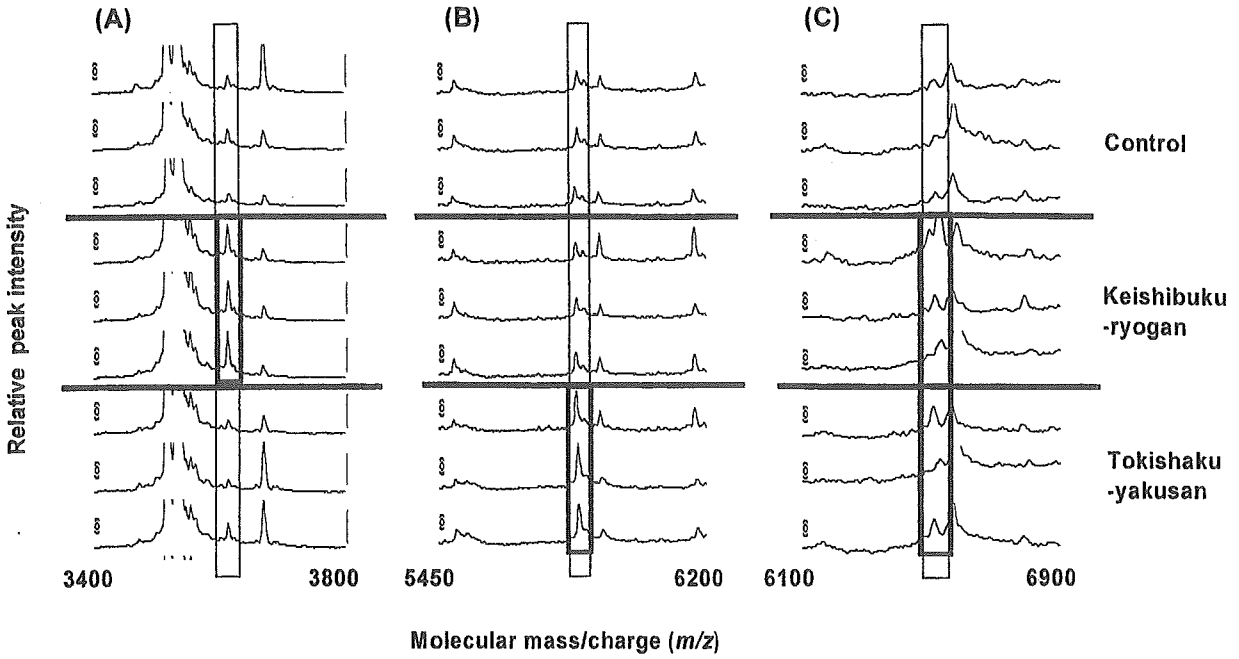

Figure 4 Typical peaks recognized by administration of keishibukuryogan and tokishakuyakusan. A) A typical peak elevated by administration of keishibukuryogan. B) A typical peak elevated by administration of tokishakuyakusan. C) A typical peak elevated by administration of both keishibukuryogan and tokishakuyakusan.

Table 2 Summary of *m/z* values and peak intensities changed significantly in the diabetic rats by keishiburyogan and tokishakuyakusan

	Peak Intensity (mean ± S.D.)		
m/z	Control	Keishibukuryogan	Tokishakuyakusan
3264.772	0.208 ± 0.102	0.309 ± 0.213	0.463 ± 0.400
3280.847	1.296 ± 0.187	1.138 ± 0.210	1.274 ± 0.372
3612.813	8.356 ± 2.445	11.998 ± 4.389	8.844 ± 3.864
3899.419	0.957 ± 0.146	1.022 ± 0.335	1.256 ± 0.183
4433.000	1.229 ± 0.302	1.506 ± 0.459	1.722 ± 0.623
4459.540	0.364 ± 0.142	0.519 ± 0.128	0.524 ± 0.185
4490.796	0.114 ± 0.093	0.221 ± 0.096	0.193 ± 0.104
5837.196	3.937 ± 0.696	4.124 ± 0.887	5.006 ± 0.690
5854.691	1.642 ± 0.202	1.742 ± 0.249	1.835 ± 0.203
6506.587	1.149 ± 0.313	1.385 ± 0.280	1.618 ± 0.665

Underline : $p<0.05$ vs. control by Mann-Whitney *U* test.

to the control group (Table 1).

Changes in plasma protein profiling of diabetic rats after oral administration of keishibukuryogan and tokishakuyakusan. We investigated the influence of keishibukuryogan and tokishakuyakusan on the expression patterns of plasma proteins in WBN/Kob rats at 67 weeks of age. Spectral analysis of samples (control group, n=7; keishibukuryogan group, n=7; tokishakuyakusan group, n=7) was performed in duplicate using the ProteinChip software program. Approximately 230 peaks per spectrum were detected in the 2000-10000 Da mass range. Representative spectra of plasma proteins of WBN/Kob rats are shown in Fig. 4. As summarized in Table 2, administration of keishibukuryogan led to significant changes in the intensities of the five peaks (*m/z* 3280, 3612, 4459, 4490 and 6506), and that of tokishakuyakusan led to significant elevation in the intensities of eight peaks (m/z 3264, 3899, 4433, 4459, 4490, 5837, 5854, and 6506), compared with control diabetic rats. Three of the peaks (m/z 4459, 4490 and 6506) were elevated by the administration of both keishibukuryogan and tokishakuyakusan.

Discussion

The WBN/Kob rat is a spontaneously insulin-dependent diabetic rat, in which the diabetes is caused by pancreatitis. This animal has a long life span, so several hyperglycemia-induced complications have been observed.[1] Several vascular dysfunctions have also been observed in WBN/Kob rats, such as endothelial dysfunction.[7] In this regard, some Kampo formulations for overcoming oketsu have been used to treat gynecological, psychiatric, and dermatological diseases *etc.* For arteriosclerosis, vascular protective effects that improve blood circulation have been reported for formulations used for overcoming oketsu.[8,9] Recently, we demonstrated that keishibukuryogan improved vascular dysfunction in WBN/Kob rats through improvement of endothelial function, suppression of vasocontraction, and decreasing blood viscosity.[2]

In the present study, we examined the effects of tokishakuyakusan in WBN/Kob rats in comparison with the effects of keishibukuryogan. The results were that keishibukuryogan was effective at improving endothelial function more than tokishakuyakusan, while tokishakuyaku-

san was effective at improving blood fluidity more than keishibukuryogan. Because the different Kampo formulations caused different responses in vascular function even in the same model, it is thought that the responses of the body also vary according to the various kinds of Kampo formulations. This variety in body responses is explained by the Kampo diagnosis of "Sho".

Since Kampo formulations are generally prepared from the combination of several crude drugs, these drugs are believed to have harmonization effects, which results in different effects than each individual crude drug. Therefore, in order to evaluate the influence of Kampo formulations on various diseases that result from multiple factors, an inclusive analytical method, such as ProteinChip technology, may be useful for profiling biological mixtures and identifying multiple biomarkers associated with diseases. We recently reported that hachimijiogan prevented renal dysfunction-induced hyperglycemia in WBN/Kob rat, and that several plasma proteins may be involved in the progression of disease and the efficacy of hachimijiogan.[10] Here we identify one of these proteins and evaluate the relationship between the efficacy of hachimijiogan and expression profiling.

The expression pattern of plasma proteins by SELDI profiling revealed that five peaks in the keishibukuryogan-treated group and eight peaks in the tokishakuyakusan-treated group were significantly changed in the 2000-10000 Da mass range compared to the control group (Table 2). Three peaks at *m/z* 4459, 4490 and 6506 were observed to be commonly elevated after the oral administration of keishibukuryogan and tokishakuyakusan. On vascular functions and blood fluidity particular effects were seen with keishibukuryogan and tokishakuyakusan. Further studies will be needed to examine the correlation between these effects and expression patterns of plasma proteins in detail. In Kampo medicine, keishibukuryogan is used for the patient who is Yang and hyperfunctional, while tokishakuyakusan is used for the patient who is Yin and hypofunctional. Thus, the differences of "Sho" for both medicines may be related to the differences in expression profiling of proteins in plasma as well as the mechanism of action.

Table 3 shows the summary of our present and previous studies on the expression patterns of plasma proteins in spontaneously diabetic rats after oral administration of three Kampo medicines using SELDI-TOF-MS. Administration of hachimijiogan significantly decreased the increased levels of six plasma proteins as compared with the control group.[10] These six plasma proteins associated with hachimijiogan were evidently different at the *m/z* level from those of formulations for overcoming oketsu, keishibukuryogan and tokishakuyakusan (Table 3). As increase in the intensities of three peaks at *m/z* 4459, 4490 and 6506 was commonly observed in both keishibukuryogan and tokishakuyakusan groups, the expression of these proteins may be specific or characteristic in the formulations for overcoming oketsu. These results may provide an important basis for clarifying "Sho" and the determination of "Sho"-directed formulations. On the other hand, shakuyaku and bukuryo are commonly contained in keishibukuryogan and tokishakuyakusan; keishi, botanpi and bukuryo in keishibukuryogan and hachimijiogan; and takusha and bukuryo in tokishakuyakusan and hachimijiogan. Thus, further study will be needed to examine whether the combination of crude drugs contained in the three formulations can be associated with the changes in expression patterns of plasma proteins after administration of these formulations.

Table 3 Changes in expression of plasma proteins of spontaneously diabetic WBN/Kob rats administered keishiburyogan, tokishakuyakusan and hachimijiogan

Group / *m/z*	Keishibukuryogan	Tokishakuyakusan	Hachimijiogan
3264		↑	
3280	↓		
3612	↑		
3899		↑	
4433		↑	
4459	↑	↑	
4490	↑	↑	
4679			↓
4733			↓
4808			↓
5837		↑	
5854		↑	
6506	↑	↑	
9058			↓
9323			↓
9465			↓

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Japanese abstract

自然発症糖尿病モデルであるWBN/Kobラットに代表的な駆瘀血薬である桂枝茯苓丸と当帰芍薬散を長期間投与し，血管機能とタンパク発現に及ぼす影響を検討した。方法は，WBN/Kobラット（雄，24週令）を18週間飼育し糖尿病発症を確認した後，対照群，3％桂枝茯苓丸（KB）群，3％当帰芍薬散（TS）群の3群に分け，さらに25週間飼育した。飼育後，胸部大動脈を摘出しOrgan bath法を用いacetylcholine（Ach）による血管弛緩作用，xanthine/xanthine oxidase（X/XOD）投与による血管収縮作用等を検討した。同時に，血液流動性，血漿脂質，NO代謝物等の測定とSELDI-TOF-MSによる血漿プロテオーム解析を施行した。結果は，対照群とKB，TS群の3群間において，体重と血糖値に有意な差を認めなかった。Achによる内皮依存性血管弛緩率はKB群で対照群に対し有意に弛緩率の増加を認めた。X/XOD投与による血管収縮率はTS群で，PLA_2投与による血管収縮率はTS，KB群の両群で対照群に対し収縮率の減少を認めた。血液流動性はTS群で対照群に対し改善傾向を認め，NO代謝物はKB，TS群の両群で対照群に対し有意に減少した。血漿プロテオーム解析により，対照群に比較しKB群では5個，TS群で8個のタンパク質の有意な変動を認めた。以上のことから，2種類の代表的な駆瘀血薬は，一部異なる作用機序で血流改善に影響を及ぼし，発現するタンパク質にも差異が認められた。作用機序とタンパク質発現との関連は今後検討を要するが，これらの多成分系の方剤による生体の複雑な反応性の差異が「証」の成立に影響していると考えられた。

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