Toxicology 339 (2016) 34–39

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Reactivity measurement in estimation of benzoquinone and benzoquinone derivatives' allergenicity

Wilbes Mbiya[a,*], Itai Chipinda[b], Reuben H. Simoyi[a], Paul D. Siegel[b]

[a] Department of Chemistry, Portland State University, Portland, OR 97207-0751, United States
[b] Allergy and Clinical Immunology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV 26505-2888, United States

ARTICLE INFO

Article history:
Received 23 March 2015
Received in revised form 27 October 2015
Accepted 11 November 2015
Available online 2 December 2015

Keywords:
Allergic contact dermatitis
Benzoquinone
Local lymph node assay
Michael addition
SNV
Nucleophilic vinylic substitution
Stimulation index

ABSTRACT

Benzoquinone (BQ) and benzoquinone derivatives (BQD) are used in the production of dyes and cosmetics. While BQ, an extreme skin sensitizer, is an electrophile known to covalently modify proteins via Michael Addition (MA) reaction whilst halogen substituted BQD undergo nucleophilic vinylic substitution (SNV) mechanism onto amine and thiol moieties on proteins, the allergenic effects of adding substituents on BQ have not been reported. The effects of inserting substituents on the BQ ring has not been studied in animal assays. However, mandated reduction/elimination of animals used in cosmetics testing in Europe has led to an increased need for alternatives for the prediction of skin sensitization potential. Electron withdrawing and electron donating substituents on BQ were assessed for effects on BQ reactivity toward nitrobenzene thiol (NBT). The NBT binding studies demonstrated that addition of EWG to BQ as exemplified by the chlorine substituted BQDs increased reactivity while addition of EDG as in the methyl substituted BQDs reduced reactivity. BQ and BQD skin allerginicity was evaluated in the murine local lymph node assay (LLNA). BQD with electron withdrawing groups had the highest chemical potency followed by unsubstituted BQ and the least potent were the BQD with electron donating groups. The BQD results demonstrate the impact of inductive effects on both BQ reactivity and allergenicity, and suggest the potential utility of chemical reactivity data for electrophilic allergen identification and potency ranking.

1. Introduction

Allergic contact dermatitis (ACD) is a prevalent occupational disease caused by a wide range of chemicals (Payne and Walsh, 1994; Roberts et al., 2011). ACD which is estimated to affect 1–4% (Smith and Hotchkiss, 2001) of the general world-wide population, accounts for 30–>50% of occupational skin disorders depending on the industry. Over 13 million workers in the US are believed to be at risk from exposure to potential skin sensitizers and the level of compensation due to occupational contact dermatitis has been estimated to be greater than $1 billion/yr (Mathias and Morrison, 1988). ACD is also a significant health hazard of concern to developers of cosmetic, personal care, chemical, pharmaceutical, and medical device products (Mckim et al., 2010; Roberts and Aptula, 2008).

Existing animal based assays such as the murine local lymph node assay (LLNA) (Basketter et al., 2000), which is based on the proliferation of lymph node cells in the induction phase or the mouse ear swelling test (MEST) (Gad, 1994) and the guinea pig maximization test (GPMT) (GPMT, 2002) which are based on the observation of the allergic responses in the elicitation phase of ACD are widely used to identify skin sensitizing chemicals and to measure the relative sensitization potential of contact allergens. Predictive animal tests such as the LLNA that have the capacity to identify sensitizers before they are placed on the market have been highly successful (Basketter and Maxwell, 2007). However, the mandated reduction/elimination of animals testing for cosmetics use in Europe has led to an increased need for alternatives methods for prediction of skin sensitization potential (Roberts et al., 2007). Most researchers moved to the LLNA due to its animal welfare

Abbreviations: ACD, Allergic contact dermatitis; BQ, p- benzoquinone; BQD, benzoquinone derivatives; CBQ, chlorobenzoquinone; 2,5-DCBQ, 2,5-dichlorobenzoquinone; EDG, electron donating groups; EWG, electron withdrawing groups; ICD, irritant contact dermatitis; LLNA, local lymph node assay; EC3, estimated concentration that produces a 3 fold increase in lymph node cell proliferation over the vehicle control; 2-MBQ, 2 methyl- benzoquinone; MA, Michael addition; NBT, nitrobenzenethiol; SI, stimulation index,; 2-tBBQ, 2-tertbutyl benzoquinone; SNV, nucleophilic vinylic substitution.

* Corresponding author.
E-mail addresses: wilbesm@gmail.com, mbiya@ohsu.edu (W. Mbiya).

http://dx.doi.org/10.1016/j.tox.2015.11.002
0300-483X/

benefits while efforts to develop robust alternative non-animal test methods still continue. For example, a published dataset consisting of quantitative test results for compounds tested in the LLNA is being used to develop and evaluate (validating or invalidating) alternative approaches (Roberts et al., 2011).

Hundreds of chemicals, belonging to different reaction mechanistic domains, have been shown to possess the ability to induce skin sensitization. One group of these chemicals is benzoquinone (BQ) and the benzoquinone derivatives (BQD). While BQ and BQD are used in the production of dyes (Boga et al., 2013; Shimada et al., 2015) they can be reduced to hydroquinones which are used in cosmetics (Dlova et al., 2015; Matsubayashi et al., 2002; Shin and Park, 2014; Uddin et al., 2011), leading to potential skin exposure. BQ is an extreme skin sensitizer EC3 = 0.013% (EC3 is the estimated concentration that produces a 3 fold increase in lymph node cell proliferation over the vehicle control) (Roberts and Aptula, 2009) but the effects of inserting substituents on the BQ ring have not been studied in animal assays. BQ and BQD are known to covalently modify proteins via the Michael addition (MA) reaction and/or through nucleophilic vinylic substitution (SNV), depending on whether it is attached to either electron donating groups (EDGs) or electron withdrawing groups (EWGs) that are good leaving groups (Mbiya et al., 2012).

The seven BQD test chemicals studied with expected sites of nucleophilic attack on the ring are shown in Figure 1. The reported (Mbiya et al., 2012) reactivity constants (k_a) for BQ and BQD were used to predict the LLNA EC3 values for the BQD after which LLNA studies were then performed for BQ and BQD to determine the EC3 values. LLNA studies also served to test the predictive power of the reactivity data thus evaluating the potential utility of the reactivity approach as an alternative method for skin sensitization testing.

2. Experimental

2.1. Chemicals

100 mM phosphate buffer (pH 7.4) was made using monosodium phosphate and disodium phosphate, acetic acid, sodium acetate, acetonitrile (ACN), 4-nitrobenzenethiol (NBT). All test chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise stated.

3. Methods

3.1. Approach for QSAR analysis

The k_a values reported in Mbiya et al., (2012), where BQ and BQD were reacted with NBT, were adopted. The structures of test compounds are given in Fig. 1 and the arrows indicate the reaction sites. The log *P* values were calculated from structure using ADMET® (MedChem-Designer) Version: 2.0 software (Dearden, 2007).

3.2. Local lymph node assay

Female BALB/c mice were purchased from Taconic (Hudson, NY, USA). Animals were 6–8 weeks old upon arrival and allowed to acclimate for a minimum of 10 days. Animals were housed in the Association for Assessment and Accreditation of Laboratory Animal Care International-accredited animal facility at National Institute for Occupational Safety and Health (NIOSH), Morgantown, WV, USA. Animals were housed under controlled environmental conditions in High Efficiency Particulate Act (HEPA)-filtered ventilated polycarbonate cages on autoclaved hardwood beta-chip bedding and provided Teklad 7913 food and autoclaved tap water ad libitum. All animal procedures were reviewed and approved by the NIOSH Animal Care and Use Committee.

The LLNA was performed according to the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) standard method (Haneke et al., 2001) to determine the allergenic potency of test chemicals. The LLNA test was conducted in three independent blocks, each of which contained solvent controls, assay of 2 BQD and BQ as the positive control. After randomly grouping mice into groups ($n = 4$), mice were dosed with 25 μL/ear of a test chemical in acetone olive oil (AOO; 4:1). The negative vehicle control group was dosed with AOO. The vehicle and test chemicals were applied on the dorsum of both ears. Two hundred μL of 20 μCi ^{3}H-thymidine in 0.01 M phosphate buffered

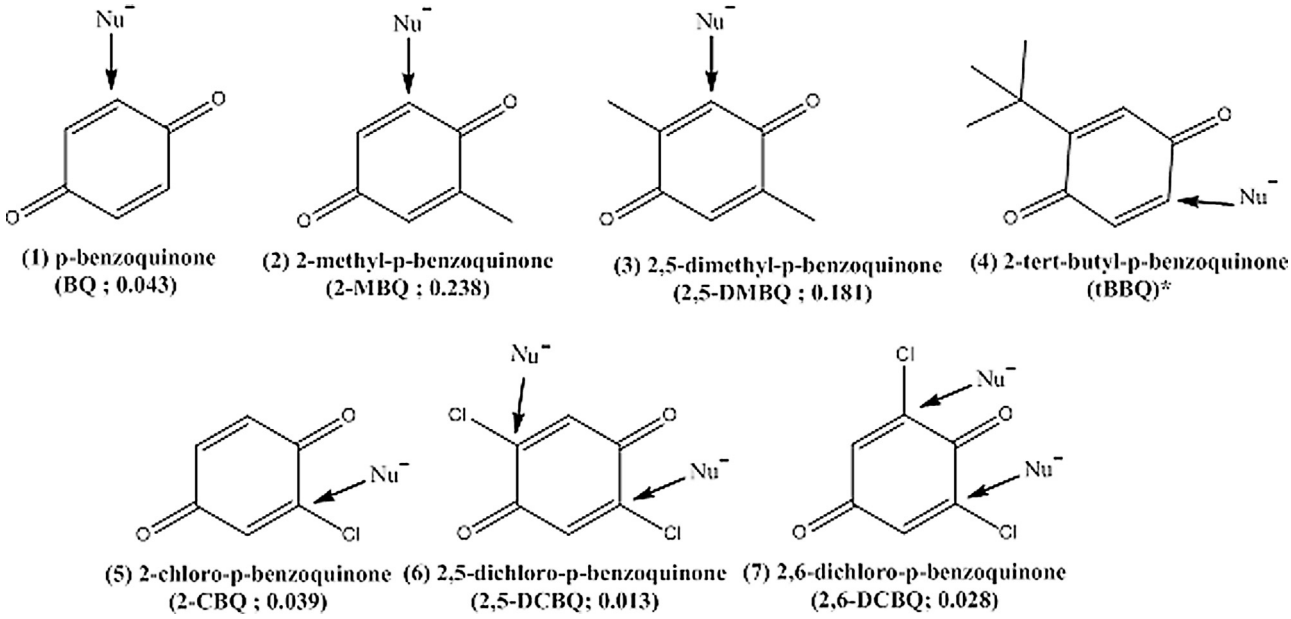

Fig. 1. BQ and BQD test chemicals and their corresponding observed EC3 values. The arrows are the sites of nucleophilic attack.