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Original Article

Effects of Some Kampo Medicines on Sorbitol Dehydrogenase

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Abstract: We reported previously that some kampo medicines have aldose reductase (AR) inhibiting activities. Then, we studied the effects of these drugs on sorbitol dehydrogenase (SDH) from rat lenses or sheep liver. SDH is another enzyme constituting the polyol pathway. An 0.1 mg/ml concentration of Hatimi-ziô-gan slightly stimulated rat lens SDH by 11.7%, and the same concentration of Keihi inhibited it by 34.7%. Keisi-ka-zyutubu-tô, Sokei-kakketu-tô, Gosya-zinki-gan, Shakuyaku, Kanzo, Sojutsu, Kakobushimatsu, Taiso and Shokyo had no effect on SDH activities. Nevertheless, since these kampo medicines inhibit AR activities, kampo medicines reduce the sorbitol content in tissues by inhibiting AR and not by stimulating SDH.

Key words: Sorbitol dehydrogenase, Aldose reductase, Kampo medicines, Polyol pathway, Diabetic complications

INTRODUCTION

The increased intracellular sorbitol accumulation by the polyol pathway has been implicated in the pathogenesis of diabetic complications.[1,2)] The polyol pathway consists of two enzymes, aldose reductase (AR), by which glucose is reduced to sorbitol, and sorbitol dehydrogenase (SDH), by which sorbitol is metabolized to fructose.[2-4)]

Recent studies suggest that the AR inhibitors improve or prevent diabetic complications. We reported previously that some kampo medicines and their crude drugs, the constituents of kampo medicines, have AR inhibiting activities.[5)] We, then, studied the effects of these drugs on SDH from rat lenses or sheep liver, since SDH activity increases in diabetic subjects who were given kampo medicines.[6)]

Abbreviations; Keisi-ka-zyutubu-tô (Gui-Zhi-Jia-Shu-Fu-Tang), 桂枝加朮附湯; Sokei-kakketu-tô (Shu-Jing-Huo-Xie-Tang), 疎経活血湯: Gosya-zinki-gan (Niu-Che-Shen-Qi-Wan), 牛車腎気丸; Hatimi-ziô-gan (Ba-Wei-Di-Huang-Wan, 八味地黄丸

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MATERIAL AND METHODS

Materials; The following kampo medicines and their crude drugs, the constituents of kampo medicines, were generous gifts of Tsumura Pharmaceutical Co., Ltd. (Tokyo, Japan): Keisi-ka-zyutubu-tô (Gui-Zhi-Jia-Shu-Fu-Tang), Sokei-kakketu-tô (Shu-Jing-Huo-Xie-Tang), Gosya-zinki-gan (Niu-Che-Shen-Qi-Wan), Hatimi-ziô-gan (Ba-Wei-Di-Huang-Wan), Shakuyaku (Paeoniae radix), Kanzo (Glycyrrhizae radix), Sojutsu (Atractylodis Lanceae rhizoma), Keihi (Cinnamoni cortex), Taiso (Zizyphi fructus), Shokyo (Zingiberis rhizoma), and kakobushimatsu (Aconiti tuber). The constituents of each of the kampo medicines are indicated in Table 1. The extractions were obtained by boiling the herbs in water for 60 min; the extracts were then spray-dried into powders.

Table 1. The constituents of kampo medicines

Herbs	Kampo medicines			
	Keisi-ka-zyutubu-tô	Sokei-kakketu-tô	Gosya-zinki-gan	Hatimi-ziô-gan
Cinnamoni cortex (*Cinnamonum cassia* BLUME)	4		4	1
Paeoniae radix (*Paeonia lactiflora* PALLAS)	4	2.5		
Atractylodis Lanceae rhizoma (*Atractylodes lancea* DE CANDOLLE)	4	2		
Zizyphi fructus (*Zizyphus jujuba* MILLER var. *inermis* REHDER)	4			
Glycyrrhizae radix (*Glycyrrhiza glabra* LINNE' var. *glandulifera* REGEL et HERDER)	2	1		
Zingiberis rhizoma (*Zingiber officinale* ROSCOE)	1			
Aconiti tuber (*Aconitum carmichaeli* DEBEAUX)	0.5		1	0.5
Rehmanniae radix (*Rahmannia glutinosa* LIBOSCHITZ var. *purpurea* MAKINO)		2	5	6
Cnidii rhizoma (*Cnidium officinale* MAKINO)		2		
Angelicae radix (*Angelica acutiloba* KITAGAWA)		2		
Persicae semen (*Prunus persica* BATSCH)		2		
Hoelen (*Poria cocos* WOLF)		2	3	3
Achyranthis radix (*Achyranthes fauriei* LEVEILLE' et VANIOT)		1.5	3	
Aurantii nobilis pericarpium (*Citrus unshiu* MARKOVICH)		1.5		
Sinomeni Caulis et rhizoma (*Sinomenium acutum* REHDER et WILSON)		1.5		
Ledebouriellae radix (*Ledebouriella seseloides* WOLFF)		1.5		
Gentianae scabrae radix (*Gentianae scabra* BUNGE)		1.5		
Angelicae dahuricae radix (*Angeeica dahurica* BENTHAM et HOOKER)		1		
Clematidis radix (*Clematis chinensis* OSBECK)		1.5		
Notopterygii rhizoma (*Notopterygium forbesii* BOISS)		1.5		
Corni fructus (*Cornus officinalis* SIEBOLD et ZUCCARINI)			3	3
Dioscoreae rhizoma (*Dioscorea japonica* THUNBERG)			3	3
Plantaginis semen (*Plantago asiatica* LINNE')			3	
Alismatis rhizoma (*Alisma orientale* JUZEPCZUK)			3	3
Moutan cortex (*Paeonia suffruticosa* ANDREWS)			3	2.5

The numbers indicated mean the ratio of the combinations of herbs.

SDH activities; SDH activity was measured in reagents containing 0.1 M sodium pyrophosphate pH 9.5, 40 mM sorbitol, 1 mM NAD and 50 μl of enzyme fractions, with or without 100 μl of various concentrations of drugs in a total volume of 1.5 ml. Reactions were initiated by the addition of enzyme fraction, and the increase in fluorescence was monitored using Hitachi F3000 fluorescence spectrophotometer (Hitachi, Japan). It had an excitation wave length of 366 nm and an emission wave length of 452nm. The fluorescence was linealy increased up to 2 min. The enzyme activity was calculated from the Δfluorescence/min for each reaction.

We used rat lens homogenate and purified sheep liver SDH (Boehringer Mannheim, Mannheim, West Germany) as enzymes. Lenses from male Wistar rats were homogenized in 135 mM sodium, potassium, phosphate buffer, pH 7.0, containing 0.5 mM phenylmethyl-sulfonyl fluoride, 10 mM 2-mercaptoethanol, and centrifuged at 105,000 $\times$ g for 30 min. The supernatant was then used as an enzyme

fraction.

Experiments in rat lens enzyme were performed in triplicate at least, and we analyzed two different experiments. When purified sheep liver SDH was used, experiments were performed in duplicate.

Results

Three kampo medicines, Sokei-kakketu-tô, Keisi-ka-zyutubu-tô and Gosya-zinki-gan had no significant effect on rat lens SDH activities at a concentration of 0.1 mg/ml. Hatimi-ziô-gan slightly but significantly stimulated rat lens SDH by 11.7% (Table 2). At a concentration of 0.001 mg/ml, these four drugs had no effect on SDH activities.

Table 2. Effects of kampo medicines on sorbitol dehydrogenase activities (rat lens)*

Kampo medicines	Concentrations of drugs	
	0.1 mg/ml	0.001 mg/ml
Without drugs (10)	100%	100%
Sokei-kakketu-tô (8)	101.2±3.3	101.1±0.7
Keisi-ka-zyutubu-tô (10)	99.6±4.1	104.1±0.9
Gosya-zinki-gan (10)	103.7±2.4	98.7±2.4
Hatimi-ziô-gan (8)	111.7±2.8q	106.6±2.5

*: The enzyme activities were expressed in relative activity compared to that without drugs.
q: significant vs. without drugs, $p<0.01$
Number in parenthesis represents the number of experiments.

Table 3. Effects of crude drugs on sorbitol dehydrogenase activities (rat lens)*

Crude drugs	Concentrations of drugs	
	0.1 mg/ml	0.001 mg/ml
Without drugs (10)	100%	100%
Shakuyaku (9)	104.0±2.5	99.4±2.5
Kanzo (6)	88.0±7.5	100.1±3.0
Sojutsu (6)	103.0±1.7	100.4±2.7
Keihi (9)	65.3±2.6q	98.7±10.7
Kakobushimatsu (6)	94.5±6.1	103.7±4.2
Taiso (6)	99.4±2.9	99.2±2.2
Shokyo (6)	99.1±3.2	101.9±5.1

*: The enzyme activities were expressed in relative activity compared to that without drugs.
q: significant vs. without drugs. $p<0.001$
Number in parenthesis represents the number of experiments.

Table 4. Effects of kampo medicines on sorbitol dehydrogenase activities (sheep liver)*

Kampo medicines	Concentrations of drugs	
	0.1 mg/ml	0.001 mg/ml
Without drugs	100%	100%
Sokei-kakketsu-tô	92.2	98.8
Keisi-ka-zyutubu-tô	88.2	104.7
Gosya-zinki-gan	92.5	96.9
Hatimi-ziô-gan	90.6	101.8

*: The enzyme activities were expressed in relative activity compared to that without drugs.

Table 5. Effects of crude drugs on sorbitol dehydrogenase activities (sheep liver)*

Crude drugs	Concentrations of crude drugs	
	0.1 mg/ml	0.001 mg/ml
Without drugs	100%	100%
Shakuyaku	96.5	103.1
Kanzo	81.9	100.4
Sojutsu	102.8	98.5
Keihi	79.0	106.2
Kakobushimatsu	99.6	101.9
Taiso	100.1	98.3
Shokyo	90.8	98.3

*: The enzyme activities were expressed in relative activity compared to that without drugs.

In Table 3, the effects of seven crude drugs on rat lens SDH are shown. Keihi significantly decreased enzyme activity to 65.3±2.6%, while the remaining six crude drugs, used at a concentration of 0.1 mg/ml, had no effects.

Almost the same results were obtained when purified sheep liver SDH was used (Tables 4 and 5).

Discussion

Traditionally in Japan, some of the kampo medicines have long been used to alleviate the subjective symptoms of diabetic neuropathy.[7,8] In our previous report,[5] we showed that four kampo medicines and some crude drugs have inhibitory activities on rat lens AR. At a concentration of 0.1 mg/ml, Keisi-ka-zyutubu-tô inhibited rat lens AR by 91.3%, Sokei-kakketu-tô by 80.3%, Gosya-zinki-gan by 72.6% and Hatimi-ziô-gan by 62.6%, respectively. The same concentration of Shakuyaku and Kanzo inhibited it by 100%, Keihi by 93.9% and Sojutsu by 81.4%, respectively. Sokei-kakketu-tô, Keisi-ka-zyutubu-tô, Shakuyaku and Kanzo also inhibited the accumulation of sorbitol in human red blood cells *in vitro*. Therefore, kampo medicines inhibit AR activities.

Recently, Akazawa *et al.*[6] reported that erythrocyte sorbitol contents were reduced in diabetic patients who were given Gosya-zinki-gan, Keisi-ka-zyutubu-tô or Sokei-kakketu-tô. This finding supported our previous results. They also reported increased activities of SDH of erythrocytes in diabetic patients who were given Hatimi-ziô-gan or Keisi-ka-zyutubu-tô. In our *in vitro* experiments, however, only Hatimi-ziô-gan, at a concentration of 0.1 mg/ml, increased SDH activity by 11.7%. The other three kampo medicines had no effect on SDH activity. We are in some doubt as to whether Hatimi-ziô-gan also exerts its effect *in vivo*, since its effect was observed only at a high concentration. With regard to crude drugs, only Keihi, at a concentration of 0.1 mg/ml, inhibited SDH activity. We conclude that the kampo medicines and crude drugs, except for Keihi, have almost no effect on SDH activities, but do inhibit AR activities.[5] Therefore, it is likely that these kampo medicines inhibit sorbitol accumulation but do not interfere with the metabolism of previously accumulated intracellular sorbitol.

So, our data again suggest that these kampo medicines reduce sorbitol content by inhibiting AR rather than stimulating SDH.

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