowing for strengthening spleen. Retinoic acid, also known as vitamin A acid, is a derivative of vitamin A that can regulate the expression of apoptotic genes such as *Rb*, *C-erbB-2*, thereby preventing the expression of some oncogenes and cytokines and playing its role in cancer prevention and treatment [2]. Weifuchun combined with retinoic acid can be used to treat PLGC. By combing the effect of strengthening body resistance and eliminating evil for traditional medicine and the advantages of western medicine (targeted effect, fast onset and exact efficacy), it has the characteristics of two-way adjustment and multiple-target effects. In this study, the ORR in the treatment group was

significantly higher than the control group, suggesting that weifuchun combined with retinoic acid could be used for the treatment of PLGC.

Rb gene is a tumor suppressor gene that is involved in the cell cycle regulation discovered firstly. The *Rb* gene deletion and mutation, and Rb protein phosphorylation inactivation can cause active cell division and proliferation, which is involved in the occurrence of a variety of tumors [7]. In recent years, it has been found that *Rb* gene was abnormally reduced in PLGC [8], suggesting that the abnormal expression of *Rb* gene can be used as an indicator of gastric mucosal carcinogenesis. *C-erbB-2* gene is the normal component of the cell genome, which is activated with a variety of pathogenic factors such as inflammatory stimulation, oxygen free radicals, N-nitroso compounds, etc., showing carcinogenic activity. It was found that the expression of gene *C-erbB-2* was significantly increased in PLGC [9]. In this study, after treatment, the positive expression rate of *Rb* in the treatment group was significantly higher than that in the control group, and the positive expression rate of *C-erbB-2* in the treatment group was significantly lower than that in the control group, suggesting that, Weifuchun combined with retinoic acid could restrictively up-regulate tumor suppressor gene *Rb* and down-regulate the cancer gene *C-erbB-2*, which was consistent with the results in relevant reports [10].

In summary, Weifuchun combined with retinoic acid has a therapeutic effect for Patients with PLGC, which may play a role through up-regulating the tumor suppressor gene *Rb* and down-regulating gene *C-erbB-2*.

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