Toxicology 394 (2018) 11–18

Contents lists available at ScienceDirect

Toxicology

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

p-Benzoquinone-induced aggregation and perturbation of structure and chaperone function of α-crystallin is a causative factor of cigarette smoke-related cataractogenesis

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ARTICLE INFO

Keywords:
p-Benzoquinone
Cigarette smoke
Cataract
αA-crystallin
Chaperone

ABSTRACT

Cigarette smoking is a significant risk factor for cataract. However, the mechanism by which cigarette smoke (CS) causes cataract remains poorly understood. We had earlier shown that in CS-exposed guinea pig, p-benzoquinone (p-BQ) derived from CS in the lungs is carried by the circulatory system to distant organs and induces various smoke-related pathogeneses. Here, we observed that CS exposure caused accumulation of the p-BQ-protein adduct in the eye lens of guinea pigs. We also observed accumulation of the p-BQ-protein adduct in resected lens from human smokers with cataract. No such accumulation was observed in the lens of never smokers. p-BQ is a strong arylating agent that forms Michael adducts with serum albumin and haemoglobin resulting in alterations of structure and function. A major protein in the mammalian eye lens is αA-crystallin, which is a potent molecular chaperone. αA-crystallin plays a key role in maintaining the integrity and transparency of the lens. SDS-PAGE indicated that p-BQ induced aggregation of αA-crystallin. Various biophysical techniques including UV–vis spectroscopy, fluorescence spectroscopy, FT-IR, bis-ANS titration suggested a perturbation of structure and chaperone function of αA-crystallin upon p-BQ modification. Our results indicate that p-BQ is a causative agent involved in the modification of αA-crystallin and pathogenesis of CS-induced cataract. Our findings would educate public about the impacts of smoking on eye health and help to discourage them from smoking. The study might also help scientists to develop new drugs for the intervention of CS-induced cataract at an early stage.

1. Introduction

Cataracts, the world's leading cause of blindness, are an enormous global public health problem. Although cataracts develop most commonly due to aging, there are various risk factors that are associated with cataractogenesis. The risk factors are heredity, over exposure to sunlight (particularly due to higher ultraviolet B radiation), diabetes, high use of steroids and particularly cigarette smoking (Robman and Taylor, 2005; Tarwadi and Agte, 2011; Wu et al., 2010; Flaye et al., 1989; West et al., 1989; Klein et al., 1993; Cheng et al., 2000; Foster et al., 2003; Ye et al., 2012). A smoker's risk of developing cataracts increases with the intensity of smoking and is more severe in heavy smokers than in light smokers (Christen et al., 1992; Flaye et al., 1989; Hankinson et al., 1992; Klein et al., 1993; West et al., 1989). However, the molecular mechanism by which cigarette smoke (CS) causes cataract remains poorly understood. CS is a complex mixture of about 4000 compounds (Robman and Taylor, 2005) and till date the chemical (s) involved in the pathogenesis of CS-induced cataract is unknown. Although effective treatment options are available to restore vision, identifying the risk factor(s) in CS would help to establish preventive measures as primary intervention. This would also reduce significant financial as well as the clinical burden.

Cigarette smoking affects almost all organs of the body (U.S. Department of Health and Human Services, 2010). Using a guinea pig model we had earlier shown that p-benzoquinone (p-BQ) derived from CS is a causative factor for various smoke-related diseases (Wang et al.,

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https://doi.org/10.1016/j.tox.2017.11.018
Received 7 September 2017; Received in revised form 15 November 2017; Accepted 27 November 2017
Available online 28 November 2017
0300-483X/

2006; Banerjee et al., 2008; Ghosh et al., 2012; Das et al., 2012; Ganguly et al., 2017). p-BQ is not present in CS. It is produced from p-benzosemiquinone (p-BSQ) of CS in the lungs and is carried by the circulatory system to distant organs and induces the smoke-related diseases (Wang et al., 2006; Banerjee et al., 2008; Ghosh et al., 2012; Das et al., 2012; Ganguly et al., 2017). p-BQ is not only a redox cycling agent that generates reactive oxygen species producing oxidative stress (Das et al., 2011), but also a strong arylating agent that forms Michael adducts with proteins (Wang et al., 2006; Das et al., 2011; Bolton et al., 2000; Ghosh et al., 2016; Sharma et al., 1998). Previous studies demonstrated that p-BQ derived from CS forms Michael adducts with serum albumin and hemoglobin in vivo and causes alteration of structure and function of the proteins (Banerjee et al., 2008; Sharma et al., 1998). The higher predisposition of smokers towards cataract formation may indicate that like other organs p-BQ may also reach the eye and cause alteration of structure and function of lens proteins.

The human eye lens is a transparent refractive medium consisting of densely packed proteins. Over 90% of the lens proteins are crystallins that maintain the transparency of the lens (Hoehenwarter et al., 2006; Sharma and Santhoshkumar 2009). The major component of the crystallin proteins is α-crystallin and the others being various β- and γ-crystallins (Sharma and Santhoshkumar, 2009). α-crystallin has two chains A and B in the ratio of 3:1. αA-crystallin is a potent molecular chaperone that prevents stress or external agent-induced insoluble aggregate formation and plays a key role in maintaining the integrity and transparency of the lens (Sharma et al., 1998; Wang and King, 2010; Horwitz, 1992). It is reported that loss of chaperone function of α-crystallin along with uncontrolled aggregation of the crystallin proteins in the lens causes scattering leading to the formation of cataract (Wang and King, 2010; Horwicz, 1993, 1992).

In this communication, we show that CS exposure leads to accumulation of p-BQ-protein adduct in the eye lens of guinea pigs. We also show that in contrast to resected cataract samples from never smokers, cataract samples from smokers contain p-BQ-protein adducts. Previously we observed that p-BQ induced aggregation of haemoglobin resulting in alterations of structure and function (Ghosh et al., 2016). It is reported that uncontrolled aggregation and loss of chaperone function of α-crystallin causes scattering that interfere with vision (cataract) (Horwitz, 2003).

Since αA-crystallin is found almost exclusively in the lens (Sharma and Santhoshkumar, 2009), in this study we expressed recombinant αA-crystallin in E. coli and using various biophysical studies investigated the effect of p-BQ on aggregation and the structure and chaperon function of αA-crystallin. Our results demonstrate that p-BQ is a causative agent involved in the pathogenesis of CS-induced cataract.

2. Experimental procedures

2.1. Ethics approval

Samples of resected cataract after surgery from current smokers and never smokers with cataract were provided by the Regional Institute of Opthalmology, Medical College, Kolkata 700073, India that was approved by the Institutional Ethics Committee. All participants in the study were provided with printed information regarding the study and informed consent was obtained prior to collection of samples. Experiments with human samples were approved by the Institutional Bioethics Committee for Human Research Studies, University of Calcutta, following the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. All animal experiments (detailed below) were approved by the Institutional Animal Ethics Committee, University of Calcutta, and were in strict conformity with NIH guidelines.

2.2. Materials

Sephacryl S300-HR, Dithiothreitol (DTT), IPTG (Isopropyl β-D-thiogalactoside), DNase and lysozyme were obtained from Sigma (St. Louis, MO, USA). p-Benzoquinone (pBQ) was procured from Himedia [RM-489] and freshly crystallized from n-hexane before use. All other reagents such as buffers and salts were of analytical grade. Polyclonal antibody to p-BQ raised in rabbit after immunization with p-BQ-bovine serum albumin conjugate was supplied by Abexome Biosciences, Bangalore, India.

2.3. Exposure of guinea pigs to cigarette smoke (CS)

Male short hair guinea pigs weighing 350–450 g were used for all experiments. The guinea pigs were fed vitamin C-restricted diet to minimize the vitamin C level of tissues. This is because vitamin C is not only a potential inhibitor of CS-induced protein oxidation, but also vitamin C ($E_0 = +0.08$ V) reduces p-BQ ($E_0 = +0.71$ V) and thereby inactivates p-BQ. Sufficient tissue vitamin C level would prevent p-BQ-induced pathogenesis. The guinea pigs were subjected to cigarette smoke exposure from 5 Kentucky research cigarettes (3R4F)/day @ 2 puffs/cigarette/animal in a smoke chamber for 56 days as before (Banerjee et al., 2008; Das et al., 2011, 2012; Ghosh et al., 2012).

2.4. Protein isolation from the human lens

Human lens nuclei were obtained after capsular cataract surgery. Smoking history of patients was recorded and samples were taken from male smokers in the age group 55–65 years having a smoking history of 10–20 cigarettes per day for 30–40 years. The lenses were homogenized in ice-cold lysis buffer containing 50 mM Tris-HCl (pH-7.4), 150 mM NaCl, 2 mM EDTA, 50 mM NaF, 0.1% Triton X and protease inhibitor cocktail, then centrifuged at 4 °C at 10,000 g for 30 min. The supernatant was assayed for protein concentration and subjected to immunoblotting.

2.5. Protein isolation from guinea pigs lens

After smoke exposure for 56 days, the guinea pigs were euthanized under deep anesthesia using i.p. injection of ketamine hydrochloride (100 mg/kg body weight) and the eye lenses were dissected out carefully and washed in PBS. The lenses were homogenized in lysis buffer as mentioned above; the supernatant was used as the soluble fraction. The pellet obtained was extracted with the same lysis buffer with 2% SDS and centrifuged similarly. The supernatant thus obtained was used as the insoluble fraction. Both the soluble and insoluble fractions were assayed for protein concentration and subjected to western blot analysis.

2.6. Western blot analysis

Protein samples (15 μg) were resolved in 12% SDS-PAGE and transferred to PVDF membrane and blocked with 10% non-fat dry milk. Polyclonal antibody to p-BQ was used for the detection of protein-p-BQ adduct formation. The membrane was developed with goat anti-rabbit IgG-HRP secondary antibody (Cell Signaling Technology) followed by detection of anti-p-BQ antibody using chemiluminescence (LumiGLO Reagent and Peroxide, Cell Signaling Technology).

2.7. Overexpression and purification of αA- crystallin

Recombinant αA-crystallin was over-expressed and purified as mentioned before (Biswas and Das, 2004). Briefly αA-crystallin cDNA cloned in the pAED4 vector was transformed into *E.coli* BL21-DE3 strain. Cultures grown in LB medium at 37 °C were induced with IPTG. The protein was isolated from the soluble fraction of the cell lysate