

## Herbal Medicine and False-Positive Results on Lymphocyte Transformation Test

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In vitro mitogenic activity of 16 herbs and 3 Kampo (herbal medicine) formulae have been reported in experimental studies. It is not known how many herbs and Kampo formulae in total have mitogenic activity. Lymphocyte transformation test (LTT) is generally utilized to diagnose drug-induced liver injury. In LTT, mitogenic activity is assessed by measuring <sup>3</sup>H-thymidine incorporation. The objective of the present study was to determine which herbs and which Kampo formulae caused false-positivity on LTT. We examined 2496 summaries of all admission records from 1979 to 1999 in our department. We selected patients in whom liver injuries were diagnosed as definitely unrelated to Kampo medication. In these patients, LTT was performed for some herbs contained in the suspect Kampo medicines, resulting in positive LTT for 17 herbs: Evodiae Fructus (Goshuyu), Zizyphi Fructus (Taiso), Ginseng Radix (Ninjin), Zingiberis Rhizoma (Shokyo), Hoelen (Bukuryo), Aconiti Tuber (Bushi), Angelicae Radix (Toki), Cnidii Rhizoma (Senkyu), Rehmanniae Radix (Jio), Ephedrae Herba (Mao), Anemarrhenae Rhizoma (Chimo), Cinnamomi Cortex (Keihi), Bupleuri Radix (Saiko), Artemisiae Capillari Spica (Inchinko), Persicae Semen (Tonin), Moutan Cortex (Botanpi) and Paeoniae Radix (Shakuyaku). These results were considered false-positive, because the results were observed in the “definitely unrelated” patients. Mitogenic activity inherent to some herbs and Kampo formulae may sometimes cause false-positivity on LTT in clinical situations. These examples suggest that LTT for Kampo formulae may be unreliable as a diagnostic method for drug-induced liver injury.

**Key words**—Kampo medicine; lymphocyte transformation test; false-positive; mitogen; liver injury

### INTRODUCTION

In vitro mitogenic activity of some herbs and Kampo (Japanese herbal) formulae have been reported in experimental studies. To date, 16 herbs<sup>1–3,5–8)</sup> and 3 Kampo formulae<sup>4,9,10)</sup> have been reported to have such activity. In the lymphocyte transformation test (LTT), mitogenic activity was assessed by measuring <sup>3</sup>H-thymidine incorporation.<sup>11)</sup> Although LTT was prevalently utilized for the diagnosis of drug-induced liver injury particularly in Japan,<sup>12–14)</sup> the problem of false-positivity on LTT has scarcely been studied.

It is not known how many herbs or Kampo medicines in total have mitogenic activity. There are fewer reports of mitogenic activity or false-positivity in LTT in comparison to a large number of herbs and Kampo medicines. We examined 2496 summaries of all admission records from 1979 to 1999 in our department. Among liver injuries once suspected of

being related to Kampo medicine in each admission period, several events were diagnosed as “unrelated” to the use of Kampo medicine in this study. In the “unrelated” patients, positive LTT for herbs was considered a false-positive result. We reported which herbs caused this false positivity on LTT in clinical situations.

### PATIENTS AND METHODS

**Patients** We retrospectively examined the summaries of all admission records between 1979 and 1999, and identified all patients in whom liver injury was suspected to be attributable to Kampo medicines, even if the suspicion was transient during the hospital admission period. The diagnoses of these patients were reexamined according to the criteria described below. After reexamination of the diagnosis, we selected patients in whom liver injuries were finally diagnosed as “definitely unrelated” to Kampo medication; patients diagnosed with liver disorders in 2000 were excluded from this study because the follow-up period was not sufficient for causality assessment.

**Criteria** All diagnoses determined during the admission period were reviewed by studying admission records and by following up the clinical course of the liver disorders after discharge. Whether there was a causal relationship between the use of Kampo medication and the occurrence of liver injury was assessed according to the criteria described by Haller et al.,<sup>15)</sup> independently from the results of the LTT. Namely, the patients with liver injury were divided into 6 groups: definitely related, probably related, possibly related, insufficient information, probably unrelated and definitely unrelated to Kampo medication. The assessment was made based on 5 factors: time to onset, course after cessation of a suspect drug, concomitant drugs, search for non drug causes, and response to readministration,<sup>16)</sup> but no scoring system was employed. When the course of events was highly inconsistent with the known course of drug-induced liver injury, or when hepatitis did not recur upon readministration of the “suspect” Kampo medicine, the liver disorder was considered “definitely unrelated” (for example, in cases of liver disorders that persisted long after the use of a Kampo medicine had been discontinued).<sup>15)</sup>

**Lymphocyte Transformation Test** Results of lymphocyte transformation tests (LTTs) for herbs were reviewed in the “definitely unrelated” group. LTTs were performed during the admission period as described previously<sup>17)</sup> by Bio Medical Laboratories (BML, Inc. Japan). Briefly, peripheral blood mononuclear cells were isolated from human peripheral blood by density gradient centrifugation, and washed in Hanks’ balanced salt solution. The cells were resuspended in culture medium supplemented with 10 % plasma from the patient to be tested and 5 % serum from AB+ donors. Various concentrations of herbs, which were filter-sterilized before use, were incubated with  $5 \times 10^5$  cells/ml for 3 days. Phytohemagglutinin was employed as a positive mitogen control. The proliferative response was assessed by measuring  $^3\text{H}$ -thymidine incorporation. Radioactivity was measured by liquid scintillation spectrometry. The stimulation index (S.I.) was defined as “counts per minute (cpm) with allergen/cpm of negative control”. The LTT was considered positive if the stimulation index was 1.8 or more, according to the criteria recommended by BML. Positive LTT observed in the definitely unrelated patients were considered “false-positive”.

Table 1. False-Positive LTTs for Herbs

Latin name (Japanese name)	S.I.
Evodiae Fructus (Goshuyu)	2.8
Zizyphi Fructus (Taiso)	2.4
Ginseng Radix (Ninjin)	4.3
Zingiberis Rhizoma (Shokyo)	4.1
Hoelen (Bukuryo)	2.0
Aconiti Tuber (Bushu)	22.4
Angelicae Radix (Toki)	3.8
Cnidii Rhizoma (Senkyu)	5.2
Rehmanniae Radix (Jio)	2.9
Ephedrae Herba (Mao)	2.3
Anemarrhenae Rhizoma (Chimo)	7.4
Cinnamomi Cortex (Keihi)	1.9
Bupleuri Radix (Saiko)	3.6
Artemisiae Capillari Spica (Inchinko)	3.6
Persicae Semen (Tonin)	2.3
Moutan Cortex (Botanpi)	3.4
Paeoniae Radix (Shakuyaku)	5.4

## RESULTS (Table 1)

Among all admission records, 9 liver injuries were considered definitely unrelated to the use of Kampo medicine. In these patients, LTT was performed for some herbal components of the “suspect” Kampo medicines. LTT results were positive for 17 herbs contained in the Kampo formulae that were once suspected to cause liver injury (Table 1).

## DISCUSSION

LTT showed false positive results for 17 herbs contained in the Kampo formulae (Table 1) that were once suspected to cause liver injury. Of these 17 herbs, the mitogenic effect of Evodiae Fructus (Goshuyu), Bupleuri Radix (Saiko), Toki (Angelicae Radix) and Shakuyaku (Paeoniae Radix) has been reported in experimental studies.<sup>2,3)</sup> Thirteen additional herbs were newly reported to have such effects in the present study. We are not aware of any clinical reports concerning false-positive phenomenon on LTT for herbs, except for our reports.<sup>18,19)</sup>

Previous experimental studies reported mitogenic activities of 16 herbs (Table 2)<sup>1-3,5-8)</sup> and 3 Kampo formulae (Table 3).<sup>4,9,10)</sup> Considering the false-positive phenomenon shown in this study in addition to the mitogenic effects reported in the previous reports, numerous herbs may have mitogenic effects. In Japan, 141 Kampo formulae are covered by the Na-

Table 2. Previous Reports on *in vitro* Mitogenic Effect of Herbs

Latin name	Japanese name	Reference No.
Glycyrrhizae Radix	Kanzo	3, 5, 6, 7
Paeoniae Radix	Shakuyaku	3
Bupleuri Radix	Saiko	2, 8
Plantaginis Semen	Shazenshi	2
Lithospermi Radix	Shikon	2
Evodiae Fructus	Goshuyu	2
Angelicae Dahuricae Radix	Byakushi	2
Mori Cortex	Sohakuhi	2
Benincasae Semen	Togashi	2
Angelicae Radix	Toki	2
Sophorae Radix	Kujin	1, 2, 8
Sanguisorbae Radix	Chiyu	3
Astragali Radix	Ogi	5, 7
Atractylodis Lanceae Rhizoma	Sojutsu	5, 7
Pinelliae Tuber	Hange	8
Zedoariae Rhizoma	Gajutsu	8

Table 3. Previous Reports on *in vitro* Mitogenic Effect of Kampo Formulae

Chinese name	Japanese name	Reference No.
Jia wei gui pi tang	Kami-kihi-to	10
Shi quan da bu tang	Juzen-taiho-to	4
Da chai hu tang	Dai-saiko-to	9

tional Health Insurance. Most of these 141 Kampo formulae contain the herbs presented in Table 1 or 2, and only 3 formulae do not contain such herbs.

In Japan, the LTT is thought to be useful for diagnosing drug-induced liver injury,<sup>13,14)</sup> and the most widely used criterion for drug-induced liver injury in Japan<sup>12)</sup> attaches importance to LTT results. The criterion states that “liver injury after administration of a drug and positivity on LTT for an incriminated drug” indicates the drug is “definitely related”.

In the lymphocyte transformation test (LTT), a mitogenic activity was assessed by measuring <sup>3</sup>H-thymidine incorporation.<sup>11)</sup> The mitogenic effect of some herbs and Kampo formulae may have misled Japanese physicians on several occasions, because few physicians were aware of such activity of Kampo formulae. Actually, we encountered 2 cases of autoimmune liver disease misdiagnosed by false-positivity on LTT for Kampo formulae.<sup>18)</sup> We previously reported that positive LTT for some Kampo medi-

cines was frequently observed in patients receiving Kampo medicine who did not develop any allergic reactions.<sup>19)</sup> The mitogenic activity inherent to some herbs and Kampo medicines may sometimes cause false-positivity on LTT in clinical situations.

Several herbs commonly contain certain kinds of tannins,<sup>20)</sup> presumably for the purpose of protecting from noxious insects and bacteria.<sup>21)</sup> Tannin-containing compounds have been reported to exert various biological activities such as antibacterial<sup>22)</sup> and antiviral effects.<sup>23–25)</sup> Ohtsu<sup>26)</sup> reported that the mitogenic substance in *Bupleurum chinense* is a polyphenol. Tannin is attached to the polyphenol group as indicated by its structure with the “hydroxyl group”.<sup>20)</sup> These findings might permit speculation that the mitogenic effect of several herbs may be attributable to tannin components. Or other substances like lectin may cause the mitogenic effect. Further experimental study for substances attributable to the mitogenic effect is warranted.

In summary, we presented positive LTTs for 17 herbs contained in Kampo formulae. The results were considered false-positive, because it was observed in the “definitely unrelated” group. These examples suggest that LTT for Kampo medicine may be an unreliable method of diagnosing drug-induced liver injury.

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