Available online at www.sciencedirect.com
ScienceDirect
Toxicology 241 (2007) 47–57
TOXICOLOGY
www.elsevier.com/locate/toxicol

In vivo hydroquinone exposure impairs allergic lung inflammation in rats

S.M.D. Macedo[a,b], S.C.M. Vaz[a], E.L.B. Lourenço[a,c], M. da Glória de Sousa[a], A.P. Ligeiro-Oliveira[d], J.M.C. Ferreira Jr.[e], S.R. Almeida[a], W. Tavares de Lima[d], S.H.P. Farsky[a,*]

[a] *Department of Clinical and Toxicological Analyses, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil*
[b] *Department of Health Science, Regional Integrated University of Alto Uruguai and Missões, Erechim, Brazil*
[c] *Paranaense University, Umuarama, Brazil*
[d] *Department of Pharmacology, Institute of Biomedical Science, University of São Paulo, São Paulo, Brazil*
[e] *Immunochemistry Laboratory, Butantan Institute, São Paulo, Brazil*

Received 13 February 2007; received in revised form 25 July 2007; accepted 5 August 2007
Available online 19 August 2007

Abstract

Hydroquinone (HQ) is naturally found in the diet, drugs, as an environmental contaminant and endogenously generated after benzene exposure. Considering that HQ alters the immune system and its several source of exposures in the environment, we hypothesized that prolonged exposure of HQ could affect the course of an immune-mediated inflammatory response. For this purpose, male Wistar rats were intraperitoneally exposed to vehicle or HQ once a day, for 22 days with a 2-day interval every 5 days. On day 10 after exposure with vehicle or HQ, animals were ovalbumin (OA)-sensitized and OA-aerosolized challenged on day 23. HQ exposure did not alter the number of circulating leukocytes but impaired allergic inflammation, evidenced by lower number of leukocytes in the bronchoalveolar lavage fluid 24 h after OA-challenge. Reduced force contraction of *ex vivo* tracheal segments upon OA-challenge and impaired mesentery mast cell degranulation after *in situ* OA-challenge were also detected in tissues from HQ exposed animals. The OA-specificity on the decreased responses was corroborated by normal trachea contraction and mast cell degranulation in response to compound 48/80. In fact, lower levels of circulating OA-anaphylactic antibodies were found in HQ exposed rats, as assessed by passive cutaneous anaphylaxis assay. The reduced level of OA-anaphylactic antibody was not dependent on lower number or proliferation of lymphocytes. Nevertheless, lower expression of the co-stimulatory molecules CD6 and CD45R on OA-activated lymphocytes from HQ exposed rats indicate the interference of HQ exposure with signaling of the humoral response during allergic inflammation. Together, these data indicate specific effects of HQ exposure manifested during an immune host defense.
Published by Elsevier Ireland Ltd.

Keywords: Hydroquinone; Allergic inflammation; Ovalbumin; Anaphylactic antibodies; CD45R; CD6; Mast cell degranulation

1. Introduction

Hydroquinone (HQ) is a naturally occurring agent in plants or plant derived products (Deisinger et al., 1996), synthetically produced to be used in chemical industries, black and white photographic developers, and in cos-

* Corresponding author at: Av. Prof. Lineu Prestes, 580 Bloco 13 B, São Paulo 05508-900, Brazil. Tel.: +55 11 3091 2197; fax: +55 11 3815 6593.
E-mail address: sfarsky@usp.br (S.H.P. Farsky).

0300-483X/$ – see front matter. Published by Elsevier Ireland Ltd.
doi:10.1016/j.tox.2007.08.085

metic products as a skin lightening agent (Stanfield et al., 2006). Moreover, the main portion of environmental contamination occurs through cigarette smoke or benzene exposure in petroleum refining, petrochemical and chemical industries (Dimitrova et al., 2005) or by traffic exhaust fumes (Darrall et al., 1998; Snyder, 2002, 2004). Experimental assays have proposed that the immunosuppressive effects of benzene intoxication are associated with its phenolic bio-products, of which HQ is a pivotal compound (Li et al., 1996, 1997; McCue et al., 2000; Snyder, 2002, 2004).

Accordingly, after benzene exposure, HQ is endogenously produced via oxidation reactions catalyzed by cytochrome P450 enzymes in the liver (Snyder, 2002, 2004). Once circulating, HQ passively reaches the bone marrow where it is metabolized and might account for the myelotoxicity in benzene intoxication (Henderson, 1996; Snyder, 2002, 2004). Taking this into consideration, HQ is potentially a toxic agent capable of affecting immune cell responses. In fact, *in vitro* assays have demonstrated that HQ blocks the production of lymphocytes and impairs their proliferation (Li et al., 1996, 1997; Poirier et al., 2002; McCue et al., 2000, 2003). The mechanisms involved are not completely understood and are related to inhibition of DNA synthesis or blastogenesis, alteration of cell cycle entry and progression through the G(1) phase (McCue et al., 2000, 2003), in addition to inhibition of cytokine production. Conversely, it was shown that HQ sensitizes mice by enhancing B cells in the popliteal lymph node (Ewens et al., 1999) and significantly increasing *in vitro* and *in vivo* IL-4 production by stimulated CD4+ T cells, accompanied by an increment in circulating immunoglobulin (Ig) E levels (Lee et al., 2002). Also, Kim et al. (2005) showed that HQ may enhance allergic immune responses by inhibiting *in vitro* IL-12 production by LPS-activated macrophage, in which the mechanism proposed is the suppression of NFκB activity by inhibiting the degradation of the IκB protein.

The allergic response is triggered by mast cell-derived mediators, released after the antigen cross-links the IgE on the mast cells surface. In the sensitization phase, antigen is presented by antigen-presenting cells (APC, dendritic cells, macrophages and Langerhans cells) to CD4 lymphocytes, which signal to the B lymphocytes to produce immunoglobulins (Murphy and Reiner, 2002; Careau et al., 2002; Ansel et al., 2003). Upon antigen challenge, the sensitized mast cell activates multiple signaling pathways causing its degranulation with a consequent release of a wide range of mediators accounting for leukocyte infiltration, plasma extravasation, airway smooth muscle contraction and mucus secretion (Galli et al., 2005; Okayama and Kawakami, 2006). In this context, it is known that accessory surface receptors and adhesion molecules in APC, T and B cells are involved in mediating the highly regulated phosphorylation and dephosphorylation of tyrosine residues on target proteins, regulating antigen presentation, lymphocyte proliferation, cytotoxicity, humoral activities and apoptosis (Iezzi et al., 1998; Burastero and Rossi, 1999; Kiefer et al., 2002). Therefore, the successful immune response is dependent on a coordinated cascade of events mediated by complex membrane and intracellular events (Burastero and Rossi, 1999; Kiefer et al., 2002).

As HQ is an environmental contaminant and displays toxicity to immune system, here we investigated the role of prolonged HQ exposure on allergic lung inflammation triggered by unrelated antigen to HQ. Data obtained show that HQ exposure impairs the expression of CD45R and CD6 co-stimulatory molecules on activated spleen lymphocytes and diminishes the levels of OA-anaphylactic antibodies and the subsequent mast cell degranulation in sensitized rats challenged with OA. These effects might be important mechanisms to account for the impaired allergic inflammation, here characterized by decreased airway reactivity and leukocyte infiltration.

2. Materials and methods

2.1. Chemicals

Pentobarbital sodium (Cristália, Brazil); FITC-labeled anti-rat CD86, CD40, CD6 and PE-labeled anti-rat CD45R and CD80 were purchased from BD Biosciences (USA); Heparin (Liquemine®, Roche, Brazil); Ovalbumin (chicken egg albumin crude, grade II), Concanavalin A from *Canavalia ensiformis* (type IV), RPMI 1640 medium, θ-dianisidine, hexadecyltrimethyl ammonium bromide, compound 48/80, EDTA and HQ were purchased from Sigma (St. Louis, MO, USA); Chloral hydrate (Quims, Brazil) ammonium chloride, aluminum hydroxide, blue toluidine, Evans blue, May-Grünwald and Giemsa dyes, H_2O_2 and ethanol were purchased from Merck (USA); [^{3}H] thymidine (Amershan Pharmacia Biotech UK Limited, UK); Fetal calf serum (Gibco-BRL, MD, USA).

2.2. Animals

Adult male Wistar rats weighing 180–220 g were used. Animals were kept under a light/dark cycle (12 h on, 12 h off), allowed a standard pellet diet and water *ad libitum*. All animal procedures were performed according to approved protocols and in accordance with recommendations for the proper use and care of laboratory animals.