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Volunteer study and serum protein profiling to understand inflammatory response induced by Satsuma mandarin

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ABSTRACT

It has been observed that consumption of a certain amount of Satsuma, lychee, and longan often caused a symptom characterized by dry or sore throat, gum swelling and even mouth ulcer, which significantly impaired the life quality of a large population. We define the adverse reaction to Satsuma as Satsuma-induced syndrome (SIS). Volunteers were assigned to oral Satsuma challenge in an open manner. The results showed that SIS was characterized with symptoms affecting the throat, oral cavity, face, gastrointestinal system and eye either individually or in combination. A comparative proteomic study was performed to investigate the differences of serum proteins in the Post-SC (after Satsuma challenge) and Pre-SC (before Satsuma challenge) serum samples of 15 volunteers with severe SIS. Ten proteins were identified to be differentially expressed ($P < 0.05$). Of these, levels of complement component C9 precursor were elevated significantly in the Post-SC serum samples and were further verified by enzyme-linked immunosorbent assay, indicating that the complement system may be activated and plays a significant role in inflammatory response. Meanwhile, serum samples were subjected to immobilized metal affinity capture (IMAC3) protein chip surfaces and tested by surface-enhanced laser desorption/ionization-time of flight-mass spectrometry. The data were analyzed by Ciphergen ProteinChip Software. A diagnostic model was constructed to discriminate the SIS from normal samples, using principal component analysis. A total of 50 detected biomarkers were found to be different with statistical significance ($P < 0.05$). The multivariate logistic analysis demonstrates a complete distinction between the two groups. Our findings suggest that these assays may provide potential biomarkers for the diagnosis of SIS.

1. Introduction

Despite the health benefits of low risks of degenerative diseases, cardiovascular diseases and cancers from horticultural products (Benavente-Garcia & Castillo, 2008; Lian, Hu, Russell, & Wang, 2006), adverse reactions to fruits are becoming an increasingly common problem with the increasing consumption of fruits. As the most widely planted and consumed fruit in the world, citrus is the main allergenic plant food in public perception surveys of food allergy (Woods, Abramson, Bailey, & Walters, 2001). In citrus fruit, three allergens have been isolated so far: Cit s 1 (orange germin-like glycoprotein), Cit s 2 (profilin), and Cit s 3 (lipid transfer protein). Over 60%, 87% and 40%–60% of patients allergic to oranges show specific IgE to Cit s 1, Cit s 2 and Cit s 3, respectively (Ahrazem et al., 2005; 2006; Lopez-Torrejon et al., 2005).

It has long been observed that consumption of a certain amount of Satsuma, lychee or longan causes a symptom characterized by dry or sore throat, gum swelling and even mouth ulcer, which significantly impaired the life quality of a large population. Satsuma-induced syndrome (SIS) is likely to be a multifactorial condition involving a number of different mechanisms, although the prominence of any particular factor may vary from patient to patient. Research into SIS has always been hampered by a lack of standardization in terms of definition and severity of disease.

Modern biology can measure the expression levels of thousands of mRNA to determine the physiological state of a cell or tissue (Lockhart & Winzeler, 2000). However, there are increasing evidences showing that the correlation between mRNA and protein abundance is unpredictable, implying that the direct measurement of protein expression is essential for the understanding of the physiological state of a cell or tissue (Gygi, Corthals, Zhang, Rochon, & Aebersold, 2000).

Human plasma contains thousands of distinct proteins including classical “plasma proteins” and all tissue proteins. Therefore, plasma proteome can hold the promise of disease diagnosis and is a rich source for biomarker discovery (Anderson & Anderson, 2002). However, a small group of proteins of high abundance constitute 99% of the total plasma proteins, and the remaining 1% proteins are those of very low

Abbreviations: SC, Satsuma challenge; SIS, Satsuma-induced syndrome; Pre-SC, before Satsuma challenge; Post-SC, after Satsuma challenge; C9, complement component C9 precursor.

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abundance. The wide dynamic range of more than 10 orders of magnitude in concentration makes the detection of low-abundance proteins extremely challenging. Therefore, the depletion of high-abundance proteins is a significant step in the plasma proteome profiling (Li et al., 2005; Tirumalai et al., 2003). In a previous study, the high-abundance proteins in the pooled sera were depleted through an immunoaffinity method, and then the sera were subjected to sodium dodecyl sulfonate-polyacrylamide gel electrophoresis (SDS-PAGE), and finally analyzed by LC-ESI-MS/MS. The results showed that C9 protein could serve as a biomarker for squamous cell lung cancer (Narayanasamy et al., 2011). Another proteomic technique of the surface-enhanced laser desorption/ionization-time of flight-mass spectrometry (SELDI-TOF-MS) is increasingly being used to identify the biomarkers in serum indicative of various cancers, such as prostate, ovarian, breast and liver cancers (Adam et al., 2002; Li, Zhang, Rosenzweig, Wang, & Chan, 2002; Petricoin et al., 2002; Schwegler et al., 2005).

There are few population-based studies that have investigated SIS. We thus examined SIS symptoms by volunteer study. In order to investigate the prevalence of SIS, a standard questionnaire was designed to evaluate the SIS symptoms and a scoring system was set for the criteria of SIS. Then, a comparative proteomic study was applied to investigate the volunteers' serum using SELDI-TOF-MS or two-dimensional gel electrophoresis (2-DE) coupled with mass spectrometry to deeply understand SIS in this study.

2. Materials and methods

2.1. Oral Satsuma challenge

Based on sufferers' experience, SIS is a dose-dependent food adverse reaction. We set up a dose of 1.5 kg Satsuma fruit for male and 1 kg for female volunteers, which was 2–3 times of normal consumption.

In 2009, 195 volunteers participated in the Satsuma challenge (SC) in the open manner (individual dada of volunteers are summarized in Table E1). (1) Day 1, from 7 p.m. to 9 p.m., volunteers finished the demanded dose at one time. (2) Days 2–4, at 8 a.m., volunteers went to Huazhong Agricultural Hospital for clinical observation. SIS symptoms were recorded in designed standard questionnaires (Table E2) by experienced physicians. Forty of these volunteers (Table E3) were selected to attend the SC in 2010. SIS symptoms were recorded, the flow diagram of SC was presented in Fig. 1. The blood samples of all the 40 volunteers before Satsuma challenge (Pre-SC) and after Satsuma challenge (Post-SC) were respectively collected at 8 a.m., and were centrifuged at 3000 × *g* for 10 min at 4 °C. Then, aliquots of the serum were stored at −80 °C for further analysis. Written informed consent was obtained from all volunteers. This study was approved by the Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology Institutional Review Board.

2.2. Standard questionnaires design

We interviewed with SIS sufferers and designed a questionnaire scoring system to evaluate SIS symptoms, as shown in Table E2. All the possible symptoms listed were derived from interviews with people who have suffered from SIS. The SIS symptoms are classified into three levels. Level One (weighted by 1 point): symptoms based on mild personal feelings that are difficult to be observed by clinical examinations, such as burning sensation and dry eye; Level Two (weighted by 2 points): clear but not severe symptoms, such as hoarse throat and blistering; Level Three (weighted by 3 points): severe symptoms, such as diarrhea and ulcer. SIS patients were determined when total score ≥3.

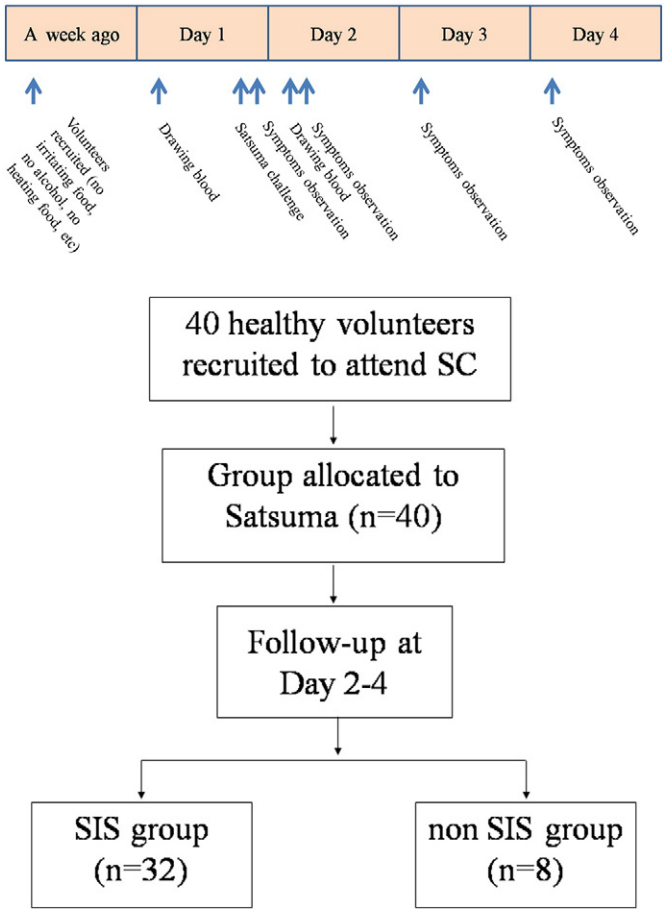

Fig. 1. SC flow diagram.

2.3. Removal of high-abundance serum proteins by Multiple Affinity Removal System

A Multiple Affinity Removal System (MARS) (Agilent Technologies, USA) was used for the removal of the six most abundant proteins (human albumin, IgG, antitrypsin, IgA, transferrin and haptoglobin) in the serum pools of the severe SIS group and its corresponding control, according to the manufacturer's protocol. Each aliquot of 100 μL diluted serum was injected onto the MARS column. The flow-through fractions from sequential injections were collected and pooled.

2.4. Desalination and concentration of the flow-through fractions by spin concentrators

The 4 mL spin concentrator (5000 Da molecular weight cut-off, part no. 5185-5991, Agilent Technologies, USA) was used to concentrate the flow-through fractions. Then, the concentrated solution was buffer-changed and transferred into a new centrifuge tube. Desalted specimen solutions which had been prepared separately were pooled.

2.5. Isoelectric focusing and second-dimensional gels

2-DE was carried out according to Pan's protocol with minor modifications (Pan, Guan, Zhu, & Deng, 2008). One milligram of total protein was applied to 17 cm liner IPG strip (pH 4–7). After 10 h passive rehydration, isoelectric focusing was conducted in Protean IEF Cell (Bio-Rad) according to the following program: 30 min at 250 V, 1 h at 1000 V for demineralization, 5 h from 1000 V to 8000 V for voltage boosting, and 7.5 h at 8000 V for focusing. IPG strips were then subjected to