Effects of Saiko-ka-ryukotsu-borei-to (Kampo medicine) on abnormal plasma lipoprotein and glucose metabolism in diabetic patients: A comparison with Dai-saiko-to

Tomio ONUMA,*[a] Masahiro TSUTSUI,[a] Akitoshi BOKU,[a] Michitaka SHIMURA,[a] Toru KIKUCHI,[a] Kazuo TAKEBE,[a] Mitsuo MASUDA,[b] Shigeru OCHIAI,[c] Osamu UEHARA,[c] Mikihiko KUDO,[d] Hitoshi YASHIRO,[e] Morio SAGARA[f]

[a] *Third Department of Internal Medicine, Hirosaki University School of Medicine,*
[b] *Internal Medicine, Aomori Municipal Hospital,* [c] *Internal Medicine, Odate Municipal Hospital,*
[d] *Internal Medicine, Hachinohe Municipal Hospital,* [e] *Internal Medicine, Itayanagi Central Hospital,*
[f] *Internal Medicine, Hirosaki Municipal Hospital*

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Abstract

This study was performed to compare the effects of Kampo midicines, Saiko-ka-ryukotsu-borei-to (TJ-12) and Dai-saiko-to (TJ-8), on abnormal plasma lipoproteins and glucose metabolism in 32 patients with non-insulin-dependent diabetes mellitus (NIDDM). The daily dosage was 7.5 g for both TJ-12 and TJ-8. The administration period was 12 weeks in both groups. In the TJ-12 group, serum total cholesterol (TC) levels after 4 and 12 weeks of treatment were significantly lower than the pre-administration TC level, and low density lipoprotein-cholesterol (LDL-C) levels after 4, 8 and 12 weeks of treatment were significantly lower than the pre-administration LDL-C level. In the TJ-8 group, these two parameters showed no change during the treatment. In the TJ-12 group, apolipoprotein (apo)-B decreased slightly during the treatment and the LDL-C/apo-B ratio decreased significantly compared with their pre-administration levels. In the TJ-8 group, neither apo-B nor LDL-C/apo-B ratio showed any change during the treatment. Triglyceride, high density lipoprotein (HDL)-C and apo AI levels showed no change during the treatment in both groups. The atherogenic index [(TC-HDL-C) / HDL-C] decreased significantly after 8 and 12 weeks of treatment in the TJ-12 group, although it did not change in the TJ-8 group. Fasting plasma glucose and hemoglobin A_{1C} showed no change during the treatment in both groups. These results suggest that TJ-12 might play a role in improving abnormal lipoproteins and reducing atherosclerosis in NIDDM.

Key words Saiko-ka-ryukotsu-borei-to (Kampo medicine), serum lipids, apolipoprotein, diabetes mellitus, atherosclerosis.

Abbreviations NIDDM, non-insulin-dependent diabetes mellitus: TJ-8, Dai-saiko-to (大柴胡湯): TJ-12, Saiko-ka-ryukotsu-borei-to (柴胡加竜骨牡蛎湯); TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein-cholesterol; apo, apolipoprotein; LDL-C, low density lipoprotein-cholesterol; FPG, fasting plasma glucose; HbA_{1C}, hemoglobin A_{1C}; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; $HepG_2$, human hepatoblastoma cell line.

Introduction

Abnormal plasma lipoprotein levels, which are often seen in patients with non-insulin-dependent diabetes mellitus (NIDDM), are an important risk factor for atherosclerosis. Some Kampo medicines are known to improve plasma lipoprotein abnormalities.

*〒036 弘前市在府町5
弘前大学医学部 第3内科 小沼富男
5 Zaifu-cho, Hirosaki-shi, Aomori 036, Japan

Both Dai-saiko-to (大柴胡湯 : TJ-8) and Saiko-ka-ryukotsu-borei-to (柴胡加竜骨牡蛎湯 ; TJ-12) contain the same herbal drug, Saiko (柴胡 ; Bupleurum falcatum) which has an effect on improving lipid metabolism. There are already some reports showing the improvement with TJ-8.[1,2] Although TJ-12 is known to have antiatherosclerotic action, its effect on abnormal serum lipids is not yet adequately understood.[3,4] Furthermore, all previous studies of the antiatherosclerotic effect of TJ-12 were performed using non-diabetic animals, and its effect on humans has not been studied. Moreover, there are no reports of the effects of TJ-12 on glucose matabolism. Therefore, we compared the effects of TJ-12 and TJ-8 on abnormal plasma lipoproteins and glucose metabolism in patients with NIDDM.

Subjects and Methods

The subjects were 32 patients with NIDDM, accompanied by hyperlipidemia, who satisfied at least one of the following : (1) serum total cholesterol (TC) over 220 mg/dl and (2) serum triglyceride (TG) over 150 mg/dl. The Sho (証) which is an indication for use of Kampo medicine given in the classics was assessed for each patient. Patients showing Chukan-Sho (中間証 ; middle resistance) were treated with TJ-12, while those showing Jitsu-Sho (実証 ; strong resistance) were treated with TJ-8. All patients provided informed consent to participation in the study. Two patients developed diarrhea immediately after the start of TJ-8 therapy. The drug was discontinued in these 2 patients. The remaining 30 patients were included in the evaluation.

Clinical characteristics in the patient for the two groups were shown in Table I. The TJ-12 group consisted of 12 patients, and the TJ-8 group of 18 patients. The body mass index was slightly lower in the TJ-12 group than in the TJ-8 group, though the difference was not significant. There was no significant difference in the duration of diabetes mellitus, the kind of diabetic treatment and the presence of complications between the two groups. There was also no significant difference in the presence of hypertension between the two groups. The predominant phenotype of hyperlipidemia was IIa in the TJ-12 group, while it was IIb in the TJ-8 group. There was no significant difference in smoking, alcohol consumption and the use of beta-blockers which relate to serum lipid levels, between the two groups.

Table I Clinical characteristics in diabetic patients administered Saiko-ka-ryukotsu-borei-to (TJ-12) and Dai-saiko-to (TJ-8).

	TJ 12	TJ 8
n (male/female)	12 (1/11)	18 (2/16)
age (yrs)	61±8[a]	65±8
BMI (kg/m^2)	23.7±3.4	25.0±3.2
duration of diabetes (yrs)	8±6	7±6
treatment of diabetes (n)		
diet:oral : insulin	7 : 3 : 2	8 : 5 : 5
complications (n)		
Retinopathy : Nephropathy : IHD	2 : 3 : 0	5 : 5 : 1
hypertension (n)	4	7
type of hyperlipidemia (n)		
IIa : IIb ; IV	11 : 1 : 0*	6 : 11 : 1
smoking (n)	0	3
alcohol intake (n)	1	2
use of beta blockers (n)	0	2
Calcium antagonists	2	3
ACE inhibitors	0	2

[a]mean ±S.D. *$p<0.01$ (vs. value of TJ-8)
IHD=ischemic heart disease, ACE=angiotensin converting enzyme

The daily dosage was 7.5 g for both TJ-12 and TJ-8. The administration period was 12 weeks in both groups. Serum TC, TG and high density lipoprotein-cholesterol (HDL-C) levels were measured immediately before and every 4 weeks after administration of drugs. Serum TC and TG levels were assayed by the enzymatic methods,[5,6] and HDL-C level was estimated after lipoproteins containing apolipoprotein (apo) B were precipitated with heparin-MnCl.[7] Low density lipoprotein (LDL-C) level was calculated by Friedewald's formula.[8] Apo AI, AII and B levels were measured before and 12 weeks after the administration by the turbidimetric immuniassay using a kit (Daiichi Kagaku Co., Japan). Immediately before and every 4 weeks after the administration, the fasting plasma glucose (FPG) level was measured by the enzymatic method using glucose oxidase and hemoglobin A_{1C} (HbA_{1C}) level was measured by high-performance liquid chromatography (Kyoto-daiichikagaku Co., Japan).

The values of each parameter were expressed in

Table II Changes in serum lipids after administration of Saiko-ka-ryukotsu-borei-to (TJ-12) and Dai-saiko-to (TJ-8).

	before	after 4 weeks	8 weeks	12 weeks
Total cholesterol (mg/dl)				
TJ-12	274.3±42.2	246.4±35.9**	248.1±35.1	239.5±30.0**
TJ-8	260.8±30.7	251.7±41.8	253.0±33.2	262.2±36.6
LDL cholesterol (mg/dl)				
TJ-12	195.5±38.9	172.8±40.5*	162.7±30.2*	160.5±29.0**
TJ-8	172.6±40.0	161.1±43.9	161.0±38.6	168.3±37.5
Triglyceride (mg/dl)				
TJ-12	106.1±32.7	101.9±28.6	115.8±16.9	114.5±43.5
TJ-8	181.2±102.6[a]	188.1±129.4	199.5±142.6	195.5±148.2
HDL cholesterol (mg/dl)				
TJ-12	57.6±15.6	53.2±10.8	62.2±18.3	56.2±11.5
TJ-8	52.0±15.0	52.9±16.4	52.1±16.0	54.8±18.2
Atherogenic index				
TJ-12	4.06±1.57	3.85±1.44	3.19±1.01*	3.46±1.25*
TJ-8	4.46±1.77	4.23±1.89	4.27±1.60	4.25±1.70

Values are maen ±S.D. Groups of TJ-12 and TJ-8 consist of 12 and 18 cases, respectively.
*$p<0.05$, **$p<0.01$ (vs. value of before), [a]$p<0.05$ (vs. value of TJ-12)

mean±S.D.. The significance of differences between pre- and post- administration measurements was tested, using paired t-test. The significance of inter-group differences was tested, using unpaired t-test or chi square test. The p-value less than 0.05 was regarded as significant.

Results

In the TJ-12 therapy group, TC levels after 4 and 12 weeks of treatment were significantly lower than the pre-administration TC level, and LDL-C levels after 4, 8 and 12 weeks of treatment were significantly lower than the pre-administration LDL-C level. In the TJ-8 therapy group, on the other hand, these two parameters showed no change during the treatment (Table II). The pre-administration TG level was significantly lower in the TJ-12 group than in the TJ-8 group. TG and HDL-C showed no change during the treatment in both groups. The atherogenic index[(TC-HDL-C) / HDL-C] decreased significantly after 8 and 12 weeks of treatment in the TJ-12 group, although it did not change in the TJ-8 group.

In the TJ-12 group, apo-B decreased slightly during the treatment and the LDL-C/apo-B ratio decreased significantly compared with their pre-administration levels. In the TJ-8 group, neither apo-

Table III Changes in serum apolipoproteins after administration of Saiko-ka-ryukotsu-borei-to (TJ-12) and Dai-saiko-to (TJ-8).

	before	after
apolipoprotein B (mg/dl)		
TJ-12	133.0±28.5	121.4±21.1+
TJ-8	128.2±19.4	129.9±26.9
LDL cholesterol / apolipoprotein B		
TJ-12	1.55±0.28	1.39±0.17*
TJ-8	1.33±0.27	1.31±0.31
apolipoprotein AI (mg/dl)		
TJ-12	147.4±25.2	138.3±12.1
TJ-8	144.0±30.8	147.4±32.0
apolipoprotein AII (mg/dl)		
TJ-12	29.8±6.4	29.4±4.2
TJ-8	34.1±5.8	37.1±7.6**
apolipoprotein AI / apolipoprotein AII		
TJ-12	5.36±2.49	4.79±0.82
TJ-8	4.27±0.84	4.00±0.53
apolipoprotein B / apolipoprotein AI		
TJ-12	0.89±0.23	0.89±0.19
TJ-8	0.90±0.28	0.89±0.27

Values are mean ±S.D. Groups of TJ-12 and TJ-8 measured apolipoprotein B consist of 8 and 13 cases, respectively. Groups of TJ-12 and TJ-8 measured apolipoprotein AI and AII consist of 8 and 13 cases, respectively.
+$p<0.1$, *$p<0.05$, **$p<0.01$ (vs. value of before),

B nor LDL-C/apo-B ratio showed any change during the treatment (Table III). Apo-AI level showed no change in both groups. Apo-AII level increased significantly during the treatment in the TJ-8 group, while it showed no change in the TJ-12 group. The apo-AI/apo-AII and the apo-B/apo-AI ratios remained unchanged in both groups.

FPG and HbA_{1C} showed no change during the treatment in both groups (Table IV).

Body weight showed no change during the treatment in both groups.

No side effects were observed in the TJ-12 group, whereas one patient in the TJ-8 group developed diarrhea and vomiting immediately after the administration. These symptoms subsided soon after the drug was discontinued. Analysis of laboratory parameters revealed slight increases in GOT and GPT after 12 weeks of treatment in two patients (one in the TJ-12 group and the other in the TJ-8 group). These laboratory parameters normalized after the drugs were discontinued. No abnormal change in renal function or peripheral blood analysis was observed in both groups.

Table IV Changes in fasting plasma glucose and hemoglobin A_{1C} after administration of Saiko ka-ryukotsu borei-to (TK-12) and Dai saiko to(TJ-8).

	before	after 4 weeks	8 weeks	12 weeks
Fasting plasma glucose (mg/dl)				
TJ-12	138.8±35.8	146.6±48.6	145.0±39.6	142.6±37.2
TJ-8	150.2±24.1	150.8±35.5	161.5±35.7	143.7±44.1
Hemoglobin A_{1C} (%)				
TJ-12	7.29±1.31	7.58±1.37	7.05±1.51	6.95±1.18
TJ-8	7.64±1.84	7.73±1.70	7.67±1.97	7.47±1.79

Values are mean ±S.D. Groups of TJ-12 and TJ-8 measured fasting plasma glucose consist of 12 and 18 cases, respectively.
Groups of TJ-12 and TJ-8 measured hemoglobin A_{1C} consist of 8 and 13 cases, respectively.

Discussion

TJ-12 was reported to suppress lipid deposition on the aortic and cardiac walls of rabbits[3] ; however, its effects on the serum lipids were not examined. The effects of TJ-12 on human serum lipids and lipoproteins are also unknown. The effect of TJ-12 in improving lipid metabolism has been attributed to saponin contained in Saiko[9] which is an herbal drug constituting the TJ-12 preparation. The same effect has also been noted for ginseng saponin[10] constituting the TJ-12. In the present study, we examined the effects of TJ-12 in patients with NIDDM. a disease often complicated by atherosclerosis or abnormal plasma lipoprotein levels which are risk factors for atherosclerosis. TJ-12 treatment resulted in antiatherosclerotic changes, such as sigificant decreases in serum TC, serum LDL-C and atherogenic index, and a slight decrease in serum apo-B.

In addition to a reduction in serum apo-B, the LDL-C/apo-B ratio which was analyzed as an index of the lipid/protein ratio of LDL also decreased following TJ-12 treatment. This change suggests a decrease in the size of LDL particles. The influence of this change on atherosclerosis remains to be clarified. When the apo-AI/apo-AII ratio was analyzed as an index of HDL subfractions, *i.e.*, HDL_2 and HDL_3, this ratio was not affected by TJ-12 treatment. This finding indicates that TJ-12 has no effect on HDL subfractions.

The mechanisms of reduction in TC, LDL-C and apo B by TJ-12 treatment are unknown. It was recently reported that TJ-12 reduced intracellular cholesteryl ester synthesis in human hepatoblastoma cell line, $HepG_2$ cells and apo-B secretion into the medium.[4] These experimental results can explain one mechanism of our data in this study.

When the effects of TJ-12 were compared with those of TJ-8, which has been shown to improve serum lipids, we found that the antiatherosclerotic effect through the improvement of plasma lipoprotein level was greater with TJ-12 than with TJ-8. In the

TJ 8 group, HDL-C did not significantly change although the apo-AII level increased; however, the clinical significance of these findings is unclear. It was recently reported that the effects of reducing intracellular cholesteryl ester synthesis and apo-B secrecion into medium were greater in $HepG_2$ cells with TJ-12 than with TJ-8.[1] These experimental results can support our data of this study showing the difference in improvement of serum lipid and lipoprotein levels between TJ-8 and TJ-12 groups. Furthermore, improvement in serum lipid and lipoprotein levels after TJ 8 treatment was very small, which is in disagreement with the findings of a previous clinical trial.[2] In the trial, decrease in levels of TC, LDL-C and atherogenic index, and increase in apo-AI, -AII and -B were reported after administration of TJ-8 for 16 weeks. Although the exact cause for this discrepancy is unknown, we cannot rule out that the following factors are related to the less marked improvement in serum lipid and lipoprotein levels in TJ 8-treated patients: (1) a higher percentage of obese patients in the TJ-8 group, and (2) a higher percentage of patients with phenotype IIb hyperlipidemia in the TJ-8 group, which may be based on the Sho assessment.

No previous study has examined the effects of TJ-12 on glucose metabolism. In the present study, TJ-12 did not affect glucose metabolism as analyzed with respect to FPG and HbA_{1C}.

In summary, the effects of reducing hyperlipidemia and the resultant antiatherosclerotic effect were greater with TJ-12 in NIDDM patients showing Chukan-Sho than with TJ 8 in ones showing Jitsu-Sho. These results suggest that TJ-12 might play a role in improving abnormal lipoproteins and reducing atherosclerosis in NIDDM patients showing Chukan-Sho.

和文抄録

インスリン非依存型糖尿病（NIDDM）30例に合併した血中リポ蛋白および糖代謝異常に対する柴胡加竜骨牡蛎湯（TJ-12）の効果について，大柴胡湯（TJ-8）の場合と比較検討した。いずれも投与量は 7.5 g/日とし，投与期間は 12 週間とした。TJ-12 群において，血清総コレステロール（TC）は投与前と比べて投与 4 および 12 週後に有意に低下し，LDL-C は投与 4，8，12 週後に有意に低下した。TJ-8 群において，それらはいずれも投与後一定の変動を認めなかった。TJ-12 群において，アポリポ蛋白（apo）B は投与前と比べて投与後に低下傾向を示し，LDL-C/apoB 比は投与後に有意に低下した。TJ-8 群において，それらはいずれも投与後一定の変動を認めなかった。血清トリグリセリド，HDL-C, apo AI は両群において投与後に一定の変動を示さなかった。atherogenic index［(TC－HDL-C)/HDL-C］は，TJ-12 群が投与前と比べて投与 8，12 週後に有意に低下し，TJ-8 群は投与後一定の変動を認めなかった。空腹時血糖，ヘモグロビン A_{1C} は TJ-12 群，TJ-8 群のいずれにおいても投与前後で一定の変動を認めなかった。以上より，TJ-12 は TJ-8 と比較して，NIDDM に合併した高脂血症をより改善させ，抗動脈硬化的に作用することが示唆された。

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