LONG-TERM ADMINISTRATION OF "SHO-SAIKO-TO" INCREASES CYTOCHROME P-450 mRNA LEVEL IN MOUSE LIVER

Keisuke KOJIMA, Hajime MIZUKAMI, Takako TAZAWA, Mitsuhiro NOSE, Makoto INOUE, and Yukio OGIHARA

Faculty of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya 467, Japan

Simplified differential display of mRNA was applied to isolate and identify genes transcriptionally regulated in mouse liver by sho-saiko-to administration. A cDNA fragment up-regulated by sho-saiko-to was isolated and characterized. cDNA sequencing and subsequent database analysis revealed that the fragment showed significant sequence similarity with mouse testosterone 16-alpha-hydroxylase (cytochrome P-450 \textit{16a}) cDNA. The increased level of mRNA expression of cytochrome P-450 \textit{16a} in association with sho-saiko-to administration suggests the molecular mechanism of the chemopreventive effect of sho-saiko-to. This result indicates the usefulness of the mRNA differential display technique to investigate the molecular mechanism of Kampo medicine.

KEY WORDS  PCR; differential display; sho-saiko-to; transcriptional regulation; cytochrome P-450 \textit{16a} cDNA

"Sho-saiko-to" has been used for curing various inflammatory diseases including hepatitis and is currently one of the most important prescriptions in Kampo medicine in Japan. Based on recent \textit{in vivo}, \textit{in vitro}, and clinical studies on its pharmaceutical and chemopreventive effects, sho-saiko-to is presumed to improve the biological defense mechanism in liver, but its mechanism is not yet fully understood.\textsuperscript{1-10} This technique involves the reverse transcription of a subset of the mRNA population with an anchored oligo(dT) primer followed by the polymerase chain reaction (PCR) using both the anchored oligo(dT) primer and a specific primer for each gene.

Molecular cloning and characterization of genes of which expression is modulated by sho-saiko-to may not only provide us with important insights into the fundamental mechanism of pharmacological action of sho-saiko-to, but also open new avenues for diagnosis and therapy of sho-saiko-to application.

The cDNA subtraction method has been used to isolate genes that are differentially expressed.\textsuperscript{11} This technology, however, has the disadvantages of being unsuitable for detecting genes with very low expression levels and of being laborious. In contrast, with the differential display (DD) technique, genes expressed at low levels can be isolated and minimal quantities of RNA are required for analysis. This method is essentially an mRNA fingerprinting originally developed by Ling and Pardoe and has been successfully used for isolating and identifying differentially expressed genes in eukaryotic cells.\textsuperscript{12} Fig. 1. Northern Blot Analysis of A1-2-5

A cDNA clone coding for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is used as a control. Twenty micrograms of total RNA was used for hybridization.
Fig. 2. (a) mRNA Expression of A1-2-5 in the Livers of Mice Treated without (left four lanes) and with Sho-saiko-to (right four lanes) and (b) The Intensities of the Bands Hybridized with A1-2-5 Probe. Twenty micrograms of total RNA was loaded into each lane. The arbitrary numbers were obtained by image analysis using LAS-1000 (Fuji Film). Each column represents the mean ± S.E.M. *p<0.05 vs control.

oligo(dT) primer and a short random primer, producing sets of cDNA fragments for identifying differentially expressed cDNAs. In order to identify genes that display transcription changes in mouse liver due to long-term administration of sho-saiko-to, we utilized the DD technique modified by Yoshida et al.\(^{13}\)

Six-week-old female ICR mice (Japan SLC Co.) were administered sho-saiko-to prepared as usual in our laboratory at a dose of 0.46 g/kg/day (five times the human dose) in drinking water for three months. Livers excised from sho-saiko-to-treated mice and control mice (five each) were pooled and used for preparing total RNA using the RNA Extraction Kit (Pharmacia). First-strand cDNA synthesis was performed using the Ready-To-Go T-Primed First-Strand Kit (Pharmacia) with the total RNA as a template. The second-strand cDNA was synthesized by PCR using an arbitrarily chosen short primer (12mer, a single primer was used for each reaction). From comparison of the displays of fingerprint generated using 22 primers between sho-saiko-to-treated mice and
control mice, we were able to identify 16 putative differentially expressed bands. These cDNA fragments were cloned into the pGEM-T vector (Promega) and characterized further. The mRNA expression of these 16 cDNA fragments were investigated by Northern blot analysis. Total RNA from the livers was separated in a 1.2% formaldehyde-agarose gel, transferred to a Hybond-N+ membrane (Amersham), and hybridized with digoxigenin-labeled cDNA fragments prepared using a PCR-DIG probe synthesis kit (Boehringer).

The results showed that of the 16 cDNA clones only A1-2-5 exhibited elevated mRNA expression in the liver of sho-saiko-to-treated mice (Fig. 1). To confirm the up-regulated mRNA expression of A1-2-5 associated with sho-saiko-to administration, total RNA was individually prepared from the liver of four sho-saiko-to-treated mice and four control mice and subjected to Northern blot analysis (Fig. 2). The relative amounts of hybridizable mRNA in the livers of sho-saiko-to-treated mice were significantly higher than those in the control mice (p<0.05, Student's t test), as estimated by image analysis using LAS-1000/Image Gauge (Fuji Film).

The A1-2-5 clone was subjected to DNA sequencing using the Thermo Sequenase fluorescent-labelled primer cycle sequencing kit (Amersham) and a homology search of the GenBank database (BLAST) was carried out. A1-2-5 displayed significant homology to mouse testosterone 16-alpha-hydroxylase (cytochrome P-45016α,14) later abbreviated as Cyp 2d-915) cDNA with 99% identity (Fig. 3). Induction of cytochrome P-450-linked monoxygenase activity in rat liver microsomes by sho-saiko-to was reported by Ohnishi et al.16

To our knowledge, this is the first report describing the isolation and identification of a gene of which transcription is up-regulated by sho-saiko-to. The present results clearly indicate the usefulness of mRNA DD in investigating the molecular mechanism of sho-saiko-to and may act as a catalyst for expansion of this technology to investigation of various other Kampo medicines at the molecular level.

REFERENCES

(Received January 8, 1998; accepted February 24, 1998)