

Lymphocyte Transformation Test for Medicinal Herbs Yields False-Positive Results for First-Visit Patients

Naoki Mantani,^{1,2*} Toshiaki Kogure,¹ Jun'ichi Tamura,³ Yutaka Shimada,²
and Katsutoshi Terasawa²

Departments of Integrated Japanese Oriental Medicine,¹ and General Medicine,³ Gunma University School of Medicine, Maebashi, and Department of Japanese Oriental Medicine, Faculty of Medicine, Toyama Medical and Pharmaceutical University, Toyama,² Japan

Received 10 October 2002/Returned for modification 4 December 2002/Accepted 2 January 2003

We performed lymphocyte transformation tests (LTTs) for toki (*angelicae radix*) and ogon (*scutellariae radix*) on first-visit patients who had never taken Kampo medicines. LTTs for both herbs were positive in 12 of 14 patients, suggesting that LTTs for these herbs are unreliable for the diagnosis of Kampo medicine-induced liver injury.

In vitro mitogenic activity of 16 herbs (5, 6, 12, 14, 17, 18) and three Kampo (Japanese herbal) medicines (3, 16, 19) has been demonstrated in experimental studies. In addition to those reports, we have encountered false-positive results in lymphocyte transformation tests (LTTs) of 17 herbs contained in Kampo formulae (9). In the LTT, mitogenic activity is quantified by measuring [³H]thymidine incorporation (2). Because the LTT is widely used in Japan for the diagnosis of drug-induced liver injury (4, 13; Anonymous, Report of 3rd Meeting of Drug and Liver, p. 96-98, 1978), liver injury has sometimes been misdiagnosed as Kampo medicine-induced liver injury (7). Few physicians were aware of such activities of Kampo medicines (8). The problem of false positivity in the LTT has scarcely been studied under clinical conditions (8). It is unknown whether positivity in the LTT for medicinal herbs is frequently observed or not in actual clinical situations.

toki (*angelicae radix* [*Angelica acutiloba* Kitagawa]) and ogon (*scutellariae radix* [*Scutellaria baicalensis* Georgi]), which are contained in numerous Kampo medicines, may be considered representative medicinal herbs. A report has demonstrated the possibility that the incidence of liver injury related to ogon may be relatively higher than that related to other herbs contained in Kampo medicines (10). In the present study, we conducted toki and ogon LTTs on patients who had taken no Kampo medicine in their lives and consulted our department for the first time.

We enrolled consecutive patients during the period of July 2001 to March 2002. Patients who were being treated with corticosteroids or immunosuppressive drugs or who had experienced drug allergy were excluded. Informed consent was obtained from all enrolled patients before examination by LTT. Toki and ogon purchased from Tochimoto (Osaka, Japan) were used. Each herb (5 g) was added to 600 ml of water and boiled for 30 min. The boiled-down extract was submitted

to Bio Medical Laboratories, Inc. (BML), along with blood samples obtained from the enrolled patients.

To examine the effects of herbs under actual clinical conditions, LTTs for toki and ogon were performed by BML (a laboratory company widely used by clinics and hospitals in Japan) as described previously (1). Briefly, mononuclear cells were isolated from the peripheral blood of each patient by density gradient centrifugation and washed in Hanks' balanced salt solution. The cells were resuspended in RPMI 1640 culture medium supplemented with 10% plasma from the patient to be tested and 5% serum from AB-positive donors. Serial dilutions (1/190, 1/570, 1/1,710, 1/5,130, 1/15,390, and 1/46,170) of the extract from the two test herbs were filter sterilized before use and then incubated with 5×10^5 cells/ml for 3 days. Phytohemagglutinin was employed as a positive mitogen control. The proliferative response was assessed by measuring [³H]thymidine incorporation. Radioactivity was measured by liquid scintillation spectrometry. The stimulation index (SI) was defined as the counts per minute obtained with the allergen divided by the counts per minute of the negative control. The LTT was considered positive if the SI was 1.8 or greater according to the BML criteria, which have been set up for Western medicines. Other LTT criteria in Japan also adopt a similar SI value as a cutoff point (11, 15).

As shown in Table 1, 14 patients were enrolled. Most of the patients who consulted our department were from various areas of Japan, but one (patient 6) came from Bangladesh. The lymphocytes obtained from all of the blood samples responded sufficiently to the T-cell mitogen phytohemagglutinin (Table 1). LTTs for both toki and ogon were positive in 12 (85.7%) out of 14 patients; in 2 patients, the LTT results for both herbs were negative. The SI values of most patients peaked at a dilution of 1/1,710 or 1/5,130. In the two patients with negative results, the counts per minute of the negative control were relatively high compared with those of all of the other patients, except patient 11. Therefore, the LTT result may have been influenced by, at least in part, the counts per minute of the negative control. The mean SIs of toki and ogon were 3.0 and 3.1, respectively. This result suggests that Kampo medicines are likely to be positive in the LTT if judged by the usual

* Corresponding author. Mailing address: Department of Integrated Japanese Oriental Medicine, Gunma University School of Medicine, 3-39-15 Showa-machi, Maebashi, Gunma 371-8511 Japan. Phone: 81-27-220-8666. Fax: 81-27-220-8666. E-mail: nmantani@showa.gunma-u.ac.jp.

TABLE 1. LTTs for Toki and Ogon in first-visit patients

Patient	Age (yr)	Sex ^a	Disease	SI		Control cpm	PHA ^b SI
				Toki	Ogon		
1	30	F	Anxiety	7.4	8.0	67	2,313.3
2	53	F	Rheumatoid arthritis	2.0	2.1	163	825.4
3	72	F	Rheumatoid arthritis	2.5	2.9	111	1,919.4
4	76	M	Neurosis	3.9	1.8	254	1,022.0
5	48	F	Hypertension	3.6	3.6	165	982.5
6	37	M	Lumbago	2.4	2.7	277	814.1
7	67	F	Sciatica	4.5	4.7	91	2,372.7
8	45	F	Heberden's nodes	1.9	3.5	116	972.3
9	54	F	Hypertension	1.1	1.2	428	488.6
10	72	M	Nephritis	3.6	2.7	79	2,503.5
11	26	M	Gastric ulcer	2.9	3.2	710	183.4
12	50	M	Depression	1.2	1.3	427	379.4
13	24	M	Shoulder stiffness	2.1	2.3	296	827.9
14	24	M	Functional dyspepsia	2.9	2.9	171	980.4

^a F, female; M, male.

^b PHA: phytohemagglutinin.

criteria in Japan. Laboratory tests, including white blood cell population counts and liver tests, were performed on 12 patients, and no eosinophilia or liver injury was observed. According to the Kampo diagnosis (sho) of each patient, various Kampo medicines were administered after examination of the LTT result. No liver injury or other adverse reactions were observed thereafter.

Whether the establishment of original criteria for Kampo medicines would be useful or not should be carefully assessed. We recently reported that the SI for each Kampo medicine in patients with Kampo-induced liver injury did not differ from that in patients with other liver diseases (10). Therefore, the establishment of original criteria of LTT for Kampo medicines may not be useful for the diagnosis of Kampo-induced liver injury.

In summary, LTT results for toki and ogon were positive in 12 of 14 enrolled patients who had not taken any Kampo medicines. Although drug-induced liver injury has been diagnosed on the basis of LTT results in Japan, we are convinced that the LTT is unreliable for the diagnosis of Kampo-induced liver injury. The clinical course, exclusion of alternative causes, and reaction to readministration are useful in the diagnosis of Kampo-induced liver injury (10).

This study was performed at Toyama Medical and Pharmaceutical University Hospital, Toyama, Japan.

REFERENCES

- Bircher, A. J. 1995. The lymphocyte transformation test in the diagnosis of immediate type hypersensitivity reactions to penicillins, p. 31–37. *In* C. Surber, P. Elsner, and A. J. Bircher (ed.), *Exogenous dermatology*. Karger, Basel, Switzerland.
- Holland, P., and A. M. Mauer. 1964. Drug-induced in-vitro stimulation of peripheral lymphocytes. *Lancet* **20**:1368–1369.
- Ikemoto, Y., Y. Mizoguchi, T. Arai, S. Yamamoto, and S. Morisawa. 1984. Effect of Sho-saiko-to and Dai-saiko-to on antibody production in vitro. *J. Tradit. Med.* **1**:235–242.
- Kawasaki, A., Y. Mizushima, H. Kunitani, M. Kitagawa, and M. Kobayashi. 1994. A useful diagnostic method for drug-induced pneumonitis: a case report. *Am. J. Chin. Med.* **22**:329–336.
- Kumazawa, Y., Y. Nakatsuru, C. Nishimura, and Y. Otsuka. 1984. Separation of T-mitogenic lectin from Sophorae radix. *J. Tradit. Med.* **1**:182–183. (In Japanese.)
- Kumazawa, Y. 1986. Immunopromotive substances. *Pharmacia Rev.* **21**:105–118. (In Japanese.)
- Mantani, N., T. Kogure, M. Kainuma, Y. Shimada, and K. Terasawa. 2001. Two cases of autoimmune liver disease misdiagnosed by lymphocyte stimulating test for Kampo medicines. *Acta Hepatol. Jpn.* **42**:460–464. (In Japanese.)
- Mantani, N., T. Kogure, M. Kainuma, Y. Shimada, and K. Terasawa. 2002. Review of the literature on Kampo-induced liver injury: in relation to problems of false-positivity on lymphocyte transformation test. *Acta Hepatol. Jpn.* **43**:32–37. (In Japanese with English abstract.)
- Mantani, N., S. Sakai, T. Kogure, H. Goto, N. Shibahara, T. Kita, Y. Shimada, and K. Terasawa. 2002. Herbal medicine and false-positive results on lymphocyte transformation test. *Yakugaku Zasshi* **122**:399–402.
- Mantani, N., T. Kogure, S. Sakai, H. Goto, N. Shibahara, T. Kita, Y. Shimada, and K. Terasawa. 2002. Incidence and clinical features of liver injury related to Kampo (Japanese herbal) medicine in 2,496 cases between 1979 and 1999: problems of the lymphocyte transformation test as a diagnostic method. *Phytomedicine* **9**:280–287.
- Namihisa, T., K. Yamaguchi, and N. Kitami. 1974. Acute hepatic disorders induced by drugs—clinical and laboratory observation of 30 cases. *Jpn. J. Gastroenterol.* **71**:256–270.
- Namba, T., K. Sawa, Y. Hashimoto, Y. Ozaki, M. Hattori, Y. Naruse, and S. Kagamimori. 1989. Studies on development of immunomodulation drugs 1—lymphocyte-mitogenic activity of crude drugs used as anti-allergic drugs in traditional medicine. *J. Tradit. Med.* **6**: 32–39. (In Japanese with English abstract.)
- Onji, M. 1995. Approach to drug-induced liver injury. *J. Jap. Soc. Internal Med.* **84**:200–205. (In Japanese.)
- Tachibana, Y., and K. Kawanishi. 1992. Mitogenic activities in the protein fractions of crude drugs. *Planta Med.* **58**:250–254.
- Uchida, S., and N. Kitami. 1989. Fundamental studies on the lymphocyte stimulation test on patients with drug-induced allergic hepatic injury. *Acta Hepatol. Jpn.* **30**:439–443. (In Japanese with English abstract.)
- Yamada, H., H. Kiyohara, J. C. Cyong, N. Takemoto, Y. Komatsu, H. Kawamura, M. Aburada, and E. Hosoya. 1990. Fractionation and characterization of mitogenic and anti-complementary active fractions from Kampo medicine "Juzen-taiho-to." *Planta Med.* **56**: 386–391.
- Yamada, H., H. Kiyohara, Y. Cho, J. C. Chong, N. Takemoto, H. Kawamura, Y. Komatsu, M. Aburada, and H. Hosoya. 1990. Influence of herbal components on immunomodulatory effect of Juzen-taiho-to. *J. Tradit. Med.* **7**:288–289.
- Yamada, H., H. Kiyohara, N. Takemoto, J. F. Zhao, H. Kawamura, Y. Komatsu, J. C. Cyong, M. Aburada, and E. Hosoya. 1992. Mitogenic and complement activating activities of the herbal components of Juzen-taiho-to. *Planta Med.* **58**:166–170.
- Yamada, H. 1997. Immunomodulatory effects of Kampo medicine, p. 41–55. *In* K. Takagi and M. Kimura (ed.), *Kampo Yakurigaku*. Nankoudo, Tokyo, Japan. (In Japanese.)