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Systems biochemical responses of rats to Kansui and vinegar-processed Kansui exposure by integrated metabonomics

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ABSTRACT

Ethnopharmacological relevance: The dried root of Kansui (*Euphorbia kansui* L.) is an effective and commonly used traditional Chinese medicine. Even so, Kansui cannot be satisfactorily applied clinically because of toxic side effects. In China, the most common Kansui-processing method uses vinegar to reduce its toxicity. The present study was designed to investigate the toxic effects caused by Kansui and evaluate detoxification of Kansui by vinegar processing of Kansui.
Materials and method: Thirty male Sprague Dawley (SD) rats were randomly assigned to five groups of six rats. Two experimental groups were oral gavaged with 7.875 and 15.75 g Kansui/kg body weight, two treated with 7.875 and 15.75 g VP-Kansui/kg body weight for 14 d, and the control group concurrently subjected to oral gavage with only distilled water. On day 14, plasma, liver and kidney tissues were collected from all rats for biochemistry assessments, histopathological examination, and NMR analyses.
Results: The metabonome of rats treated with Kansui and vinegar-processed (VP-) Kansui was found to differ from that of controls. In liver extracts, the variational metabolites included elevated concentrations of isoleucine, leucine, valine, glutamate, and phenylalanine, with decreased taurine, glucose, and glycogen. However, changes in lysine, methionine, choline, phosphorylcholine, and tyrosine were only observed in Kansui-treated rats. In kidney extracts, prominent changes included elevations in isoleucine, leucine, valine, methionine, creatine/creatinine, and phenylalanine as well as decreased glutamine. Only Kansui treatment induced variations in alanine, lysine, acetate, choline, and phosphorylcholine.
Conclusion: Perturbations in endogenous metabolites induced by Kansui correlated with disturbances in glycolysis and amino acid and lipid metabolism, while biochemical pathway disorders caused by VP-Kansui only involved glycolysis and amino acid metabolism. All results were confirmed by histopathological examination of liver and kidney tissues and clinical biochemistry analyses.

1. Introduction

The dried root of Kansui (*Euphorbia kansui* L.) is an effective and commonly used traditional Chinese medicine (TCM), which is widely applied for treating edema, ascites, and asthma (Chinese Pharmacopeia, 2010). Currently, newly described pharmacological activities of Kansui include tumor inhibition (Chang et al., 2010; Wang et al., 2012), anti-viral effects (Zheng, 2004), immune system regulation (Nunomura et al., 2006), modulatory effect on IFN-γ (Khiev et al., 2012), and diabetes treatment (Guo et al., 2012). Even so, Kansui cannot be satisfactorily applied clinically because of toxic side effects, such as inflammation (Shu et al., 2010), skin irritation (Diao, 2007), tumor promotion (Matsumoto et al., 1992; Zheng et al., 1998), and liver and kidney lesions (Deng et al., 2008; Han, 1980; Tang et al., 2012; Yang et al., 2006). In China, the most common Kansui-processing method uses vinegar to reduce its toxicity such that it has been officially incorporated in Chinese Pharmacopoeia (Chinese Pharmacopeia, 2010). Yan et al. (2012) performed preliminary studies of the mechanism of Kansui detoxification, when processed by stir-baking with vinegar, on normal liver cells LO2. The detoxification of vinegar-processed (VP)-Kansui has been evaluated to some degree by observations of

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its inflammation, purgative, and skin irritation effects (Ding et al., 2008; Geng et al., 2008; Huang et al., 2008;). However, both the mechanistic details of Kansui toxicity to liver and kidney as well as of the detoxification of VP-Kansui remain uncertain and have not been fully explained from a holistic perspective.

Metabonomics, which provides insight into the integrated function of a complex biosystem at a systemic level (Nicholson and Wilson, 2003), has been utilized in examining the generation, progression, and recovery from toxic lesions (Beckwith-Hall et al., 1998; Holmes et al., 2010; Nicholls et al., 2001). NMR has been one of the most utilized approaches in metabonomic analyses owing to its advantages, such as rapidity, noninvasiveness, and objectivity. An integrated metabonomic approach that analyzes different biological sample profiles can monitor holistic biochemical changes (Craig et al., 2006; Sheedy, 2013) and is widely used in medical toxicity analyses. For instance, an integrated metabonomic approach has been applied to investigate systemic biological responses to chronic perfluorododecanoic acid exposure (Ding et al., 2009a, 2009b). The metabolic effects of acute carbon tetrachloride treatment in multiple rat organs, including liver, kidney, lung, and spleen, have also been studied by Jiang et al. (2012) using NMR spectroscopy in conjunction with multivariate data analysis. An integrated metabonomic technique is coincident with the integrity and systematics of TCM, and is suitable for revealing toxicity mechanisms in TCM from a comprehensive perspective (Li et al., 2009; Nicholson, 2006; Tang et al., 2012; Wang et al., 2005). The toxicological effects of the TCM realgar have been investigated by comparing and combining the NMR profiles of urine, serum, and liver tissue aqueous extracts (Wei et al., 2009).

According to our previous study (Tang et al., 2012), urinary metabonomic analyses combined with traditional toxicity assessments verify the toxicity of Kansui to liver and kidney. Thus, the mechanistic details regarding the effects of Kansui on these primary target organs were further explored in this study. Furthermore, the global biochemical signature effected by VP-Kansui was captured and the mechanism of reducing Kansui toxicity by vinegar-processing also investigated.

2. Methods and materials

2.1. Kansui and VP-Kansui aqueous solution preparation

Kansui was purchased from the Medicinal Materials Market of Qingping (Guangzhou, China), and identified by Prof. Jizhu Liu (Department of Chinese Medicine of Guangdong Pharmaceutical University). VP-Kansui was produced by immersing Kansui in 30% vinegar and then stir-fry at 100 °C until dry, referencing the Chinese Pharmacopeia (2010). The Kansui and VP-Kansui aqueous solutions were concentrated and prepared according to our previous article (Tang et al., 2012). Thus, 1 g/mL aqueous solutions of Kansui and VP-Kansui were prepared and stored at 4 °C.

2.2. Animal experiments and sample collection

Thirty male Sprague Dawley (SD) rats (180–220 g, seven weeks old) were purchased from the Medical Laboratory Animal Center of Guangdong and housed at a certified animal experimental laboratory, with a 12 h light/dark cycle, constant 25 ± 1 °C, and food and water ad libitum. After one-week acclimatization, the rats were randomly assigned to five groups of six rats. Two experimental groups were oral gavaged with 7.875 and 15.75 g Kansui/kg body weight, two treated with 7.875 and 15.75 g VP-Kansui/kg body weight for 14 days, and the control group concurrently subjected to oral gavage with only distilled water. On day 14, plasma and liver and kidney tissues were collected from all rats for biochemistry assessments, histopathological examination, and NMR analyses. All samples were stored at −80 °C.

2.3. Clinical biochemistry assessments and histopathology examination

Clinical biochemical analyses of plasma samples were performed by the Guangdong Provincial People's Hospital on a Beckman DxC 800 automatic analyzer (Beckman Coulter, Inc., Brea, CA, USA), including assays for alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea nitrogen (BUN), glucose (GLUC), and creatinine (CREA). Data were statistically analyzed using SPSS16.0 software. Formalin-fixed liver and kidney biopsies from Kansui and VP-Kansui-treated rats were embedded in paraffin wax, sectioned to 3–4 μm thickness, and stained with hematoxylin and eosin (H&E) for assessment by the Department of Pathology, Guangdong Pharmaceutical University (Quangzhou, China). Generally, two or five slices from each sample were examined.

2.4. Sample preparation

Liver and kidney tissues (~50 mg each) were extracted twice with 1 mL 50% aqueous acetonitrile using a mortar and pestle. After centrifugation at 15,000 × *g* at 4 °C for 10 min, the combined supernatants were lyophilized, after removing acetonitrile under a stream of nitrogen gas with warming, and the residue resuspended in 600 μL of phosphate buffer (0.2 mol/L Na_2HPO_4/NaH_2PO_4, pH 7.4). Following a second centrifugation, as above, each supernatant was transferred into 5 mm NMR tubes and 100 μL D_2O, containing 0.05% sodium 3-trimethylsilyl-(2,2,3,3-$^2H_4$)-1-propionate (TSP), added.

2.5. ^1H NMR spectroscopy

^{1}H-NMR spectra of liver and kidney extracts were collected at 298 K on a Bruker Avance 500 MHz spectrometer equipped with a Bruker inverse probe (Bruker Biospec, Erlangen, Germany). A NOESY pulse sequence (recycle delay-G1-90 °C-t1-90 °C-G2-tm-90 °C-acquisition) was applied to suppress the residual water signal in both liver and kidney extracts. Free induction decays were collected with 64 transients into 64k data points, using a spectral width of 10 kHz with a mixing time of 100 ms and a recycling delay time of 2 s. All free-induction decays were multiplied by an exponential function with a line-broadening factor of 0.3 Hz before Fourier transformation.

2.6. Pattern recognition

All spectra were manually phase and baseline-corrected, and then bucketed and automatically integrated using a self-developed automation routine in the MestreNova software (http://mestrelab.com/software/). Each ^{1}H-NMR spectrum was segmented into regions of 0.005 ppm, with the chemical shifts of liver and kidney extract spectra referenced to TSP at δ 0.00. The integrals of these buckets covered the region δ 0.5–9.0 and were input as variables for pattern recognition. Variations in water suppression efficiency in the spectra were removed by excluding the regions from δ 4.6–5.1. Prior to pattern recognition analysis, each bucketed region was normalized to the total sum of the spectral integrals to compensate for concentration variation effects (Holmes et al., 1994).

All ^{1}H-NMR spectra were submitted to multivariate pattern recognition using orthogonal projections to latent structures-discriminant analysis (OPLS-DA) (Trygg, 2002; Trygg and Wold, 2003) in the Simca-P$^+$ 11.0 software (Umetrics, Umeá, Sweden). OPLS-DA is an extension of partial least squares-discriminant analysis (PLS-DA) featuring an integrated orthogonal signal correction (OSC) filter (Trygg and Wold, 2002) to remove variability not