Journal of Trace Elements in Medicine and Biology 31 (2015) 53–60

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

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

TOXICOLOGY

Hesperidin ameliorates heavy metal induced toxicity mediated by oxidative stress in brain of Wistar rats

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ARTICLE INFO

Article history:
Received 14 November 2014
Accepted 16 March 2015

Keywords:
Cadmium
Heavy metal
Hesperidin
Rats
Neurotoxicity
Antioxidant

ABSTRACT

Cadmium (Cd) induces neurotoxicity owing to its highly deleterious capacity to cross the blood brain barrier (BBB). Recent studies have provided insights on antioxidant properties of bioflavonoids which have emerged as potential therapeutic and nutraceutical agents. The aim of our study was to examine the hypothesis that hesperidin (HP) ameliorates oxidative stress and may have mitigatory effects in the extent of heavy metal-induced neurotoxicity. Cd (3 mg/kg body weight) was administered subcutaneously for 21 days while HP (40 mg/kg body weight) was administered orally once every day. The results of the current investigation demonstrate significant elevated levels of oxidative stress markers such as lipid peroxidation (LPO) and protein carbonyl (PC) along with significant depletion in the activity of non-enzymatic antioxidants like glutathione (GSH) and non-protein thiol (NP-SH) and enzymatic antioxidants in the Cd treated rats' brain. Activity of neurotoxicity biomarkers such as acetylcholinesterase (AchE), monoamine oxidase (MAO) and total ATPase were also altered significantly and HP treatment significantly attenuated the altered levels of oxidative stress and neurotoxicity biomarkers while salvaging the antioxidant sentinels of cells to near normal levels thus exhibiting potent antioxidant and neuroprotective effects on the brain tissue against oxidative damage in Cd treated rodent model.

Introduction

Heavy metals, a poorly defined group of elements which primarily includes transition metals, some metalloids, lanthanides and actinides, may enter human body and have previously been implicated in causing various adverse health effects. Research on heavy metals have established the prime routes of exposure to be ingestion of food plants cultivated on soils laden with these contaminants and water followed by inhalation of dust and air and topical exposure in agriculture, manufacturing, pharmaceutical and industrial settings [1,2]. The biological manifestations of heavy metal intoxication are linked to their chemical properties. One such adverse biological manifestation among many others is the neurotoxicity associated to the exposure of heavy metals which has lately caught the attention of researchers.

Cadmium (Cd) is a serious environmental toxicant which is widely distributed and has the potential to affect cellular antioxidant defenses, damage oxidative DNA repair systems, play a vital role in differentiation and apoptosis with the underlying cause being the heightened production of reactive oxygen species (ROS) which may act as a signaling molecule in apoptosis [3–5]. Cd though widely recognized for its ROS generation potential is a non-redox active bivalent metal which produces free radicals indirectly by increasing the concentration of free Fe most likely by the latter's replacement in various proteins as Cd itself has only one oxidation state and hence unable to generate free radical directly. Oxidative stress indirectly induced by cadmium manifests in the form of inhibition of antioxidative components of the cell such as superoxide dismutase (SOD) which may show both increase and decrease of levels, an inconsistency which is best explained by different exposure conditions [6,7].

This metal has been reported to alter the functioning of mitochondrial function too primarily through the mechanism of lipid peroxidation (LPO) of the mitochondrial membrane which results in the undermining of the mitochondrial membrane integrity [8]. ROS induced by Cd leads to mitochondrial membrane depolarization [9]. This loss of membrane potential is a crucial event in the intrinsic pathway of apoptosis as loss of potential causes mitochondrial pores and hence the opening of gates on cytochrome c which escapes to the cytoplasm initiating the programmed cell death pathway [10]. Several reports have been shown that Cd induces cell death through apoptosis in many tissues and cells [11–13].

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http://dx.doi.org/10.1016/j.jtemb.2015.03.002
0946-672X/

Among all others, brain is one of those organs which is highly susceptible to the adverse effects of oxidative stress as it bears plenty of polyunsaturated fatty acids in its membranous fence which are a soft target for LPO. Studies have shown that low levels of Cd cross through the brain barrier into the brain in adult rats and higher levels gain access if ethanol is used to deliver it to the brain and has the point of vantage to induce oxidative stress in brain cells. Thus Cd is able to induce neurotoxicity with a varied spectrum of alterations to the normal functioning of the neurons and other associated entities like action of neurotransmitters primarily depending on its highly deleterious capacity to cross the blood brain barrier (BBB).

Extensive research is being done to evaluate several natural antioxidants for their therapeutic effects in heavy metals induced toxicities [14]. Flavonoids are natural substances that comprise a large group of naturally occurring compounds with low molecular weight and variable phenolic structures which are present in food and medicinal plants. One of the widely distributed and easily available flavonoid found abundantly in citrus fruits is hesperidin (HP). The antioxidant activity of HP has been, like all other flavonoids, attributed to its chemical structure as this flavonoid derives its antioxidant property from a hydroxyl group that it bears at the position 3′ of the ring B. The pharmacological effects of HP have been repeatedly subjected to analysis in order to screen for its beneficial properties [15]. It has been found to possess anti-inflammation and analgesic activity along with the ability to reduce superoxide in *in vitro* electron transfer reaction.

The pharmacological property of HP which bears special relevance to our study is that it has the capacity to cross the BBB just like other common flavonoids and produce effects on the CNS. Mammoth amounts of evidence have accumulated out of works of investigators which shows that flavonoids have effects on memory, cognition and neurodegeneration [16]. Studies show that flavonoids have potential to protect neurons against injury induced by neurotoxins and neuroinflammation and ability to improve cerebrovascular blood flow [17]. No study has still been reported where HP has been tested against Cd in rodent brain models. Hence the purpose of this study was to measure and analyze the ameliorative capacity of HP in case of cadmium induced neurotoxicity in adult male rats.

Materials and methods

Materials

Bovine serum albumin (BSA), butylated hydroxy toluene (BHT), 1-chloro-2, 4-dinitrobenzene (CDNB), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), epinephrine, oxidized glutathione (GSSG), reduced glutathione (GSH), hydrogen peroxide (H_2O_2), nicotinamide adenine dinucleotide phosphate (NADPH), o-phosphoric acid (OPA), thiobarbituric acid (TBA), trichloro acetic acid (TCA) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). 2,4-Dinitrophenyl hydrazine (DNPH), ethylenediaminetetraacetic acid (EDTA), and sulfosalicylic acid (SSA) were purchased from Merck Limited (Mumbai, India). Guanidine hydrochloride and sodium azide were obtained from Hi-Media Labs (Mumbai, India). HP was obtained from Sigma Chemicals Co. and $CdCl_2$ was purchased from Merck Limited.

Animals

In this study male Wistar rats (250–280 g body weight) were used. Rats were obtained from the animal house of Jamia Hamdard (Hamdard University). The standard guidelines of Institutional Animal Ethics Committee were obeyed during the whole experiment and the study was approved by them. Rats were kept at temperature $30 \pm 1\,^{\circ}C$ with relative humidity at $65 \pm 10\%$ and at a photoperiod of 12 h light/dark cycle. Standard pellet rodent diet and water were provided to the animals *ad libitum*.

Experimental design

To evaluate the neurotoxicity caused by HP and the protective effect of $CdCl_2$ under *in vivo* conditions, the animals were divided into four groups ($n = 6$ per group) Control, HP, $CdCl_2$ and $CdCl_2$ + HP groups. All groups were treated for a period of 21 days. HP was administered orally once every day at a dose of 40 mg/kg b.wt., while $CdCl_2$ was injected subcutaneously once every day at a dose of 3 mg/kg b.wt. In $CdCl_2$ + HP group, HP was administered prior to $CdCl_2$ injection. Control group was treated both orally and subcutaneously with saline once every day.

The following doses were selected on the basis of previous study and literature reports [18,19]. After the end of the experimental period all animals were sacrificed by decapitation. The whole brain were quickly excised, rinsed in ice-cold phosphate buffer and kept chilled until homogenization.

Homogenate preparation

The brain tissue were homogenized in 0.1 phosphate buffer, pH – 7.4 to obtain 10% homogenate using a Potter–Elevehjam homogenizer giving 6–8 strokes at medium speed keeping the sample under ice.

Post mitochondrial supernatant (PMS) preparation

Homogenate was subjected to differential centrifugation in refrigerated centrifuge at temperature of $4\,^{\circ}C$. It was centrifuged at 10,000 rpm for 20 min. The resulting pellet is the primary mitochondrial pellet and the supernatant is 10% post mitochondrial supernatant. PMS was used for the estimation of various biochemical analysis.

Oxidative stress indices

LPO

LPO was measured using the procedure of Chaudhary and Parvez [20]. Determination of LPO is based on the thiobarbituric acid reacting species (TBARS), which largely include malondialdehyde. The rate of LPO was expressed as μmoles of TBARS formed/h/g tissue using a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

PC

The oxidative damage to proteins was measured according to the method described by Waseem and Parvez [21]. The quantification of carbonyl groups was based on the reaction with DNPH. DNPH reacts with protein carbonyls to produce the corresponding hydrazone. The carbonyl content was measured spectrophotometrically at 340 nm. The results were expressed as nmoles of DNPH incorporated/mg protein based on the molar extinction coefficient of $22{,}000\ M^{-1}\ cm^{-1}$.

Non-enzymatic antioxidant assays

GSH. GSH was assessed by the method of Ashafaq et al. [22]. The reaction is based on the fact that the thiol group of GSH reacts with the —SH reagent (DTNB) to form thionitro benzoic acid. The reduced glutathione concentration was calculated as nmoles GSH/mg protein using a molar extinction coefficient of $1.36 \times 10^4\ M^{-1}\ cm^{-1.}$

Non-protein thiol (NP-SH). NP-SH was assessed by the method of Ashafaq et al. [22] which measures the entire pool comprising