EXTRACT OF ASTRAGALUS MEMBRANACEUS AND LIGUSTRUM LUCIDUM DOES NOT PREVENT CYCLOPHOSPHAMIDE-INDUCED MYELOSUPPRESSION

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ABSTRACT

There is increasing evidence that many Chinese medicinal herbs are promising biological response modifiers in cancer treatment. The extract of some Chinese herbs have shown ability in stimulating the bone marrow and improving the peripheral white cell counts in rats. We studied the effect of a dried extract of two commonly used Chinese herbs, Astragalus membranaceus (AM) and Ligustrum lucidum (LL) on cytotoxic-induced myelosuppression. Wistar rats weighing 250-300g each were divided into two groups of 12. Both groups were given cyclophosphamide intravenously at 75mg/kg on day 1 of the study. Rats in the study group were fed 240 mg of crude extract of AM and LL from day 1 to day 12 of study. The daily absolute neutrophil count (ANC) and the platelet count were monitored. There was no difference between the study and the control group in terms of nadir count, time to nadir and time to recovery for both the ANC and the platelet counts. The duration of neutropenia (ANC < 1.0 x 10^9/L) was also similar in both groups. Our results showed that the extract of AM and LL does not prevent cyclophosphamide-induced myelosuppression.

Keywords: *Chinese, herbs, chemotherapy, neutropenia, thrombocytopenia*

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INTRODUCTION

There has been a great deal of interest in incorporating Chinese Traditional Medicine in the treatment of cancer patients; either as a biologic response modifier to enhance the efficacy of chemotherapy and radiotherapy[1] or in supportive care to improve the quality of life of cancer patients[2]. In the area of supportive care, Chinese medicinal herbs have been reported, mainly in Chinese literature, to be able to attenuate many of the treatment-related adverse effects like immunosuppression, bone marrow suppression and gastrointestinal toxicities[2,3].

Much work has been done in the past decade on two commonly used Chinese medicinal herbs: Astragalus membranaceus (AM), which is the root of the membranous milk vetch and Ligustrum lucidum (LL), which is the fruit of the glossy privet. Both the crude extract of AM and LL have been shown in laboratory studies to have immuno-modulatory activities[4-7]. When given to cyclophosphamide-treated mice, some Chinese medicinal herbs were found to increase the white blood cell count[7].

A dried crude extract of AM and LL has been developed by the Cancer Institute of the Chinese Academy of Medical Science in recent years. This extract is presently manufactured by the Number 2 Pharmaceutical Factory of Yangji City in China. Its purported actions include strengthening of the immune function and protection of the bone marrow and adrenal cortical function. It can also help cancer patients undergoing chemotherapy and radiotherapy to recover their normal organ functions[3].

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We conducted an animal study to find out the effect of this preparation of AM and LL extract on chemotherapy-induced myelosuppression.

MATERIALS AND METHODS

Animals

Wistar rats weighing between 250 - 300g were obtained from the Animal Holding Unit of the National University of Singapore. Twenty-four rats were divided into two groups of twelve, one group designated study group and the other, control group. These rats were housed at room temperature in separate cages. Food pellets and water were given ad libitum.

AM and LL extract

The commercially pre-packed dried extract of AM and LL was used. An aqueous form of the extract was prepared by dissolving 15 g (one sachet) of the dried extract in 50 ml of distilled water giving a concentration of 300 mg/ml. Each rat in the study group was tube-fed 0.8 ml (240 mg) of the extract daily from day 1 to day 12 of the study. This dose was approximately equivalent to the daily recommended dose (30 g/day) for adults. The control group was given water.

Chemotherapy

The study was conducted in two phases. In phase I, no chemotherapy was given. The effect of AM and LL on the blood counts was studied. In Phase II, cyclophosphamide at 75 mg/kg were given intravenously via the dorsal foot vein to all rats on day 1 of the study. This dose was chosen based on a dose ranging study where two dose levels ie 50 mg/kg and 100 mg/kg were examined. At 50 mg/kg, the mean nadir absolute neutrophil count (ANC) was 2 x 10^9/L. This degree of neutropenia was considered inadequate. At 100 mg/kg, the mean nadir absolute neutrophil count attained was 0.06 x 10^9/L. Five out of six rats died during the neutropenic phase. The high mortality encountered with this dose level made it impossible to examine the recovery from neutropenia.

Evaluation

Complete blood counts with differential counts were monitored daily. 0.1 ml of venous blood was taken from the tail vein each day and analysed by the H1-Technicon Blood Cell Counter. The mean counts of the two groups were compared using the unpaired t-test. Time to nadir and time to recovery were measured in terms of days. The statistical procedures were performed with StatView II version 1.03 (Abacus Concepts Inc.)

RESULTS

There was no difference in the baseline characteristics of the two groups of rats as shown in Table I. At the end of the study all twenty-four rats were alive and well.

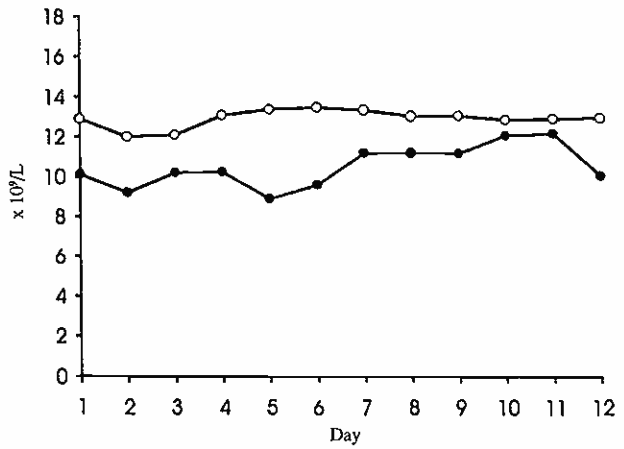
Table I – Baseline characteristics of the rates

	Test	Control
Numbers	12	12
Weight (grams)	302±31	295±16
White cell count (x10^9/L)	16.81±4.07	16.91±3.83
Platelet (x10^{12}/L)	784±83	775±128
Hematocrit (%)	43.4±1.4	42.7±2.0

Absolute neutrophil counts

In phase I of the study, there was no significant change in the ANC of the rats in the study group over a twelve-day period. The daily mean ANC was somewhat lower in the rats given the AM and LL extract (Fig 1). There was no difference between the control and study groups (p>0.1).

Fig 1 – Daily total white cell count of rats fed with AM and LL extract (∘) versus the control (•).



In phase II of the study, a rapid decline in the ANC after the injection of the cyclophosphamide was seen. It reached a nadir at about day 6 and recovered with a reactive granulocytosis (Fig 2a). There were no differences in mean nadir, time to nadir and time to recovery between the study and control groups (Table II). Time to recovery was defined as the number of days taken for the counts to rise from the nadir to 3.0 x 10^9/L or more. Although the reactive granulocytosis was more pronounced in the study group, the difference did not reach statistical significance. The durations of neutropenia, defined as the number of days when ANC was less than 1.0 x 10^9/L, were also similar.

Platelet counts

The decline in the platelet count was more gradual than the ANC (Fig 2b). The nadir was reached at about day 7. There were no differences in the mean nadir, time to nadir and time to recovery between the two groups (Table II). In contrast to ANC, the reactive thrombocytosis was similar in both groups.

Table II – White cell count (WCC), absolute neutrophil count (ANC) and platelet count in the first cycle of treatment.

	Test	Control	p
WCC (x10^9/L)			
Nadir	0.970±0.2	0.960±0.28	0.93
Day 12	23.820±13.73	17.360±4.50	0.14
Days to nadir	5.5±0.5	5.7±0.45	0.41
ANC (x10^9/L)			
Baseline	4.45±1.35	4.33±1.39	0.84
Nadir	0.12±0.05	0.09±0.03	0.38
Day 12	13.99±6.86	9.42±3.29	0.06
Days to nadir	5.8±0.7	6.0±0.4	0.50
Days to recovery	4.1±0.8	3.8±0.8	0.46
Days ANC < 1.0	5.0±1.6	5.4±1.2	0.57
Platelet (x 10^{12}/L)			
Nadir	214±60	191±62	0.38
Day 12	1534±296	1526±203	0.94
Days to nadir	6.9±0.3	7.0±0.3	0.17

Cycle 2

The experiment was repeated on day 22 of the study. The purpose was to see if the results obtained from the first cycle were reproducible. The dose of cyclophosphamide and AM and LL extract used were exactly the same as cycle 1. The daily mean ANC were shown in Fig 2c. After reaching the nadir on day 6, there appears to be a more rapid recovery in the study group. This was also reflected by a shorter period of neutropenia. The difference of 0.7 day was however not statistically significant at the 5 percent level (p=0.09) (Table III). A more pronounced reactive granulocytosis during the recovery phase was again observed in the study group but this also did not reach the conventional level of statistical significance (p=0.1). There was no difference in the daily platelet counts between the two groups (Fig 2d).

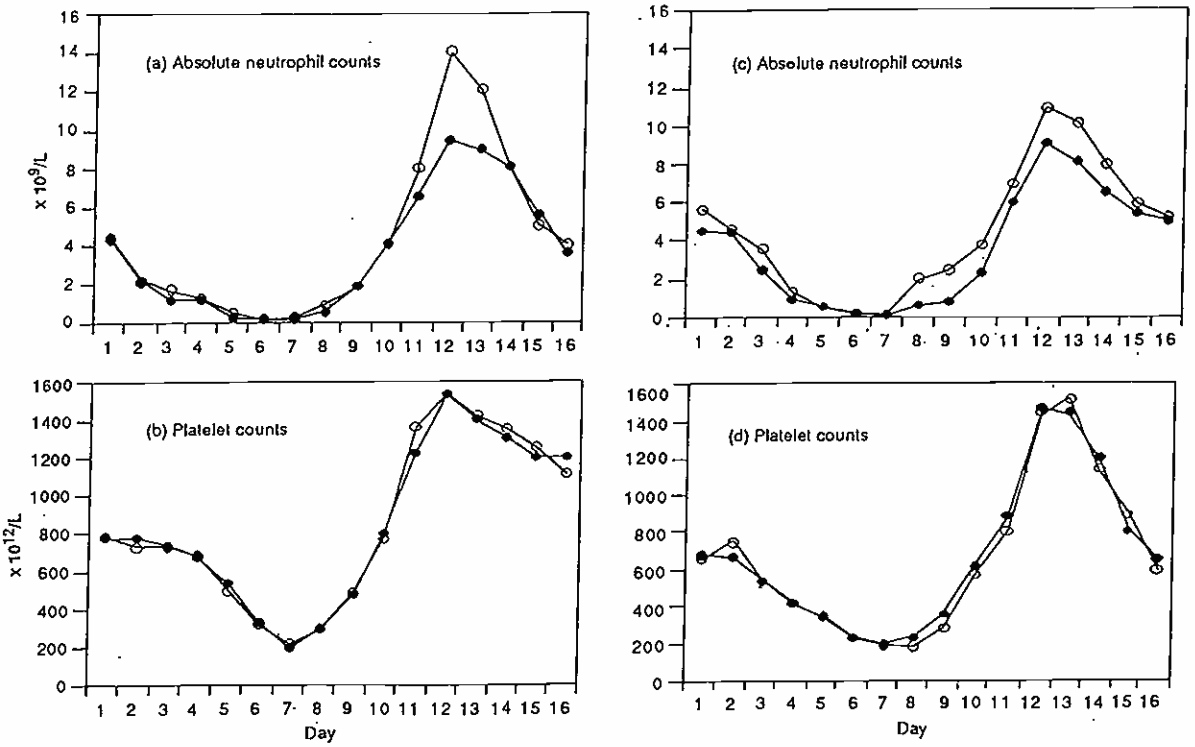
Table III – Absolute neutrophil counts in the second cycle of treatment

	Test	Control	p
Nadir (x 10^9/L)	0.12±0.12	0.11±0.11	0.75
Days to nadir	6.6±0.5	6.5±0.5	0.8
Days < 1.0 x 10^9/L	3.8±1.0	4.5±0.9	0.09

DISCUSSION

The promotion and enhancement of host defence mechanism, termed Fu-zheng in Chinese, is a basic concept in Traditional Chinese Medicine. In practice, this is translated into a two-pronged approach in the management of disease; namely tackling the disease-causing factors and supporting the host. A treatment plan is considered incomplete unless it also includes measures to enhance the ability of the host to withstand the insults from the treatment directed at the disease-causing factors and the disease itself. In the past decade, there has been an increasing interest, both within and without China, to apply this concept to cancer treatment. In many ways, Chinese medicinal herbs are ideal compounds to combine with chemotherapy and radiation therapy. They have little or no side effects, are relatively inexpensive and usually taken orally.

Fig 2 – Daily mean blood counts for cycle 1, (a) & (b), and cycle 2, (c) & (d). Control group (•), Study group (◦).



Many scientific experiments looking at the effect of AM and LL on the immunological functions have been reported. One preparation (F3) derived from the crude extract of AM was found to abrogate immunosuppression when injected into cyclophosphamide-treated rats[4]. F3 also potentiates IL-2 activity in the in vitro generation of LAK cell activity[5]. Another derivative of AM extract (F7) was also found to be able to enhance the antibody response to a T-dependent antigen in normal and immunosuppressed mice. Using chemiluminescent assay, Rittenhouse demonstrated that suppression of macrophage associated with renal cell carcinoma and bladder tumour was reversed by the extracts of AM and LL[6].

There are, on the other hand, very few published reports on the effect of AM and LL extracts, either alone or in combination, on chemotherapy-induced neutropenia or thrombocytopenia. Nevertheless, unreferenced studies cited in review articles have shown that some herbs are able to stimulate colony-forming units of bone marrow in tumour-bearing mice and an active component of AM is capable of improving the rat's peripheral white cell counts.

Our data showed that AM and LL extracts did not significantly alter the course of cyclophosphamide-induced myelosuppression. It did not delay the onset, hasten the recovery or shorten the duration of neutropenia. The ANC nadir was similar with or without the extract. There were no mortality in either group. Therefore the effect on mortality, if any, cannot be assessed.

Two interesting observations were made from the data. Firstly, the reactive granulocytosis during the recovery from neutropenia was consistently of a greater magnitude in the study group. Secondly, there was a trend towards a shorter period of neutropenia in the study group during the second cycle of treatment. It could be that AM and LL extract requires a few days of priming before an effect on neutropenia can be seen. However, this was not borne out by an earlier experiment looking specifically at the effect of priming. In that study, AM and LL extract was fed to the rats in the study group from day -5 through day 12. Cyclophosphamide was given on day 1 at a dose of 75mg/kg. There was no difference in the nadir ANC or duration of neutropenia observed[8].

It is not to be inferred from our study that AM or LL do not contain substances that may impact on bone marrow suppression. The basic method of preparing a herb extract involves suspending finely-ground parts of a plant in deionized water at fixed temperature for several hours. The supernatant was then concentrated by evaporation. Such herbal extracts contain many different chemical compounds. Some of these are probably inert thus diluting the active substances. Just as many of the immuno-modulatory activities of AM were demonstrated using one of its purified fraction, it is entirely possible that one or more of the constituents of the AM and LL extract can actually stimulate and accelerate maturation of granulocyte stem cells. Studies should be carried out on such purified fractions of AM and LL to explore their potential as hemopoietic growth factors.

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