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Altered expression of serum protein in ginsenoside Re-treated diabetic rats detected by SELDI-TOF MS

William C.S. Cho [a,*], Tai-Tung Yip [b], Wai-Shing Chung [a], Sally K.W. Lee [a], Albert W.N. Leung [c], Christopher H.K. Cheng [d], Kevin K.M. Yue [a]

[a] *School of Chinese Medicine, Hong Kong Baptist University, 7 Baptist University Road, Kowloon Tong, Kowloon, Hong Kong, China*
[b] *Ciphergen Biosystems Inc., 6611 Dumbarton Circle, Fremont, CA 94555, USA*
[c] *School of Chinese Medicine and Health Care, The Chinese University–Tung Wah Community College, Kowloon, Hong Kong, China*
[d] *Department of Biochemistry, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, China*

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Abstract

Diabetes mellitus (DM) is now a global health problem, however, its pathogenesis has not yet been fully deciphered. Even though modern medicine has great contribution to the control and treatment of DM, it is still far from success to completely cure the disease. *Panax ginseng* C.A. Meyer (ginseng) is a well-recognized traditional Chinese medicine for treating DM in Asia. In this study, high throughput proteomic approach has been adopted to investigate the antidiabetic action of 2 weeks' ginsenoside Re (Re, a major component of ginseng) administration to streptozotocin-induced diabetic rats. Employing surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) and bioinformatics, 432 cluster peaks were detected in the samples, among them 293 potential biomarkers were found to have significant differentiations between the DM and control normal rats. When the Re-treated diabetic rats were compared to the untreated ones, a protein peak was detected to have significant alteration corresponding to Re treatment. This specific protein was found to match with C-reactive protein (CRP) in the protein database, and was subsequently validated by ELISA. This is the first study demonstrated that CRP could be altered by Re treatment, indicating that Re may improve diabetes and its complications by alleviation of inflammation.

Keywords: Diabetes mellitus; Traditional Chinese medicine; *Panax ginseng*; Ginsenoside Re; SELDI-TOF MS; C-reactive protein

1. Introduction

Diabetes mellitus (DM) can affect every cell and their essential biochemical processes in the body. Through years of research it is found that the diabetic condition is not simply a matter of disorder of one or two physiological processes, but a complex condition with a multitude of metabolic imbalances. As a consequence, the conventional medical approach for diabetes treatment using insulin or oral drugs is insufficient and the patient taking these medications to prevent long-term complications remains at risk.

In traditional Chinese medicine (TCM), diabetes is considered to be a condition of disharmony in the body. There are many Chinese herbs that have been traditionally used for DM (Cho et al., 2005a). *Panax ginseng* C.A. Meyer (ginseng) is one of these herbal medicines that show promising result in the treatment of diabetes (Xie et al., 2005b). Previous studies have demonstrated that an active compound of ginseng, ginsenoside Re (Re), has significant antihyperglycemic effect (Attele et al., 2002; Xie et al., 2005a).

We have previously demonstrated that the onset of oxidative stress in diabetic rats occurred at the eighth week after streptozotocin (STZ) injection (Yue et al., 2003) and different tissues employ different antioxidant enzymes in the defense against oxidative stress during the development of diabetes (Yue et al.,

Abbreviations: CRP, C-reactive protein; DM, diabetes mellitus; Ginseng, *Panax ginseng* C.A. Meyer; HPLC, high performance liquid chromatography; *m/z*, mass to charge; PCA, principal components analysis; Re, ginsenoside Re; ROC, receiver-operator characteristic; SELDI-TOF MS, surface-enhanced laser desorption/ionization time-of-flight mass spectrometry; STZ, streptozotocin; TCM, traditional Chinese medicine; Vehicle, polyvinylpyrrolidone-10 solution

* Corresponding author. Tel.: +852 2958 5441; fax: +852 2715 6139.
E-mail address: williamcscho@gmail.com (W.C.S. Cho).

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2005). In this study, the serum protein alteration of Re-treated diabetic rats was investigated using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS).

2. Materials and methods

2.1. Chemicals

Streptozotocin of analytical grade was obtained from Sigma (St. Louis, MO, USA). Re of high performance liquid chromatography (HPLC) grade with purity >99% was obtained from Hongjiu Ginseng (Jilin, PR China). Unless otherwise stated, all chemicals are of analytical grade.

2.2. Animal models

Wild-type outbred non-diabetic male Sprague–Dawley rats weighing 250–300 g provided by Laboratory Animals Service Centre of The Chinese University of Hong Kong were used for the experiments. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC). The study was performed according to the international and national rules considering animal experiments and biodiversity rights. All the animals were treated as approved by the Hong Kong Baptist University Animal Research Committee and according to the guidelines for animal experiments established by the university.

Induction of diabetes in the overnight-fasted rats and monitoring of blood glucose concentrations were based on our established procedures previously described (Yue et al., 2003). Briefly, two injections of freshly prepared STZ in 0.1 M sodium citrate buffer at pH 4.5 were given intraperitoneally to the rats at a dose of 40 mg/kg on 2 consecutive days (Arulmozhi et al., 2004). Control animals received injections of citrate buffer only. All rats were accommodated in room with 12 h dark/12 h light cycle, and allowed pellet diet and tap water ad libitum throughout the experiment. Four weeks after STZ administration, diabetes was confirmed by the Glucose Diagnostic Kit (Sigma, St. Louis, MO, USA) on the venous blood collected from the tail vein. Only rats with fasting serum glucose levels > 300 mg/dL were allowed to enter into the study protocol as diabetic animals.

On the sixth week post-STZ injection, half of the diabetic and control rats began to receive Re treatment. The drug (150 mg) was first mixed with 50 mL HPLC grade methanol (Fisher, USA), and then dissolved in 1500 mg polyvinylpyrrolidone-10 (Sigma, St. Louis, MO, USA) in 50 mL methanol. In order to avoid oxidization of the materials in the evaporation process, the solution was evaporated under N_2 in a 50 °C water bath to dryness and then stored in a 4 °C refrigerator. Before each treatment, the dried Re was re-dissolved in 5 mg/mL polyvinylpyrrolidone-10 solution (vehicle), with drug concentration of 20 mg/mL. Each animal was orally administered with 20 mg/(kg day rat) Re for 2 weeks starting from the sixth week after STZ administration. The remaining diabetic and control rats received same amount of the vehicle alone.

2.3. Sample preparation

On the eighth week post-STZ injection, the diabetic and control rats (with or without Re treatment) were killed upon an overnight fasting. The blood samples were collected and prepared according to the protocol previously established (Yue et al., 2003). Briefly, each blood sample was allowed to clot and centrifuge at 1500 × *g* for 10 min at 4 °C. Sera were collected, aliquoted and kept frozen at −80 °C until use.

For the proteomic profiling study, serum samples were thawed and 20 μL of each serum sample was denatured by adding 30 μL of 50 mM Tris–HCl buffer containing 9 M urea and 2% 3-[3-(cholamidopropyl) dimethylammonio]-1-propanesulfonate at pH 9. The proteins were fractionated in an 180 μL anion exchange Q HyperD F 96-well filter plate (Ciphergen Biosystems Inc., Fremont, CA, USA). Proteins/peptides bound by the ion exchange beads were eluted by a pH 9 elution buffer, followed consecutively by buffers at pH 7, 5, 4, 3 and finally by an organic buffer containing isopropanol, acetonitrile and trifluoroacetic acid as described in details in our previous papers (Cho et al., 2004, 2006a, 2006b; Yip et al., 2005). This fractionation procedure significantly increases the number of protein peaks detectable from each individual sample (Fung and Enderwick, 2002).

2.4. Proteomic profiling

Quantitative serum proteomic profiles were measured with SELDI-TOF MS technology. Two types of proteinchip arrays were used to fractionate proteins in the serum, which include IMAC30 (coated with a nitriloacetic acid functional group to entrap transition metals for subsequent metal affinity binding to proteins with a hydrophobic barrier coating) and CM10 (mimic weak cation exchange chromatography with carboxylate functionality) (Ciphergen Biosystems Inc., Fremont, CA, USA).

All the proteinchip arrays were pretreated according to manufacturer's protocols. For IMAC30, 80 μL of binding buffer (100 mM sodium phosphate containing 0.5 M NaCl at pH 7) and 20 μL of each fractionated sample were applied to each well of bioprocessor and incubated on a shaker for 30 min at room temperature; whereas 90 μL of binding buffer (100 mM sodium acetate at pH 4) and 10 μL of each fractionated sample for CM10 were applied to the same setting. The chips were washed twice with the corresponding binding buffer on a Ciphergen bioprocessor, followed by two rinses in distilled water and then air-dried. Each fraction was then applied to both the IMAC30 and CM10 proteinchips. Saturated sinapinic acid (Ciphergen Biosystems Inc., Fremont, CA, USA) which served as energy absorbing molecule, prepared from manufacturer's protocol, was applied onto each proteinchip array before SELDI analysis for facilitating desorption and ionization of proteins/peptides.

The arrays were then air-dried and read in a proteinchip reader Protein Biological System IIc (Ciphergen Biosystems Inc., Fremont, CA, USA) with mass deflection at 1.5 kDa and time lag focusing. The acquisition range was set to 0–200 kDa and the instrument has a mass accuracy < 0.1% when properly calibrated, which was performed with the All-in-1 Pep-

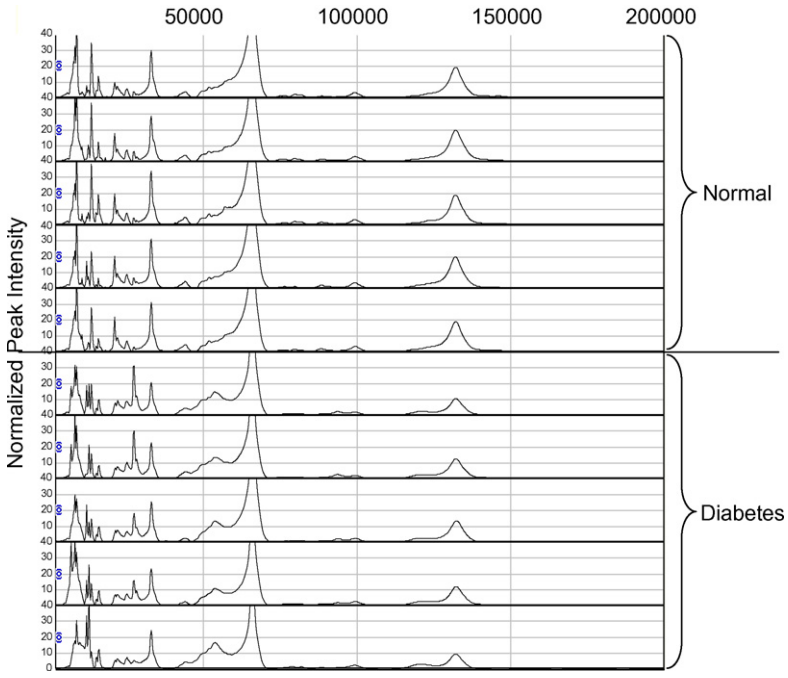

Fig. 1. Peak view of the mass spectra comparing the untreated diabetic rats with the control normal rats at the eighth week after streptozotocin injection.

tide (1–7 kDa) and Protein (7–147 kDa) Standard (Ciphergen Biosystems Inc., Fremont, CA, USA). The parameters used for spectral preprocessing and peak selection were: baseline subtraction, both the smoothing before fitting baseline and the automatic fitting width options selected (with a window of 25 data points for the former); filtering, variable width moving average filter of 0.2 times expected peak width; noise estimation: to start from 1.5 kDa; normalization by total ion current, to start from 1.5 kDa; peaks detected, to start from 2 kDa by centroid mass. Outlier spectra were excluded from further analysis if the normalization factor exceeded two standard deviations above the mean normalization factor. All the controls were randomized and run concurrently with the disease samples on the same chip. Variability analyses were performed in a spot to spot and chip-to-chip fashion for the proteinchips. The chip-to-chip and spot to spot coefficients of variation were found to be 1–16% and 1–14%, respectively (Yip et al., 2005) (Fig. 1).

2.5. Bioinformatics

CiphergenExpress Biomarker Analysis System 3.0.6 (Ciphergen Biosystems Inc., Fremont, CA, USA) was used for data mining and cluster analysis. The system consists of various powerful analytical tools, such as principal components analysis (PCA, a technique that can be used to simplify a multivariant dataset) (Jayakar, 1980), heat maps, receiver–operator characteristic (ROC, a graphical plot of the sensitivity versus 1-specificity for a binary classifier system as its discrimination threshold is varied) (Nettleman, 1988), scatter plot, Box and Whisker plot, etc. Together with Ciphergen's other computational analysis softwares, including the ProteinChip Software 3.2.1, Biomarker Wizard and Biomarker Patterns Software 4.0.1, discovering potential biomarkers among thousands of proteins become possible.

Application details of these bioinformatic tools were described in details in our previous papers (Cho et al., 2004, 2006a, 2006b; Yip et al., 2005). Briefly, the proteinchip profiling spectra from all the serum samples were collected and analyzed by the ProteinChip Software and Biomarker Wizard. The Biomarker Analysis Module in CiphergenExpress Biomarker Analysis System was then used to identify potential biomarkers. The Biomarker Patterns Software was finally applied to reveal the hierarchy of specific proteins using the classification and regression trees algorithm (Gribonval, 2005), a supervised learning process which utilizes cross-validation to minimize classification error.

In the last decade, the number of proteins recorded in public databases such as Swiss-Prot and TrEMBL has increased exponentially. One of the main applications of bioinformatics for proteomic researches is protein matching. In this study, the identification of proteins was conducted by matching the molecular mass of potential biomarkers against proteins (<0.1% difference at MW) in the Swiss-Prot knowledgebase.

2.6. C-reactive protein measurement

The quantitative measurement of C-reactive protein (CRP) in serum samples was performed using the commercial Rat CRP ELISA Test Kit (Helica Biosystems Inc., Fullerton, CA, USA) with an intra-assay variability of 4–8%. Briefly, rat serum samples for testing were diluted to 1:4000 and allowed to react with antibodies coated on specially treated micro-wells. After appropriate incubation according to manufacturer's protocol, the wells were washed to remove unreacted serum proteins and

an enzyme-labeled rabbit anti-rat CRP (conjugate) was then added to react with and tag the antigen–antibody complexes. Following another incubation period, the wells were washed again to remove unreacted conjugate. A urea peroxide substrate with tetramethylbenzidine as chromogen was added to initiate color development. Development of a blue color indicated a positive reaction while negative reactions appeared colorless or with a trace of blue. The reaction was interrupted with a stop solution that turned the blue positive reactions to yellow. Negative reactions remained colorless or with a hint of yellow. Color intensity (absorbance) was read at a wavelength of 450 nm on a spectrophotometer (Tecan Trading AG, Switzerland). Semi-quantification of absorbance was accomplished using a standard curve generated by measuring two-fold dilutions of the standard provided.

2.7. Statistical analyses

The results were analyzed by the Mann–Whitney *U*-test, $p < 0.05$ were considered statistically significant. Unless otherwise stated, all results were expressed as the mean values ± S.D.

3. Results

3.1. Changes in serum protein expression profile between diabetic rats and control normal rats

Proteomic patterns of sera from untreated diabetic rats and untreated control normal rats were first investigated and compared to identify peaks specific to the diabetic state using SELDI-TOF MS technology.

Comparing serum biomarker profiles in DM rats with those from control normal rats at the eighth week after STZ administration, 432 different biomarkers were found in the samples. There were 293 biomarkers with statistically significant differences between the diabetic and normal groups. The top eight biomarkers were selected with outstanding classification values to differentiate the diabetic samples from the controls. These biomarkers included seven overexpressed peaks at 3507, 5161, 6145, 10,012, 11,614, 25,442 and 47,739 Da, as well as one underexpressed peak at 13,936 Da in diabetic rats relative to control normal rats (Table 1 and Fig. 2).

3.2. Changes in serum protein expression profile induced by treatment with ginsenoside Re

To investigate the possible alteration in the pattern of serum protein expression underlying the antidiabetic action of Re, the serum protein profiles of Re-treated diabetic rats were compared with the control vehicle-treated diabetic rats.

Among the eight proteins/peptides differentially expressed between diabetic rats and control normal rats, the normalized peak intensities of a candidate biomarker (at 25,442 Da) were significantly decreased by 20% after Re treatment of the diabetic rats, but not in the control normal rats. This protein had its expression level altered toward the level in the diabetic rats without Re treatment (7.07 ± 1.08 versus 8.80 ± 0.75, $p < 0.005$) (Fig. 3).

3.3. Protein matching and validation

The identification of proteins was conducted by matching the molecular mass of the eight potential biomarkers against proteins in the Swiss-Prot knowledgebase. Among these eight biomarkers found to be differentially expressed between the diabetic and control normal groups, one of the proteins (at 25,442 Da) was matched to be CRP in Swiss-Prot, which was also the only protein altered after Re treatment.

The quantitative measurement of CRP in serum was subsequently performed using commercial ELISA kit to validate the identity of the matched protein; results were expressed as the mean values ± S.E. The serum CRP level in the diabetic rats was significantly higher than that of the control normal rats by 4.5% at week 6 after STZ injection (303 ± 6 μg/mL versus 290 ± 2 μg/mL, $p < 0.05$). It was found that serum CRP level in the diabetic rats (without Re treatment) at week 8 were significantly higher than those of the diabetic rats at week 6 by 6% (321 ± 5 μg/mL versus 303 ± 6 μg/mL, $p < 0.05$). When serum CRP level was measured at week 8, the level in the untreated diabetic rats was significantly higher than that of the control normal rats by 15% (321 ± 5 μg/mL versus 278 ± 13 μg/mL, $p < 0.005$) while there was no significant difference observed between the 20 mg/kg Re-treated diabetic rats and the control normal rats (286 ± 7 μg/mL versus 284 ± 13 μg/mL, $p > 0.05$). Comparing the serum CRP level in the untreated diabetic rats with that of the Re-treated ones at week 8, it was found that the serum CRP

Table 1
Potential biomarkers—diabetic rats vs. control normal rats

m/z (Da)	Normalized peak intensity		Change in DM	Difference (%)	*p*-Value (<)	Area under the ROC curve
	Diabetes	Normal				
3507	16.05 ± 3.73	9.17 ± 2.08	Increase	75	0.001	0.93
5161	8.61 ± 2.27	3.27 ± 0.65	Increase	163	0.001	0.93
6145	6.75 ± 2.40	2.46 ± 1.83	Increase	174	0.001	0.93
10012	15.36 ± 4.69	3.30 ± 0.95	Increase	365	0.001	0.99
11614	29.27 ± 4.19	21.93 ± 3.25	Increase	33	0.001	0.93
13936	11.16 ± 2.68	19.27 ± 3.77	Decrease	42	0.001	0.01
25442	8.80 ± 0.75	6.04 ± 0.98	Increase	46	0.001	0.93
47739	6.24 ± 2.39	3.97 ± 0.63	Increase	57	0.005	0.87

Normalized peak intensity is expressed as the mean values ± S.D.

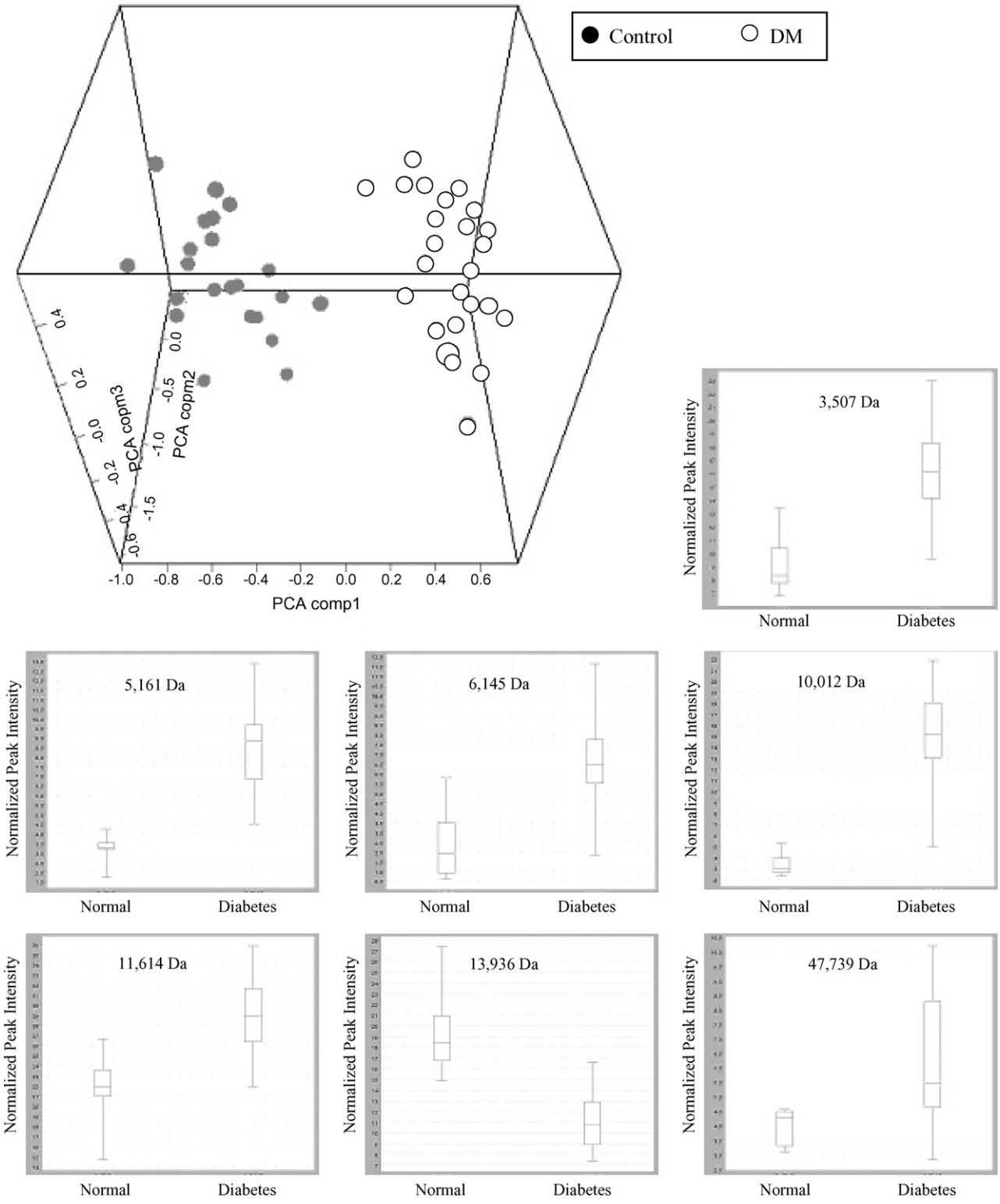

Fig. 2. Potential biomarkers to differentiate the diabetic rats from control normal rats. The results were expressed as principal components analysis plot and Box and Whisker plots. Untreated diabetic group ($N = 8$) was compared with the untreated control normal rats ($N = 10$).

level in the Re-treated diabetic rats was significantly lower than that in the untreated diabetic rats by 11% (286 ± 7 μg/mL versus 321 ± 5 μg/mL, $p < 0.05$). No significant difference in serum CRP level was observed among the three control normal groups (Fig. 4).

4. Discussion

Diabetes mellitus may be caused by the modification of protein signaling pathways and therefore it can be recognized as a proteomic disease in functional sense. The therapeutic effect

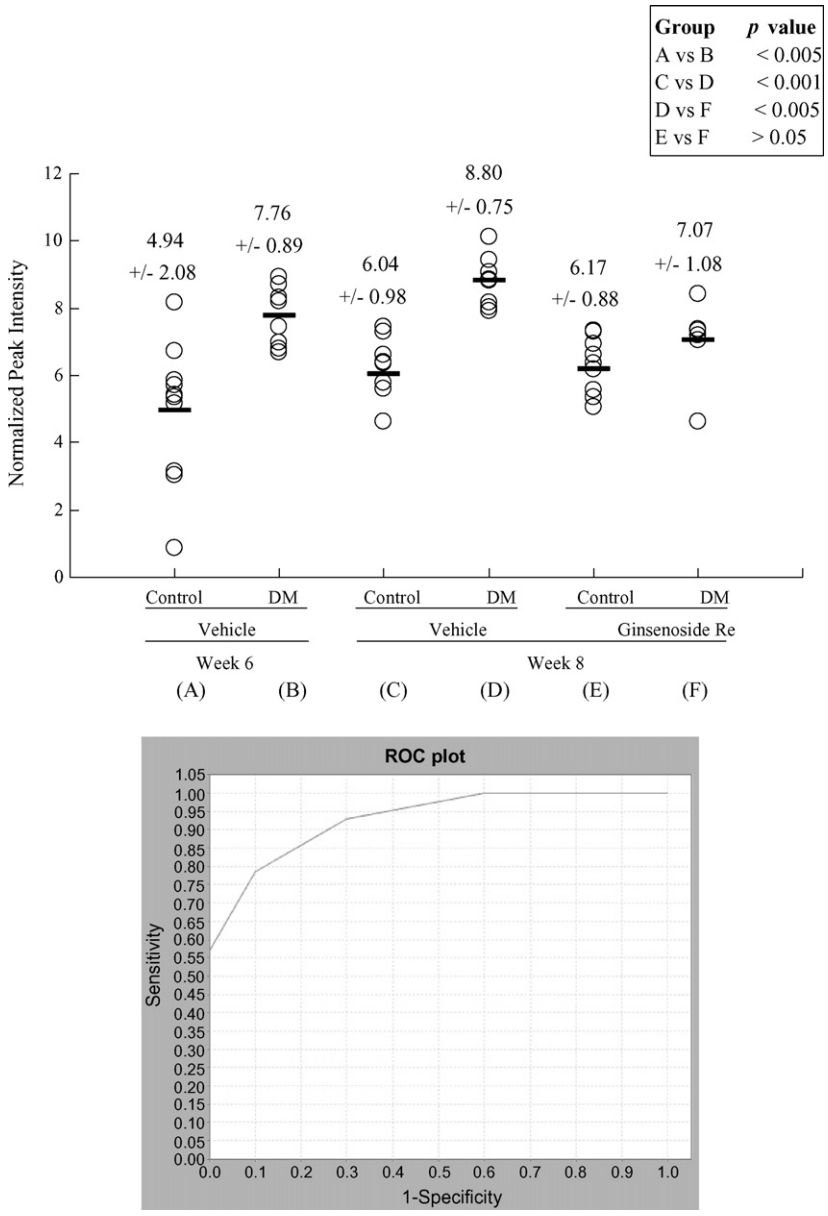

Fig. 3. Alteration in the level of the potential biomarker at 25,442 Da by proteomic profiling. The results were expressed as scatter plot where the Re-treated (20 mg/kg/day for 2 weeks) diabetic rats ($N = 8$) and vehicle-treated diabetic rats ($N = 8$) were compared with control normal rats ($N = 10$), and receiver–operator characteristic (ROC) plot where the Re-treated rats ($N = 8$) was compared with the vehicle-treated diabetic rats ($N = 8$). Area under the ROC curve is 0.93.

of insulin has revealed the clue that using hormone or protein for the treatment of DM may be the most feasible approach to cure this endocrine disorder. Proteomics is useful in deciphering the protein expression profile of metabolomic changes (Mayr et al., 2004), however, relatively few studies using high throughput proteomic technologies to investigate the DM development have been published to date.

In Asia, ginseng has been used in medicine for more than 5000 years. In China, this herbal medicine has been treasured since the dawn of written history (Yun, 2001). In the western world today, ginseng is commonly considered as an adaptogenic herb, and hence is popularly used as a health-enhancing tonic because it strengthens body functions and the immune system to help people adapt to the effects of physical stress (Liu and Xiao, 1992; Lee et al., 2005).

Previous experiments have been performed to investigate the antidiabetic actions of Re; results indicate that Re demonstrates significant antidiabetic actions (Attele et al., 2002; Xie et al., 2005a).

To explore the possible proteins involved in the antidiabetic actions of Re, the present study attempts to provide a link between the proteomic study in DM with the promising antidiabetic actions of Re. As a result, eight proteins/peptides were discovered to have highly altered expressions in the diabetic models as compared to the control normal rats. The most rewarding finding is that there is a significant reduction in the level of a potential biomarker (i.e. CRP) after treatment with Re.

The candidate protein was found to match with CRP in the Swiss-Prot knowledgebase. C-reactive protein is produced by the liver in response to inflammation, it is one of the principal

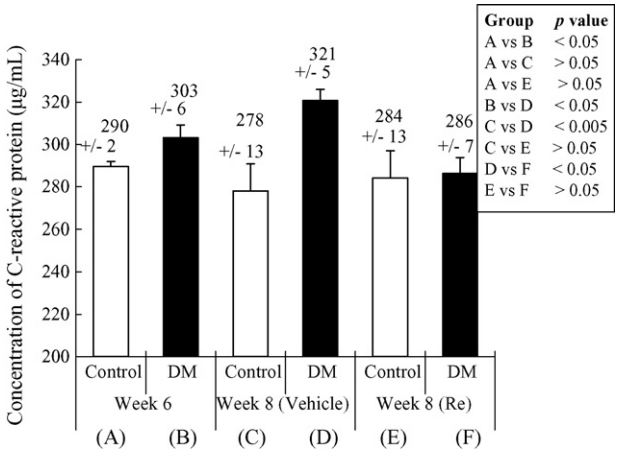

Fig. 4. Serum C-reactive protein concentrations of Re-treated (20 mg/kg/day for 2 weeks) diabetic rats ($N = 8$) and vehicle-treated diabetic rats ($N = 8$) vs. those of the control normal rats ($N = 10$). The results are expressed as bar chart showing the mean ± S.E.

members of the pentaxin family. Pentaxins are a family of penta- or decameric-serum proteins that include serum amyloid protein and CRP (Reid and Blobel, 1994). Proteins of the pentaxin family are involved in acute immunological responses.

CRP is expressed during the acute phase response to tissue injury or inflammation in mammals. The protein resembles antibody and performs several functions associated with host defence: it promotes agglutination, bacterial capsular swelling and phagocytosis and activates the classical complement pathway through its calcium-dependent binding to phosphocholine (Romero et al., 1998).

Our result was subsequently validated by commercial ELISA kit confirming the alteration in the level of the candidate protein during the development of DM. In the diabetic rats without administration of Re, at both week 6 and 8 after STZ injection, the CRP levels were significantly higher than that of the control normal rats. Previous study has shown that elevated CRP is associated with insulin resistance (Festa et al., 2000) while our data demonstrated that diabetic rats exhibit significant higher CRP levels than that of the control animals. This difference in the CRP level is even more obvious at longer time intervals (increasing from the sixth week to the eighth week after STZ injection). A major finding of the present study is that no elevation of CRP concentration was found in the diabetic rats with Re administration as compared to the control normal rats. Re treatment of the diabetic rats has resulted in a significant reduction in the CRP level. This result indicated that the intake of Re could reduce the elevation of CRP in DM. As a matter of fact, the level of CRP in the diabetic rats was actually normalized after 2 weeks of Re treatment. Interestingly, no such change was observed among the control normal groups, indicating that the effect is only apparent on the diabetic animals. This validated result is concurrent with our finding discovered by proteinchip profiling.

As a well-known indicator of inflammation, serum level of CRP is often elevated in subjects with inflammatory conditions (Schalkwijk et al., 1999). The inflammatory reaction is crucial for survival and is meant to be tailored to the stimulus and time. A full-fledged systemic inflammatory reaction results in stimulation of four major programs: the acute-phase reaction, the sickness syndrome, the pain program and the stress response, mediated by the hypothalamic–pituitary–adrenal axis and the sympathetic nervous system. Abnormalities of the systemic anti-inflammatory feedback and the failure of the adaptive systems to resolve inflammation affect the well-being of an individual, including behavioral parameters, quality of life and sleep, as well as indices of metabolic and cardiovascular health (Ridker and Haughie, 1998; Elenkov et al., 2005).

There have been various studies demonstrating that high level of CRP generally associated with development of atherosclerosis (Colhoun et al., 2002; Ilhan et al., 2005) and coronary heart diseases (Koenig et al., 1999; Folsom et al., 2002), the common macrovascular complications of DM. The finding in the present study have shown that intake of Re could reduce the level of CRP in DM, indicating that this saponin triterpenoid glycoside could alleviate macrovasculopathy by improving the status of inflammation. This is the first study demonstrating that CRP could be altered by Re treatment, indicating that Re may improve DM and its complications by reducing inflammation.

The anti-inflammatory actions of ginseng is also well recognized (Cabral de Oliveira et al., 2001) and its beneficial effect on cardiovascular diseases has aroused increasing interest (Zhou et al., 2004). This study has thus shed light on the possible therapeutic application of Re on the prophylaxis of DM-associated heart diseases such as atherosclerosis. In this connection, exploration of Chinese herbal medicines with powerful proteomic tools and bioinformatic technologies would be an ideal integration for discovering new treasure of herbal medicine and bring TCM research to a new horizon (Cho et al., 2005b).

References

Arulmozhi, D.K., Veeranjaneyulu, A., Bodhankar, S.L., 2004. Neonatal streptozotocin-induced rat model of type 2 diabetes mellitus: a glance. Indian Journal of Pharmacology 36, 217–221.

Attele, A.S., Zhou, Y.P., Xie, J.T., Wu, J.A., Zhang, L., Dey, L., Pugh, W., Rue, P.A., Polonsky, K.S., Yuan, C.S., 2002. Antidiabetic effects of *Panax ginseng* berry extract and the identification of an effective component. Diabetes 5, 1851–1858.

Cabral de Oliveira, A.C., Perez, A.C., Merino, G., Prieto, J.G., Alvarez, A.I., 2001. Protective effects of *Panax ginseng* on muscle injury and inflammation after eccentric exercise. Comparative Biochemistry and Physiology, Toxicology and Pharmacology 130, 369–377.

Cho, W.C., Yip, T.T., Yip, C., Yip, V., Thulasiraman, V., Ngan, R.K., Yip, T.T., Lau, W.H., Au, J.S., Law, S.C., Cheng, W.W., Ma, V.W., Lim, C.K., 2004. Identification of serum amyloid A protein as a potentially useful biomarker to monitor relapse of nasopharyngeal cancer by serum proteomic profiling. Clinical Cancer Research 10, 43–52.

Cho, W.C., Yue, K.K., Leung, A.W., 2005a. An outline of diabetes mellitus and its treatments by traditional Chinese medicine and acupuncture. Journal of Chinese Medicine (UK) 78, 29–37.

Cho, W.C., Yue, K.K., Leung, A.W., 2005b. Proteomics—leading the postgenome era. China Biotechnology 25, 33–38.

Cho, W.C., Yip, T.T., Chung, W.S., Leung, A.W., Cheng, C.H., Yue, K.K., 2006a. Differential expression of proteins in kidney, eye, aorta, and serum of diabetic and non-diabetic rats. Journal of Cellular Biochemistry (epub ahead of print).

Cho, W.C., Yip, T.T., Chung, W.S., Leung, A.W., Cheng, C.H., Yue, K.K., 2006b. Potential biomarkers found by protein profiling may provide insight for

the macrovascular pathogenesis of diabetes mellitus. Disease Markers 22, 153–166.

Colhoun, H.M., Schalkwijk, C., Rubens, M.B., Stehouwer, C.D., 2002. C-reactive protein in type 1 diabetes and its relationship to coronary artery calcification. Diabetes Care 25, 1813–1817.

Elenkov, I.J., Iezzoni, D.G., Daly, A., Harris, A.G., Chrousos, G.P., 2005. Cytokine dysregulation, inflammation and well-being. Neuroimmunomodulation 12, 255–269.

Festa, A., D'Agostino, R., Howard, G., Mykkanen, L., Tracy, R.P., Haffner, S.M., 2000. Chronic subclinical inflammation as part of the insulin resistance syndrome: the insulin resistance atherosclerosis study (IRAS). Circulation 102, 42–47.

Folsom, A.R., Aleksic, N., Catellier, D., Juneja, H.S., Wu, K.K., 2002. C-reactive protein and incident coronary heart disease in the atherosclerosis risk in communities (ARIC) study. American Heart Journal 144, 233–238.

Fung, E.T., Enderwick, C., 2002. ProteinChip clinical proteomics: computational challenges and solutions. BioTechniques Supplement 3, 34–38,40–41.

Gribonval, R., 2005. From projection pursuit and CART to adaptive discriminator analysis? IEEE Transactions on Neural Networks 16, 522–532.

Ilhan, F., Akbulut, H., Karaca, I., Godekmerdan, A., Ilkay, E., Bulut, V., 2005. Procalcitonin, C-reactive protein and neopterin levels in patients with coronary atherosclerosis. Acta Cardiologica 60, 361–365.

Jayakar, S.D., 1980. A principal component analysis of quantitative serum protein data. La Ricerca in Clinica e in Laboratorio 10, 243–246.

Koenig, W., Sund, M., Frohlich, M., Fischer, H.G., Lowel, H., Doring, A., Hutchinson, W.L., Pepys, M.B., 1999. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. Circulation 99, 237–242.

Lee, T.K., Johnke, R.M., Allison, R.R., O'Brien, K.F., Dobbs Jr., L.J., 2005. Radioprotective potential of ginseng. Mutagenesis 20, 237–243.

Liu, C.X., Xiao, P.G., 1992. Recent advances on ginseng research in China. Journal of Ethnopharmacology 36, 27–38.

Mayr, M., Mayr, U., Chung, Y.L., Yin, X., Griffiths, J.R., Xu, Q., 2004. Vascular proteomics: linking proteomic and metabolomic changes. Proteomics 4, 3751–3761.

Nettleman, M.D., 1988. Receiver operator characteristic (ROC) curves. Infection Control and Hospital Epidemiology 9, 374–377.

Reid, M.S., Blobel, C.P., 1994. Apexin, an acrosomal pentaxin. The Journal of Biological Chemistry 269, 32615–32620.

Ridker, P.M., Haughie, P., 1998. Prospective studies of C-reactive protein as a risk factor for cardiovascular disease. Journal of Investigative Medicine 46, 391–395.

Romero, I.R., Morris, C., Rodriguez, M., Du Clos, T.W., Mold, C., 1998. Inflammatory potential of C-reactive protein complexes compared to immune complexes. Clinical Immunology and Immunopathology 87, 155–162.

Schalkwijk, C.G., Poland, D.C., van Dijk, W., Kok, A., Emeis, J.J., Drager, A.M., Doni, A., van Hinsbergh, V.W., Stehouwer, C.D., 1999. Plasma concentration of C-reactive protein is increased in type I diabetic patients without clinical macroangiopathy and correlates with markers of endothelial dysfunction: evidence for chronic inflammation. Diabetologia 42, 351–357.

Xie, J.T., Mehendale, S.R., Li, X., Quigg, R., Wang, X., Wang, C.Z., Wu, J.A., Aung, H.H., Rue, P.A., Bell, G.I., Yuan, C.S., 2005a. Anti-diabetic effect of ginsenoside Re in *ob/ob* mice. Biochimica et Biophysica Acta 1740, 319–325.

Xie, J.T., Mehendale, S.R., Yuan, C.S., 2005b. Ginseng and diabetes. The American Journal of Chinese Medicine 33, 397–404.

Yip, T.T., Chan, J.W., Cho, W.C., Yip, T.T., Wang, Z., Kwan, T.L., Law, S.C., Tsang, D.N., Chan, J.K., Lee, K.C., Cheng, W.W., Ma, V.W., Yip, C., Lim, C.K., Ngan, R.K., Au, J.S., Chan, A., Lim, W.W., 2005. Protein chip array profiling analysis in patients with severe acute respiratory syndrome identified serum amyloid A protein as a biomarker potentially useful in monitoring the extent of pneumonia. Clinical Chemistry 51, 47–55.

Yue, K.K., Chung, W.S., Leung, A.W., Cheng, C.H., 2003. Redox changes precede the occurrence of oxidative stress in eyes and aorta, but not in kidneys of diabetic rats. Life Sciences 73, 2557–2570.

Yue, K.K., Leung, S.N., Man, P.M., Yeung, W.F., Chung, W.S., Lee, K.W., Leung, A.W., Cheng, C.H., 2005. Alterations in antioxidant enzyme activities in the eyes, aorta and kidneys of diabetic rats relevant to the onset of oxidative stress. Life Sciences 77, 721–734.

Yun, T.K., 2001. Brief introduction of *Panax ginseng* C.A. Meyer. Journal of Korean Medical Science 16, S3–S5.

Zhou, W., Chai, H., Lin, P.H., Lumsden, A.B., Yao, Q., Chen, C.J., 2004. Molecular mechanisms and clinical applications of ginseng root for cardiovascular disease. Medical Science Monitor 10, 187–192.