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Investigation of the preventive effect of Sijunzi decoction on mitomycin C-induced immunotoxicity in rats by ^{1}H NMR and MS-based untargeted metabolomic analysis

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Chemical compounds studied in this article:
ginsenoside Rg_2 (PubChem CID: 75412551)
ginsenoside Re (PubChem CID: 441921)
atractylenolide I (PubChem CID: 5321018)
atractylenolide II (PubChem CID: 14448070)
atractylenolide III (PubChem CID: 155948)
poricoic acid B (PubChem CID: 5471852)
16-deoxyporicoic acid B (PubChem CID: 16736458)
liquiritigenin (PubChem CID: 114829)
gancaonin I (PubChem CID: 480777)

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ABSTRACT

Ethnopharmacological relevance: Sijunzi decoction (SJZD) is a well known traditional Chinese prescription used for the treatment of gastrointestinal disorders and immunity enhancement. It has been found to indeed improve life quality of chemotherapy patients and extensive used in clinical conbined with chemotherapeutics for the treatment of cancer.

Aim of the study: The aim of this study was to investigate the preventive effect of the immunotoxicity of SJZD on mitomycin C (MMC) and the metabolic mechanism of action.

Materials and methods: NMR and MS-based metabolomics approaches were combined for monitoring MMC-induced immunotoxicity and the protective effect of SJZD. Body weight change and mortality, histopathological observations and relative viscera weight determinations of spleen and thymus, sternum micronucleus assay and hematological analysis were used to confirm the immunotoxicity and attenuation effects. An OPLS-DA approach was used to screen potential biomarkers of immunotoxicity and the MetaboAnalyst and KEGG PATHWAY Database were used to investigate the metabolic pathways.

Results: 8 biomarkers in plasma samples, 19 in urine samples and 10 in spleen samples were identified as being primarily involved in amino acid metabolism, carbohydrate metabolism and lipid metabolism. The most critical pathway was alanine, aspartate and glutamate metabolism.

Conclusions: The variations in biomarkers revealed the preventive effect of the immunotoxicity of SJZD on MMC and significant for speculating the possible metabolic mechanism.

1. Introduction

Mitomycin C (MMC) is a frequently used anticancer agent for the clinical treatment of gastrointestinal, breast, head and neck, cervical, and bladder malignancies (Kozuch et al., 2001; Kaasinen et al., 2016). There have also been recent reports of scar reduction in the inflammatory and proliferative phases of wound healing and in the treatment of refractory benign oesophageal strictures in adults (Li et al., 2016; Bartela et al., 2016). However, toxicity has limited its clinical usage. It is known that MMC can induce immunotoxicity including hematotoxicity and mutagenicity by forming inter-strand DNA cross-links (Shimada et al., 2015). The structure of MMC possesses three potentially active groups, benzoquinonyl, carbamoyl and ethyleniminyl moieties and the possible toxicity mechanism is shown in Fig. S1. Immunotoxicity have been reported frequently (Bregman et al., 1989; Piątkowska et al., 2008; Yeh, 2006) and several methods for

Abbreviations: SJZD, Sijunzi decoction; MMC, mitomycin C; NMR, nuclear magnetic resonance; UPLC-MS, ultra performance liquid chromatography tandem mass spectrometry; OPLS-DA, orthogonal partial least squares-discriminant analysis; EDTA, ethylene diamine tetraacetic acid; MN, micronucleus; WBC, white blood cell; RBC, red blood cell; LYM, lymphocyte; PLT, platelet; MNIMEs, micronucleated immature erythrocytes; TICs, representative total ion chromatograms; LPC, lysophosphatidylcholine; CTX, cyclophosphamide

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[1] Design and implementation of experiment.
[2] Auxiliary design of experiment.
[3] Auxiliary implementation of experiment.
[4] Overall planning and experiment design.

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attenuating the toxicity of chemotherapeutic agents have been reported in the literature. Typical methods involve the preparation of derivatives (Boamah et al., 2010), the promotion of dosage forms (Zhu et al., 2014) and the use of combinations with Chinese traditional medicine (Song et al., 2011; Cai et al., 2010).

Sijunzi decoction (SJZD) is a well known Chinese prescription first reported in the Song dynasty. Its main ingredients are four traditional herbal medicines, namely Ginseng Radix et Rhizoma, Atractylodis Macrocephalae Rhizoma, Poria and Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle. In China, SJZD has long been used for the treatment of gastrointestinal disorders, and it can effectively combat nausea, vomiting, and diarrhoea. Recently, clinical studies have shown that SJZD could effectively improve the gut microbiota disturbances in rats with spleen deficiency syndrome (An et al., 2014). Moreover, it has been found to indeed improve life quality of chemotherapy patients and extensive used in clinical conbined with chemotherapeutics for the treatment of cancer (Xiaochun et al., 2010; Shun, 2016). Emerging evidence has shown that SJZD and modified SJZD can play an important role in suppressing tumours and have a protective effect against gastrointestinal mucosa damage induced by chemotherapy. A literature report has described the effects of SJZD on cell apoptosis in mice with gastric cancer (Liu et al., 2006). The main constituents of SJZD are flavonoids, saponins, polysaccharides and organic acids such as ginsenoside Rb_1, ginsenoside Rg_2, ginsenoside Re, atractylenolide I-III, poricoic acid B, 16-deoxyporicoic acid B, pachymic acid A, pachymic acid B, liquiritin, liquiritigenin, glycyrrhizic acid and gancaonin I (Xu et al., 2013a, 2013b; An et al., 2014). The structures of several of these compounds are shown in Fig. 1. The use of a combination of MMC and SJZD has been reported involving an attenuation of toxicity, especially an anti-mutation effect, and a synergic anti-tumor effect in mice (Li et al., 2005).

Metabolomics involves characterization of a series of small molecules in a biological system, and it is also a research method commonly used to examine the mechanisms of disease at a metabolite level (Gooding et al., 2016). Untargeted metabolomics is usually performed in a semi-quantitative way, aimed at investigating changes in metabolic profiles. Another option is targeted metabolomics by which predefined metabolites are detected to verify specific metabolic mechanisms (West et al., 2016). Recently, the toxicity of drugs or adverse reactions of drugs have been widely investigated using a variety of metabolomic analytical techniques. The method can explain the mechanism of action of a phenotype in a promising and dependable way (Lin et al., 2014; Lock et al., 2017; He et al., 2012; Sanins et al., 1992; Coen, 2010). In this research, for the first time, NMR and MS-based metabolomics analyses of plasma, urine and spleen tissue of rats were carried out to examine the toxicity attenuation effects in rats treated with MMC and MMC combined with SJZD, and the rats treated with MMC were used only as a control. An OPLS-DA approach was used to screen for potential biomarkers of toxicity and the MetaboAnalyst and KEGG PATHWAY Database were used to identify possible metabolic pathways. This study aimed to examine the broadest range of metabolites and clarify the metabolic mechanism of action of the toxicity attenuating effect of SJZD to MMC and so provide a scientific basis for the clinical application of MMC and other chemotherapeutic agents.

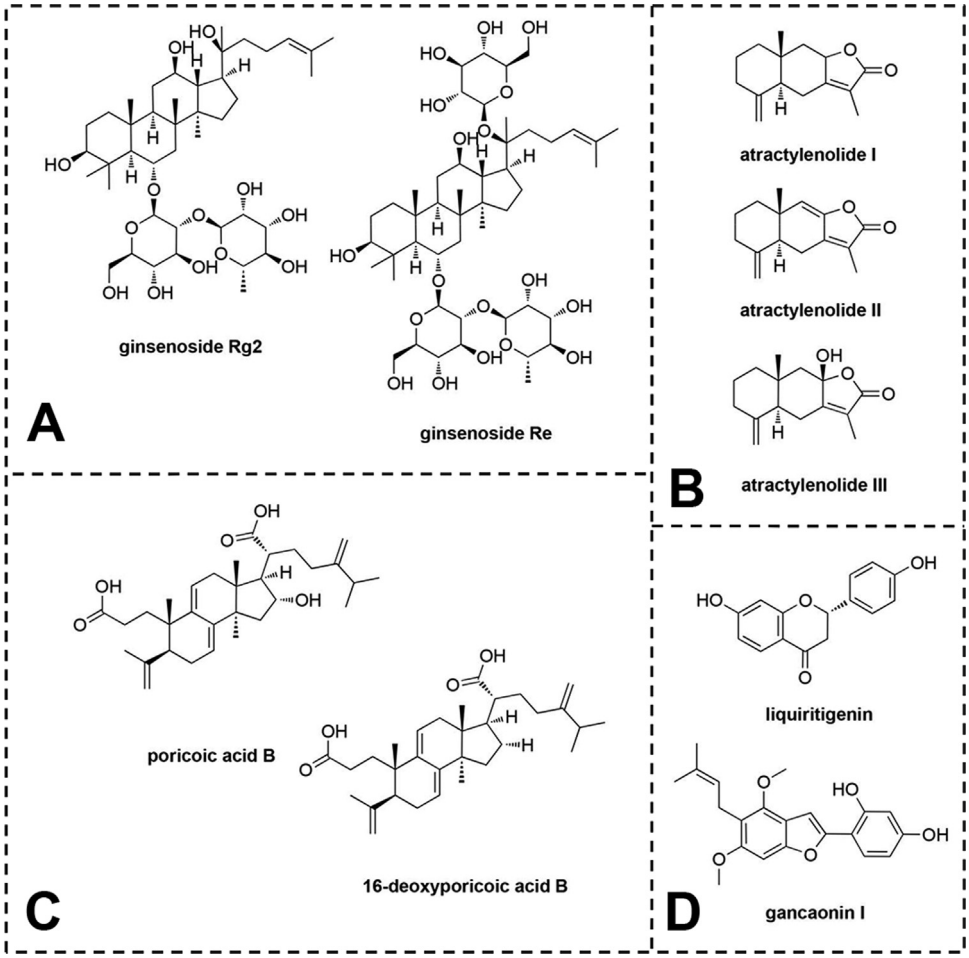

Fig. 1. The structure of several main compounds in Sijunzi decoction. A represents the typical compounds in Ginseng Radix et Rhizoma; B represents the typical compounds in Atractylodis Macrocephalae Rhizoma; C represents the typical compounds in Poria; D represent the typical compounds in Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle.