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Hepatic and renal toxicological evaluations of an industrial ovotoxic chemical, 4-vinylcyclohexene diepoxide, in both sexes of Wistar rats

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ABSTRACT

4-Vinylcyclohexene diepoxide (VCD) is an industrial occupational health hazard chemical because it induces ovotoxicity in rodents. The current study investigated the impacts of VCD on selected hepatic and renal markers of oxidative stress and inflammation in both sexes of Wistar rats. Thus, male and female rats were randomly distributed into four groups of ten rats per group, and dosed orally with VCD for 28 days. The control male and female groups of rats received corn oil only, while each of the three remaining groups of both sexes of rats received VCD (100, 250 and 500 mg/kg BW) respectively. Thereafter, biomarkers of hepatic and renal oxidative damage, inflammation and immunohistochemical expressions of iNOS, COX-2, caspase-9 and caspase-3 were evaluated. The results revealed that VCD increased markers of liver and kidney functions, oxidative damage and inflammation, and disrupted the antioxidant homeostasis of the rats ($p < 0.05$). Lastly, VCD enhanced the immunohistochemical expressions of iNOS, COX-2, caspase-9 and caspase-3 in the liver of the rats. Thus, our data imply that VCD induced toxicity in the liver and kidney of rats via the combined impacts of oxidative damage and inflammation.

1. Introduction

4-Vinylcyclohexene diepoxide (VCD) is an occupational chemical widely reported to selectively destroy ovarian and small pre-antral (primordial and primary) follicles in rodents via the acceleration of the natural, and apoptotic process of atresia (Kappeler and Hoyer, 2012). Thus, VCD is of toxicological concern to women because it can result in premature ovarian failure (*i.e.* early menopause). It is one of the metabolites of 4-vinylcyclohexene (VCH), a dimer of 1,3-butadiene generated in the course of tyre curing (Takai et al., 2003). The production process of VCD involves the epoxidation of VCH with peroxyacetic acid in an inert solvent (Wallace, 1964). Of note, VCD is in high demand in the industry because it provides commercial outlet for VCH, and it is used as a reactive diluent for several epoxy resins (Chemical Manufacturers Association, 1991; Union Carbide Corp, 1964). Importantly, human exposure to VCD occurs when it is being produced and during the manufacture of epoxy-based polyglycols and resins. In addition, the risk of occupational exposure to VCD is high in laboratories where embedding of biological tissues with the use of epoxy polymer for electron microscopy is carried out (Ringo et al., 1982). Sadly, between 1981 and 1983, up to 6200 workers, and possibly consumers, were exposed to a product contaminated with VCD (US National Institute for Occupational Safety and Health, 1993). Additionally, it was reported that some Russian workers in the rubber industry got exposed to VCH, the parent compound, with average concentrations of 271–542 mL/m^3 with peak concentrations of 677 mL/m^3. These people suffered from several ailments such as leukopenia, keratitis, headaches, rhinitis, lymphocytosis and neutrophilia (ACGIH, 1995). In mammalian liver and kidney, the metabolism of VCD occurs through bioactivation to an inactive tetrol metabolite [4-(1,2-dihydroxy)ethyl-1,2-dihydroxycyclohexane] catalysed by

Abbreviations: VCD, 4-vinylcyclohexene diepoxide; VCM, 4-vinylcyclohexene 1,2-monoepoxide; VCH, 4-vinylcyclohexene; DCFH-DA, 2′,7′-dichlorofluorescein diacetate; mEH, microsomal epoxide hydrolase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, gamma-glutamyltransferase; IL-1β, interleukin-1 beta; IL-6, interleukin-6; TNF-α, tumor necrosis factor alpha; caspases-3 and -9, cysteine-dependent aspartate-directed proteases-3 and -9; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2.

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microsomal epoxide hydrolase (mEH). Another pathway involves the conjugation of VCD with Glutathione (GSH) with the aid of Glutathione S-transferase (GST) to form VCD-SG conjugates (Scheme 1, Bhattacharya et al., 2012; Rajapaksa et al., 2007; Keating et al., 2008).

The exposure of humans to environmental contaminants can lead to the accumulation of free radicals, which in turn triggers several factors such as oxidative stress, inflammation and cell death, which may predispose to different liver and kidney diseases. In effect, oxidative stress and inflammation are linked in a multifaceted feedback sequence. In this context, the expressions of pro-inflammatory cytokines and antioxidant enzymes are stimulated due to ROS-induced activation of transcription factors which results in the mobilisation and recruitment of phagocytic leukocytes to the sites of inflammation. These leucocytes then express certain enzymes that contribute to oxidative damage, which in turn intensify tissue inflammation (Conner and Grisham, 1996; Peters et al., 2015; Reuter et al., 2010). Thus, oxidative stress and inflammation are interconnected contributory factors that can predispose to a variety of hepatic and renal diseases.

In our previous studies, we reported that VCH and its metabolites such as 4-vinylcyclohexene monoepoxide (VCM) and VCD, induced accumulation of reactive oxygen and nitrogen species (RONS) and disrupted antioxidant homeostasis using a non-mammalian model, *Drosophila melanogaster* (Abolaji et al., 2014, 2015). However, in these studies, it was not possible to directly investigate the impacts of VCD on the analogous organs of liver and kidney of the flies. Also, most of the studies on VCD were mainly focussed on its ovotoxic effects, with few carried out to investigate its effects on the liver and kidney of rats. For instance, daily treatment (i.m. injections) of four adult female cynomolgus monkeys with VCD (250 mg/kg body weight) increased the concentrations of AST and ALT, which resolved by day 15 of treatment (Appt et al., 2006). Likewise, Chhabra et al. (1990) administered VCD (between 62.5–1000 mg/kg for 13 weeks) to rats and mice and showed that in addition to the ovary, other target organs are the forestomach (hyperplasia and hyperkeratosis) and the kidney (tubular cell degeneration). In addition, the early report of the National Toxicological Program revealed that male and female rats and mice dosed by gavage with VCH for two years resulted in early mortality of most of the animals. In this same study, male and female mice dosed by gavage with VCH for two years developed inflammatory lesions in the forestomach and had lung congestions (NTP, 1986). Here, we sought to further comprehend the mechanisms of toxicity of VCD in both sexes of rats, with particular emphasis on selected hepatic and renal markers of oxidative damage, inflammation and apoptosis. Mainly, we evaluated the impacts of VCD on the antioxidant defense systems of the liver and kidney, serum inflammatory markers such as IL-1β, IL-6 and TNF-α, as well as immunohistochemical expressions of iNOS, COX-2 and caspases-9 and 3 in the liver of both sexes of rats.

2. Material and methods

2.1. Reagents and chemicals

The 4-vinylcyclohexene diepoxide (CAS: 106-86-6: 96%), 1-chloro-2,4-dinitrobenzene, 5′,5′-dithiobis(2-nitro-benzoic acid) (DTNB), 2′,7′-dichlorofluorescein diacetate (DCFH-DA), epinephrine, glutathione (GSH), thiobarbituric acid (TBA), were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other analytical grade reagents were procured from the British Drug House (Poole, Dorset, UK).

2.2. Experimental animals and study design

Adult male and female Wistar rats (150–220 g) were procured from the Central Animal House Facility of the College of Medicine, University of Ibadan, Nigeria. The rats were initially acclimatized for two (2) weeks after their purchase. Then, ten (10) rats per cage were provided with rat pellets and water *ad libitun*. The rats were kept in a well-ventilated room in plastic cages under conditions of 12-h light/12-h dark cycle and temperature 24 ± 2 °C throughout the period of acclimatization and treatment with VCD. After the approval of our institutional animal ethics committee, both sexes of rats received humane care in accordance with the protocol stated in the 'Guide for the Care and Use of Laboratory Animals' published by the National Institute of Health. Subsequently, male and female rats were randomly distributed into four groups of 10 rats per group, and were respectively treated orally with VCD for 28 days using corn oil as vehicle. Groups 1a and b, (respective male and female control rats), received corn oil only. Similarly, Groups 2a and b rats received 100 mg/kg body weight of VCD, Groups 3a and b rats received 250 mg/kg body weight of VCD, while Groups 4a and b rats received 500 mg/kg body weight of VCD respectively. These concentrations were chosen based on previous studies in which VCD doses ranging from 40 to 1000 mg/kg have been used in rats and mice for different durations (Chhabra et al., 1990; Devine et al., 2001; Kao et al., 1999; Springer et al., 1996).

2.3. Sacrifice, serum preparation, and post mitochondrial fraction collection

The rats were then sacrificed by cervical dislocation. Blood was collected by cardiac puncture technique with the aid of a sterilized needle and syringe into clean, dry, non-heparinized centrifuge tubes and allowed to coagulate, by standing for about 30 min. Serum was then separated by centrifugation of the clotted blood at 4000*g* for 10 min with a table centrifuge. The clear supernatant (serum) was collected and stored in the refrigerator. The liver and kidney were quickly removed, rinsed in ice-cold 1.15% KCl and blotted with filter paper. They were then minced with scissors in 4 and 5 vols of ice-cold 0.1 M phosphate buffer pH 7.4 respectively, and homogenized using potter-Elvehgen homogenizer. The homogenates were later centrifuged at 10,000*g* for 10 min in a cold centrifuge at 4 °C, and the supernatants (post mitochondrial fractions) were used for the biochemical assays.

2.4. Determinations of liver and renal function markers in the serum of both sexes of rats treated with VCD

The liver and kidney function markers were evaluated by using commercially available kits from Randox Laboratory Limited (UK). The activity of serum Gamma-glutamyl transferase (γ-GT) was carried out using reconstituted γ-GT diagnostic reagent kit (Szasz, 1969). The activities of aspartate and alanine aminotransferases (AST and ALT) were determined by the method of Reitmann and Frankel (1957), while creatinine level was determined using the method of Jendrassik and Grof (1983).

2.5. Assessment of liver and kidney markers of oxidative stress and antioxidant status

2.5.1. Determination of reactive oxygen and nitrogen species (RONS) in the liver and kidney of both sexes of rats treated with VCD

The liver and kidney supernatants obtained above were used to determine 2′,7′-dichlorofluorescein (DCFH) oxidation as a general index of oxidative stress (Perez-Severiano et al., 2004). The reaction mixture was made of 450 μL of 0.1 M potassium phos-