Original 和漢医薬学会誌 7, 74－80, 1990

Effects of Keishi-shakuyaku-chimo-to on erythrocyte antioxidant enzymes in patients with rheumatoid arthritis

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(Received March 14, 1990. Accepted June 29, 1990.)

Abstract

The effects of Keishi-shakuyaku-chimo-to (KSC) on inflammation, anemia and erythrocyte antioxidant enzyme activity were investigated in 9 patients of RA with anemia. Following the oral administration of KSC for about 18 months, inflammation and anemia improved significantly. The activities of erythrocyte antioxidant enzymes, *i.e.*, superoxide dismutase and catalase, were also improved significantly. The results suggest that KSC not only has a salutary effect on inflammation and anemia of RA but also on the recovery of erythrocyte antioxidant enzyme activity of RA.

Key words Keishi-shakuyaku-chimo-to (Keisi-syakuyaku-timo-tô), erythrocyte antioxidant enzyme, rheumatoid arthritis, anemia, inflammation.

Abbreviations CAT, catalase ; GSH-Pox, glutathione peroxidase ; SOD, superoxide dismutase ; OA, osteoarthritis ; RA, rheumatoid arthritis ; Keishi - shakuyaku - chimo - to (Gui-Zhi-Shao-Yao-Chih-Mu-Tang), 桂枝芍藥知母湯.

Introduction

The role of oxy radicals in inflammatory processes has recently been proposed.[1] Primary protection against oxy radicals is provided by a metalloenzyme, superoxide dismutase (SOD), which catalyzes the conversion of superoxide into hydrogen peroxide. Erythrocytes possess a number of enzymes capable of scavenging toxic reactive intermediates of oxygen metabolism, *i.e.*, SOD, catalase (CAT) and glutathione peroxidase (GSH-Pox). It is thought that these antioxidant enzymes play essential roles in the cytoprotection of red blood cells.

We have reported that the activities of these three antioxidant enzymes were significantly reduced in patients of RA with anemia in comparison with patients of OA,[2] and discussed that the decreased activity of antioxidant enzymes in erythrocytes may be a reason for the concomitant anemia in patients with RA. However, this hypothesis would need to be supported by continuous monitoring of the patients, *i.e.*, if the anemia resolved and the levels of antioxidant enzymes returned to normal with the institution of anti-inflammatory therapy.

In the present paper, we attempted to evaluate changes in erythrocyte antioxidant enzymes in patients of RA with anemia following long-term administration of Keishi - shakuyaku - chimo - to (KSC), an anti-inflammatory Kampo prescription.

Subjects and Methods

Patients : Under the criteria of the American Rheumatism Association (A.R.A.),[3] a total of 9 female cases (8 of classical RA and 1 of definite RA) were investigated. Characteristics such as age, functional class, anatomical stage, con-

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Table I Patient profile.

Case	Age	Diagnosis	Class	Stage	Contraction period (year)	Observation period (year)	Hb (g/dl)	Complication
1	55	Classical	III	IV	6	1.3	5.4	
2	58	Classical	III	II	7	1.4	8.6	
3	59	Classical	III	III	15	1.5	10.4	
4	59	Classical	III	IV	5	1.3	7.9	Hypertension
5	60	Classical	III	IV	9	1.9	9.6	Diabetes
6	63	Classical	II	IV	14	1.5	8.3	
7	66	Classical	III	IV	47	1.4	7.0	
8	73	Classical	III	IV	13	1.5	10.7	Chronic thyroiditis
9	66	Definite	II	II	10	1.5	9.5	

tracted period, observation period, and hemoglobin (Hb) recorded during new consulting at the Department of Japanese Oriental Medicine at Toyama Medical and Pharmaceutical University are listed in Table I. The mean age was 62.1 years. According to the A.R.A. anatomical stages,[4] the subjects comprised 2, 1 and 6 cases for stages II, III and IV, respectively, and according to the A.R.A. functional classes,[4] they made up 2 and 7 cases for classes II and III. The mean contracted period was 14.0±12.9 years (mean± S.D.), and the mean observation period was 17.8± 2.0 months (mean±S.D.). None of the cases had any hemorrhagic disease. All subjects in this study before KSC administration were active RA, *i.e.*, they had more than 6 swollen and tender joints, their erythrocyte sedimentation rate (ESR) was more than 30 mm/hr, and they had the symptom of morning stiffness for more than 30 minutes. Other findings at the new consulting were emaciation, dry skin and anemia.

Kampo prescriptions : The components of the Kampo prescription used in this study are listed in Table II. Each crude drug was mixed with 800 ml of water and boiled to 300 ml. The decoction was administered 3 times a day before meals.

Protocol of medication : Following physical examination, X-ray examination and blood sampling, KSC was administered daily. The dose of concurrent administration of other drugs did not vary during this testing period. Those who had been receiving corticosteroid agents changing to predonisolone of more than 5 mg per day were excluded. Among these subjects, 3 patients had been taking predonisolone. Other patients who had been receiving ordinary dosages of nonsteroidal anti-inflammatory drugs for at least 6 months before the KSC therapy were included in this study.

Blood samples : For the determination of erythrocyte antioxidant enzyme activity, 9 ml of blood was withdrawn from the cuvital vein

Table II The crude drugs comprising Kampo formulations used in this study.

Keishi-shakuyaku-chimo-to (KSC)		
桂枝芍薬知母湯		
Byakujutsu (白朮), Atractylodis Rhizoma, *Atractylodes macrocephala* KOIDZUMI	5.0 g	from China*
Keihi (桂皮), Cinnamomi Cortex, *Cinnamomum cassia* BLUME.	4.0	China*
Chimo (知母), Anemarrhenae Rhizoma, *Anemarrhena asphodeloides* BUNGE.	4.0	China*
Bofu (防風), Ledebouriellae Radix, *Ledebouriella seseloides* WOLL.	4.0	China*
Shakuyaku (芍薬), Paeoniae Radix, *Paeonia lactiflora* PALL.	3.0	Japan*
Mao (麻黄), Ephedrae Herba, *Ephedra sinica* STAPF.	3.0	China*
Kanzo (甘草), Glycyrrhizae Radix, *Glycyrrhiza glabra* L. var. *glandulifera* REG. et HERD.	2.0	China*
Shokyo (生姜), Zingiberis Rhizoma, *Zingiber officinale* ROSCOE.	1.0	China*
Bushi (附子), Aconiti Tuber, *Aconitum carmichaeli* DEBX.	1.0−5.0	China**

Note : *) Supplied by Tochimoto-Tenkaido Co., Ltd., Osaka.
**) Supplied by Uchida Wakan-Yaku Co., Ltd., Tokyo.

into a siliconized glass tube containing 100 units of heparin Na. All samples were taken before breakfast and were examined within 4 days after sampling ; they were assayed at a temperature of 4℃.

Measurement of antioxidant enzymes : The measurement of SOD activity was carried out by the method of Oyanagui.[5] CAT activity was determined according to the methods of Beer and Sizer.[6] GSH-Pox activity was determined essentially according to the method of Paglia and Valentine[7] with minor modifications. These methods were described in detail in our previous paper.[2]

Other variables : Each sample was measured for red blood cell count (RBC), Hb, hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total protein, total cholesterol, choline esterase, serum iron (Fe), total iron binding capacity, ferritin, serum copper (Cu), haptoglobin (Hp) and ESR (Westergren method). The Lansbury's activity index (LAI) was measured by standard techniques.[8]

Statistical analysis : Statistical analysis of all data was done by the paired *t* test.

Results

Changes in the activity of RA during KSC therapy

The changes in ESR, Fe, Cu, Hp and LAI following KSC administration are shown in Figs. 1 and 2. ESR improved significantly from

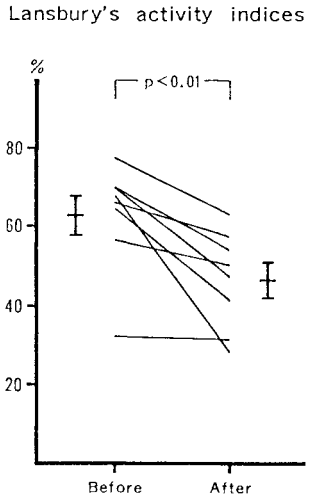

Fig. 2 Changes in Lansbury's activity indices during KSC therapy.
Each bar represents the mean±S.E. of 9 RA patients. Values significantly different from the former data.

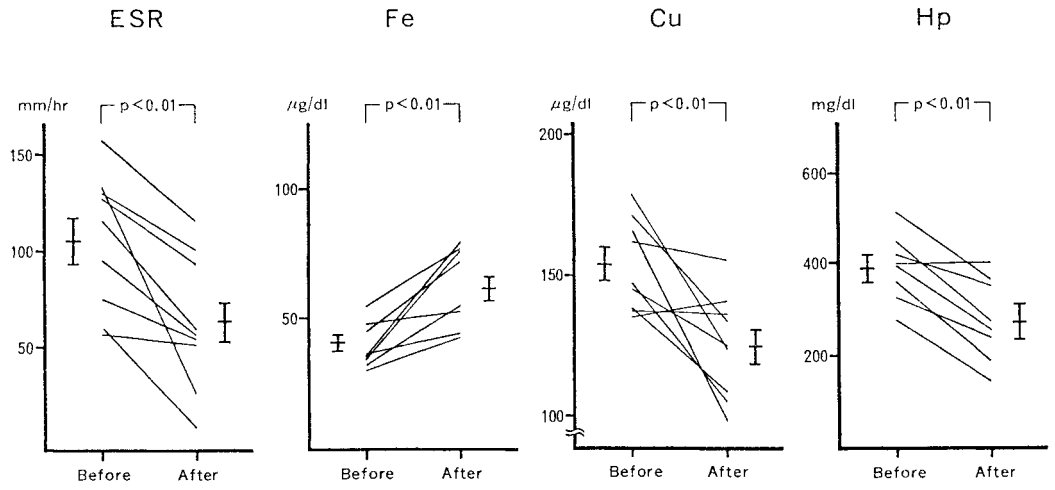

Fig. 1 Changes in erythrocyte sedimentation rate (ESR), serum iron (Fe), serum copper (Cu) and haptoglobin (Hp) during KSC therapy.
Each bar represents the mean±S.E. Values significantly different from the former data.

105.8 ± 11.8 mm/hr (mean ± S.E.) to 63.2 ± 11.3 mm/hr. Fe, Cu and Hp also improved significantly (Fig. 1). LAI also improved from 66.7 ± 5.7% to 49.4 ± 4.9% (Fig. 2).

Changes in anemia during KSC therapy

The changes in hematological parameters are shown in Figs. 3 and 4. Both Hb and Ht improved significantly from 8.6 ± 0.6 g/dl (mean ± S.E.) to 10.5 ± 0.3 g/dl and from 27.9 ± 1.4% to 32.6 ± 0.7%, respectively. However, RBC showed no significant improvement (Fig. 3). MCV, MCH and MCHC increased significantly from 76.7 ± 3.2

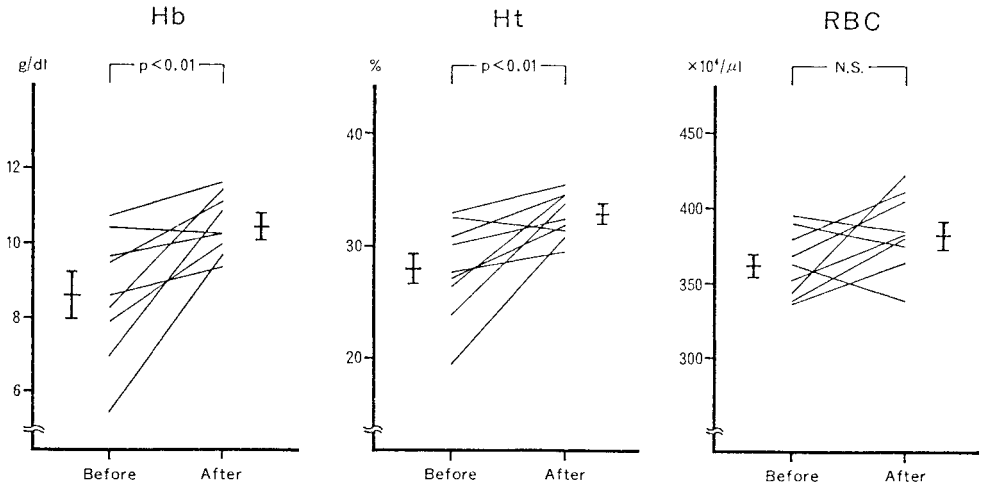

Fig. 3 Changes in hemoglobin (Hb), hematocrit (Ht) and red blood cell count (RBC) during KSC therapy.
Each bar represents the mean ± S.E. of 9 RA patients. Values significantly different from the former data without RBC.

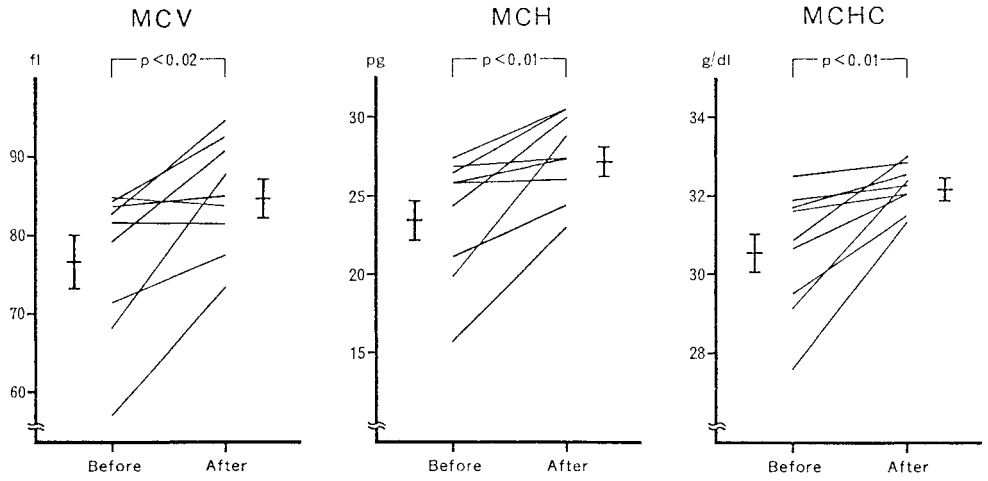

Fig. 4 Changes in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) during KSC therapy.
Each bar represents the mean ± S.E. of 9 RA patients. Values significantly different from the former data.

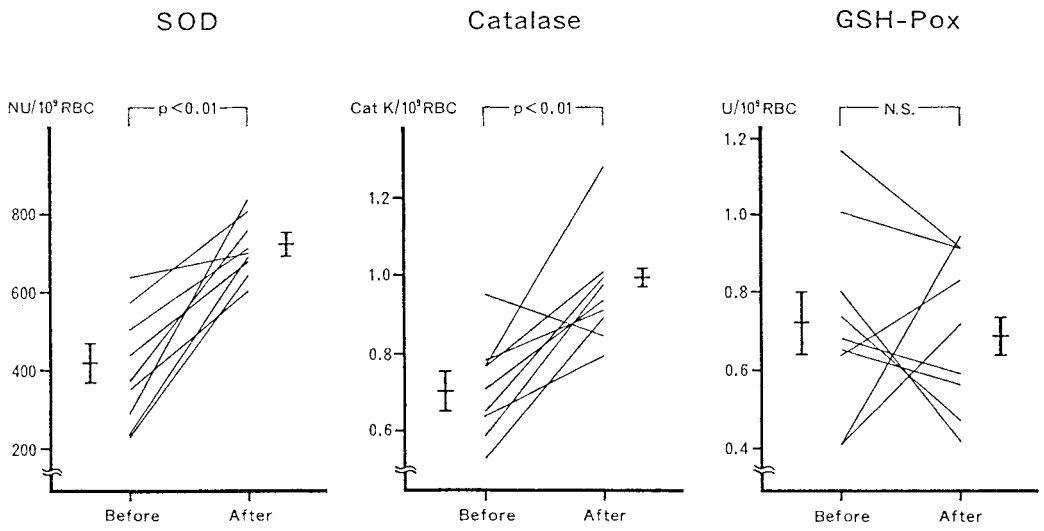

Fig. 5 Changes of three antioxidant enzymes activities *i.e.*, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Pox) in one erythrocyte during KSC therapy.

Each bar represents the mean±S.E. of 9 RA patients. Values significantly different from the former data without GSH-Pox.

fl (mean±S.E.) to 85.1±2.4 fl, from 23.6±1.3 pg to 27.5±0.9 pg and from 30.6±0.5 g/dl to 32.3±0.2 g/dl, respectively (Fig. 4).

Changes in the activities of SOD, CAT, and GSH-Pox in one erythrocyte following KSC therapy

The changes in the activities of erythrocyte antioxidant enzymes following the KSC therapy are shown in Fig. 5. The activity of Cu, Zn-SOD improved significantly from 414.4 ± 46.7 NU / 10^9 RBC to 716.7±24.4 NU/10^9 RBC. The latter data corresponded to our control data of OA.[2] The activity of CAT improved significantly from 0.71±0.04 Cat K/10^9RBC to 0.97±0.04 Cat K/10^9 RBC. The latter data was also comparable to our control data of OA.[2] However, the activity of GSH-Pox failed to show any significant change after the KSC treatment (Fig. 5).

Discussion

The causes of anemia associated with RA have been reported as follows : Abnormalities of iron metabolism,[9,12] shortening of the lifespan of red blood cells,[13] decrease of erythropoietin,[14] lack of folic acid,[15-17] dysfunction of differentiation and proliferation of bone marrow,[18] and bleeding.[19] However, no one particular theory has as yet been established.

Recently, the importance of oxy radicals such as superoxide and hydroxyl radicals as causative agents of inflammation has been recognized.[1] The significance of antioxidant enzymes that scavenge the radicals has also been referred to. It has been well known that the anemia associated with RA is best relieved not by hematinics but by bringing the disease activity under control,[20] suggesting a close correlation between anemia and the inflammatory process.

Previously, we revealed that the activities of all erythrocyte antioxidant enzymes, *i.e.*, SOD, CAT and GSH-Pox, decreased significantly in RA patients associated with anemia,[2] and presented a hypothesis that the anemia concomitant with RA is in part brought on by the reduced activities of antioxidant enzymes in the erythrocytes. Therefore, the reduced activities of these enzymes yield impaired cytoprotection.

Although this was not a controlled study, the results may suggest that the KSC therapy improves not only the inflammatory parameters such as ESR, serum Fe, serum Cu, serum Hp and LAI, but also the erythrocyte antioxidant en-

zymes activities. With the improvement of these parameters, the microcytic hypochromic anemia associated with RA changed to a normocytic normochromic one.

Therefore, our hypothesis is at least partly supported by this study. That is, there was an improvement in the cytoprotection system of erythrocytes which led to a recovery of anemia associated with RA. However, it was still unclear whether the recovery of the antioxidant enzymes resulted in normalized anemia or not. It also remains a possibility that the reduction of oxidative stress in RA following anti-inflammatory therapy causes a complete recovery of the activity of such enzymes.

Concerning the recovery of the three antioxidant enzymes activities, only GSH-Pox does not improve significantly. One of the reasons for this is thought to be that the relatively high amount of hydrogen peroxide provided by SOD consumes GSH-Pox activity rather than the CAT activity. Another possible reason is thought to be that the recovery of GSH-Pox activity may be slowed down by the deoxidation of lipid peroxide in the erythrocyte membrane.

Sinet and Blum reported that SOD and CAT were inactivated by superoxide and hydrogen peroxide.[25,26] The present results suggest the significance of oxidative stress in RA, as inflamed synovial tissues in RA provide large amounts of oxygen radicals to the circulatory blood, *i.e.*, erythrocytes. It can therefore be suggested that there is a causal relation between oxidative stress and the activity of antioxidant enzymes in erythrocytes.

Recently, antioxidant effects of medicinal plants have been reported,[27-29] and several plants composed of KSC also have antioxidant effects. Therefore, KSC may act not only as a classical anti-inflammatory agent but also as a reducer of oxidative stress.

In any case, the long-term administration of KSC results in both the suppression of inflammation and the recovery of RA concomitant anemia, in association with the recovery of erythrocyte antioxidant enzymes. For a clarification of the causal connection between the recovery of erythrocyte antioxidant enzymes and the improvement of RA related anemia, further investigations will be called for.

Acknowledgements

We express our gratitude to Mr. A. Gerz for his critical reading of this manuscript. This research was supported by the Uehara Memorial Fund.

和文抄録

慢性関節リウマチ患者9人における炎症反応，貧血，赤血球中の抗酸化酵素活性に対する桂枝芍薬知母湯の効果を観察した。桂枝芍薬知母湯は平均約18カ月間，経口投与を行った。これにより炎症反応と貧血は著明に改善した。また，桂枝芍薬知母湯投与により抗酸化酵素，すなわちSODとカタラーゼについては，その活性値が有意に上昇した。以上の結果より桂枝芍薬知母湯は，慢性関節リウマチにおける抗炎症や抗貧血の効果のみならず赤血球中の抗酸化酵素活性の改善をももたらす作用を有することが示唆された。

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