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Metabonomic analysis of quercetin against the toxicity of chronic exposure to low-level dichlorvos in rats via ultra-performance liquid chromatography–mass spectrometry

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HIGHLIGHTS

- First metabonomics study on the effect of quercetin on toxicity induced by DDVP.
- Research dose was according to the human exposed dose.
- We found quercetin has a partial protective effect on DDVP-induced toxicity.
- Quercetin can regulate the DDVP-induced toxicity at the organism metabolism level.

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ABSTRACT

This study aims to determine whether quercetin elicits a protective effect against the toxicity of chronic exposure to low-level DDVP using metabonomic technology. Rats were randomly assigned into the control, DDVP-treated, quercetin-treated, and quercetin plus DDVP-treated groups. DDVP and quercetin were given to rats daily via drinking water and gavage respectively for 90 days. Eighteen metabolites, including the biomarkers of DDVP exposure (dimethyl phosphate, DMP) and quercetin exposure (quercetin and isorhamnetina), were identified from the metabonomic profiles of rat urine using ultra-performance liquid chromatography–mass spectrometry. Compared with the control group, the DDVP-treated group showed statistically significantly increased intensities of indoxyl sulfate, estrone sulfate, cholic acid, deoxycholic acid, p-cresol, p-cresol sulfate, and orotic acid but decreased intensities of suberic acid, citric acid, sebacic acid, hippuric acid, taurine, phosphocreatine, 3-hydroxyanthranilic acid, and kynurenic acid. The tendency of the aforesaid metabolites to change was significantly ameliorated in the quercetin (50 mg/kg·bw) plus DDVP (7.2 mg/kg·bw)-treated group compared with the DDVP-treated group. However, the levels of these metabolites in the quercetin plus DDVP-treated groups were still significantly different from those of the control group. These results indicate that quercetin has a partial protective effect on DDVP-induced toxicity.

1. Introduction

Dichlorvos or dimethyl-dichloro-vinyl-phosphate (DDVP), an organophosphate insecticide introduced in 1961, is extensively used to control pests worldwide. DDVP is also widely used in the agricultural, domestic, veterinary, and public health industries in China because of its high efficiency and accessibility (Du et al., 2013). However, the extensive applications of DDVP inevitably cause environmental, soil, and crop pollution. Consequently, human exposure to low-level DDVP became chronic via contaminated food and water. As a result, the effect of DDVP on human health has aroused increasing attention from both the academia and the general public.

It is well known that DDVP is an inhibitor of acetylcholinesterase (Dere et al., 2010). Some studies have demonstrated that DDVP has hepatotoxicity, renal toxicity, neurotoxicity, immunotoxicity, reproductive toxicity, and carcinogenicity (Ajiboso et al., 2012;

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Celik et al., 2009; Desai and Desai, 2008; Lafontaine et al., 1981; Okamura et al., 2005). Substantial evidence has also proven that DDVP can induce lipid peroxidation (Ajiboye, 2010; Dirican and Kalender, 2012; Sharma and Singh, 2012). Therefore, researching how to reduce the DDVP-induced toxicity is highly necessary.

Quercetin or 3,5,7,3′,4′-pentahydroxyflavanone, a representative compound in the flavonoid family, is ubiquitous in human diets, such as vegetables, fruit, seeds, tea, cereals, pulses, and Chinese herbal medicine. Numerous studies have substantiated that quercetin has various biological actions, including antioxidation, anti-inflammatory, antianaphylaxis, and DNA protection. Epidemiological studies have also suggested that quercetin can prevent chronic diseases, such as cardiovascular disease, hypertension, and cancer (Gregory and Kelly, 2011; Zhang et al., 2010a). Many studies have proposed that the beneficial effects of quercetin are linked to its antioxidant activity (Baghel et al., 2012; Kalender et al., 2012; Zhao et al., 2011). Some animal experiments have indicated that quercetin can protect organisms against the toxicity of exogenous poisons, such as insecticides and hazardous metals (Barcelos et al., 2011; Uzun et al., 2010). However, these studies only focused at the tissue or organ level rather than at the organism level in rats. Therefore, a systemic approach is necessary to study the effect of quercetin on DDVP-induced toxicity at the organism level.

Metabonomics is defined as “the quantitative measurement of the dynamic multi-parametric metabolic response of living systems to pathophysiological stimuli or genetic modification” (Nicholson et al., 1999). The rapid improvements of the technologies enabling the determination of large numbers of biomolecules and subsequent multivariate data analysis provide the opportunity to obtain new insights in the toxicity by a biochemically based systems toxicology approach (Greef et al., 2004; Van der Greef et al., 2007). In such an approach, the biochemical changes in preferably easily accessible biological fluids such as blood or urine are detected as biomarkers for toxicity-related pathogenesis. The use of metabonomics is rapidly expanding, and it is now becoming a useful tool to investigate the biochemical effects of many toxins. To date, metabonomics has had perhaps its greatest impact in the area of toxicology. It is now recognized as an independent and widely used technique for identifying target organ toxicity through biomarkers and evaluating the toxicities of chemicals (Du et al., 2013; Wang et al., 2009).

The potential effects of chemicals are dependent on numerous factors, including the interactions among them that can alter their metabolism (Safe, 1998; Wilkinson et al., 2000). The human diet contains many natural and potential hazards; thus, the entire population is constantly exposed to a complex mixture of compounds, including the residual insecticides in food. The metabolic pathways of flavonoid and organophosphate pesticides may be similar; hence, we infer by theoretically analyzing that quercetin can possibly influence DDVP-induced toxicity at the organism metabolism level. In previous study, we assessed the effects of long-term exposure to low levels of DDVP on rats by metabonomics. Changes in the concentration of some urine metabolites were detected, the intensities of lactobionic acid, estrone sulfate, and indoxyl sulfic were statistically increased and the intensities of citric acid, suberic acid, gulonic acid, urea, creatinine, and uric acid were statistically decreased in DDVP-treated group compared with the intensities in time-matched control group. These results indicated that chronic exposure to low-level DDVP can interfere with the metabolism of carbohydrates and fatty acids, and disturb the antioxidant system, etc. in rats. The current study aims to investigate whether quercetin has the protective effect on DDVP-induced toxicity mentioned above using the same method, and provide further insight into the mechanism of the effect.

2. Materials and methods

2.1. Chemicals and reagents

The DDVP (95%) used in this study was supplied by Hebei New Century Chemical Co. Ltd. (Hengshui, China). Quercetin (95% purity) was purchased from Sigma–Aldrich (Germany). HPLC-grade methanol and acetonitrile were obtained from Dikma Science and Technology, Co. Ltd. (Canada). The drinking water was purified using the Milli-Q system (Millipore, Billerica, MA, USA). Carboxymethylated cellulose (CMC) and other chemicals were of analytical grade.

2.2. Animal handling and test design

For the quercetin treatment groups, three dose groups were designed as follows: low-dose (2 mg/kg·bw/d), middle-dose (10 mg/kg·bw/d), high-dose (50 mg/kg·bw/d) groups, and the middle-dose was the Chinese exposed dose (Zhang et al., 2010b). Quercetin was dissolved in 0.5% CMC according to the dose of the treatment group and was given to the rats via gavage. The dose of DDVP (7.2 mg/kg·bw/d) was determined according to the previous study of Yang et al. (2011). According to the dose of each group, DDVP was dissolved in drinking water and was given to the rats by drinking water ad libitum. Body weight was measured once a week during the study.

A total of 88 male Wistar rats weighing approximately 180–200 g were purchased from Vital Laboratory Animal Technology Co. Ltd. (Beijing, Chain). All animal experimental procedures were approved by the Institute of Zoology Animal and Medical Ethics Committee and were in compliance with the current Chinese legislation. All rats were housed individually in metabolic cages under the following controlled circumstances: temperature ($22 \pm 2\,^{\circ}C$), humidity (50–60%), and a 12 h light–dark cycle. Normal powder diet (AIN-93M) and drinking water were given ad libitum.

After 1 week acclimatization, the rats were divided randomly into eight groups: group I (C), normal group; group II (Q1), treated with low-dose quercetin; group III (Q2), treated with middle-dose quercetin; group IV (Q3), treated with high-dose quercetin; group V (D), treated with DDVP; group VI (DQ1), treated with low-dose quercetin plus DDVP; group VII (DQ2), treated with middle-dose quercetin plus DDVP; and group VIII (DQ3), treated with high-dose quercetin plus DDVP. The weight of each group was normalized to guarantee the reliability of the results. The rats in groups II, III, IV, VI, VII, and VIII were administered with quercetin via gavage once a day according to the dose assigned to each group, whereas the rats in groups I and V were given 0.5% CMC through the same administration method. The rats in groups V, VI, VII, and VIII were given DDVP via drinking water ad libitum, whereas the rats in groups I, II, III, and IV were only given drinking water. The treatment was administrated continually for 90 d. The water consumption of rats increased from 26 mL to 43 mL in the first 7 weeks after treatment and then settled at approximately 40 mL at the end of the study. The water consumption of the rats in different experimental groups was not significantly different with that of the rats in the control group at each time point ($p > 0.05$).

2.3. Sample collection and preparation

Urine samples were collected in metabolism cages for 24 h over ice packs at each time point (24 h pre-dose, 4-week, 8-week, and 12-week post-dose). The urine samples were centrifuged at 10,000 rpm for 10 min at $4\,^{\circ}C$ (Beckman, USA, AllegraTM64R). The resulting supernatants were stored at $-80\,^{\circ}C$. Prior to analysis, the supernatants were thawed at $4\,^{\circ}C$ and then centrifuged at 12,000 rpm for 10 min. The supernatants were collected and diluted