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Effects of total glucosides of paeony for delaying onset of Sjogren's syndrome: An animal study

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ABSTRACT

Objective: To investigate the effectiveness of total glucosides of paeony (TGP) on Sjogren's syndrome (SS) using non-obese diabetic (NOD) mice model.
Study design: Twenty-seven 8-week-old female NOD mice were assigned into TGP group, hydroxychloroquine (HCQ) group and normal saline (NS) group, receiving corresponding drugs respectively and sacrificed at 24-week-old. Saliva flow rate (SFR), ration of regulatory T cells, level of anti-SSA/SSB, histological changes in submandibular glands (SMG) and microarray analysis were assessed. The data were analyzed using SPSS.
Results: Compared to NS group, in TGP group, SFR, SMG index and the ration of regulatory T cells were significantly higher, while anti-SSA/SSB and lymphocytic foci were significantly lower. HCQ group demonstrated similar results except SMG index. Altered gene expression was found in 10.71% of TGP and 13.09% of HCQ of the profile.
Conclusion: TGP demonstrated a similar effectiveness as HCQ in delaying the onset of SS-like disease in NOD mice.

1. Introduction

Sjogren's syndrome (SS) is a chronic disorder with focal lymphocytic infiltration in salivary and lachrymal glands leading to dry mouth and dry eye. It is one of the most common autoimmune diseases around the world. The reported prevalence varies from 0.4% to 0.7% (Vitali et al. 2002; Sardenberg et al. 2010). The aetiopathogenesis resulting in the loss of immune tolerance and the aggressive inflammation in exocrine glands is largely unknown. The current therapies are mainly palliative.

Hydroxychloroquine (HCQ) is used in systemic treatment of SS (Kruize et al. 1993; Tishler et al. 1999). By interfering with antigenic processes, HCQ is beneficial to SS by improving salivary gland secretion and suppressing the production of disease-associated factors including IgG, antinuclear and rheumatoid factor (Tishler et al. 1999; Dawson et al. 2005; von Bultzingslowen et al. 2007; Thanoustavraki and James 2008). However, long-term administration of HCQ may cause ocular toxic effects like outer retinal damage and pigmentary retinopathy (Tehrani et al. 2008).

Total glucosides of paeony (TGP) are the powder substances extracted from the root of *Paeonia lactiflora* pall. Its main effective component is peoniflorin, accounting for over 90% in all substances (WU 1985). Wu et al. has reported that TGP has anti-inflammatory and antioxidative actions by suppressing the expression of intercellular adhesion molecule-1, interleukin-1, tumor necrosis factor-alpha and 3-nitrotyrosine protein (Wu et al. 2009). TGP has demonstrated its biological safety after long-term clinical use without serious side effects. Some recent studies have demonstrated the potential of TGP for treating adjuvant arthritis, collagen-induced arthritis and rheumatoid arthritis (Zheng and Wei 2005; Zhu et al. 2005; Wang et al. 2011). However, the investigation of TGP in treatment of SS is rare.

As a spontaneous model of SS, non-obese diabetic (NOD) mice have been widely used to investigate the pathological mechanism

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and management of this disease (Cha et al. 2002a; Gillespie et al. 2008; Nguyen et al. 2008). NOD mice can develop lymphocytic function causing diabetes and SS-like disease (Cha et al. 2002a,b; Jonsson et al. 2006; Jonsson et al. 2007; Chiorini et al. 2009). The present study aimed to investigate the effectiveness and safety of TGP on SS using NOD mice model by comparing to HCQ, which is used in systemic treatment of this disease with known side effects.

2. Materials and methods

2.1. Animals and treatments

Eight-week-old female NOD mice were purchased from Shanghai SLAC Laboratory Animal Co. Ltd. The animals were provided with standard rodent feed and water ad libitum and housed in the Animal Unit of Health Science Center Peking University. The experiment was approved by the Institutional Ethics Committee of Peking University.

Twenty-seven NOD mice were randomly assigned into three groups, nine in each. Four or five mice from the same group were housed in one cage. From the age of 8-week to 24-week, the mice were administered with TGP (100 mg/kg/d, Ningbo Liwah Pharmaceutical Co., Ltd., China), HCQ (50 mg/kg/d, Shanghai Zhongxi Pharmaceutical (Group) Co. Ltd., China), or normal saline (NS) every day intragastrically, respectively. The dosage was obtained according to an equivalent dose between mice and human (Shuyun and Rulian Cx 2001). The drugs were diluted in NS and delivered to the stomach of mice using an irrigation stomach needle. All the mice were sacrificed at 24 weeks of age.

2.2. Salivary flow rate measurement

Stimulated total salivary flow rate (SFR) was measured every other week. The mice were anesthetized with tribromoethanol (0.36 g/kg body weight, Alfa Aesar, Ward Hill, MA, USA) by intraperitoneal injection. After 5 min, the mice were given pilocarpine (0.5 mg/kg body weight, Sigma, USA) to stimulate the secretion of saliva. Saliva was collected for 15 min and the SFR was counted according to the protocol reported by Hu et al. (1992).

2.3. Autoantibody qualification

The blood was harvested from inner canthus at 10-, 16- and 24-week of age. The IgG class autoantibodies against the ribonuclear proteins SSA/Ro and SSB/La (Euroimmun, Lübeck, Germany) in serum were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacture's protocol.

2.4. Exponent and histological analysis of submandibular glands

After the mice were sacrificed, the submandibular glands (SMG) were dissected out within 10 min. The SMG index was obtained by comparing the weight of the submandibular glands and the mice body (SMG index = weight of the submandibular glands/weight of the mice) (Wang et al. 2007). The glands were then fixed in 10% formalin and embedded in paraffin. Five-micrometer sections were prepared and the H&E staining was performed to examine the infiltration of the tissues. Clusters of $\geq$50 lymphocytes in a 4 mm^2 area are considered abnormal. The number of lymphocytic foci was obtained using the protocol described by Qi et al. (2009).

2.5. Flow cytometric analysis

The spleen monocytes of the mice were harvested at 24-week of age. The cells were stained with anti-CD4-FITC (BD PharMingen, San Diego, CA, USA), and then incubated and permeabilized in Fix/Perm buffer (eBioscience, San Diego, CA, USA) and stained in anti-FoxP3-PE (eBioscience). The isotype-matched control antibody was used appropriately in FACS analyses. Cells information was analyzed on a FACS Calibur flow cytometer using Cell Quest software (BD PharMingen).

2.6. Microarray analysis

At 24-week of age, three mice were randomly selected from each group and their spleen T cells were subjected to microarray analysis. Eighty-four cytokine genes related to inflammation and autoimmunization including interferon, interleukin, TGF-β family and TNF superfamily were chosen for microarray analysis.

Total RNA was extracted from the 1×10^6 spleen T cells of each mouse by TRIZOL (Invitrogen life technologies, Carlsbad, CA, USA). DNase was used to remove the contaminating DNA from the RNA preparations. OD assay was measured to calculate the concentration and 1% formaldehyde-agarose gel to check the quality of RNA product. First strand cDNA synthesis and real time-polymerase chain reaction (PCR) were conducted in superarray PCR master mix (SA Biosciences, QIAGEN, Hilden, Germay). $2^{-(\Delta\Delta Ct)}$ method was used to analyze the data obtained from each array (Livak and Schmittgen 2001).

2.7. Statistical analysis

All the experimental data was analyzed by ANOVA using SPSS 11.5 software (SPSS® Base 11.5, Wacker Drive, Chicago, IL, USA). The statistical significance level was considered at $P < 0.05$.

3. Results

3.1. Determination of salivary flow rate

SFR demonstrated a declining tendency alone with the aging of the mice in all three groups. Since 10-week, mice in TGP and HCQ groups showed higher SFR than NS group (Fig. 1). There was no significant difference between TGP and HCQ group.

3.2. Quantification of serum autoantibodies

Compared to NS group, the concentrations of anti-SSA/Ro and anti-SSB/La were significantly lower in TGP and HCQ groups (Fig. 2). No significant difference was found between TGP and HCQ groups.

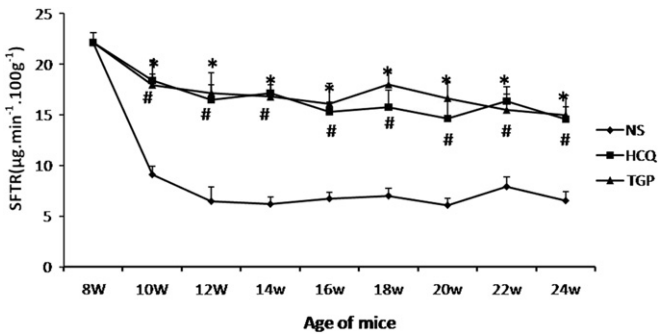

Fig. 1. Stimulated total salivary flow rates of the NOD mice in the control and treatment groups at different time points. The SFR in HCQ and TGP groups are significantly higher than in NS group. *$P < 0.05$, TGP VS. NS group; #$P < 0.05$, HCQ vs. NS group.