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Alterations of Na^+/K^+-ATPase, cholinergic and antioxidant enzymes activity by protocatechuic acid in cadmium-induced neurotoxicity and oxidative stress in Wistar rats

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ABSTRACT

Background: This study assessed the possible protective mechanisms of protocatechuic acid (PCA) against cadmium (Cd)-induced oxidative stress and neurotoxicity in rats.
Methods: Male wistar strain rats weighing between 150–160 g were purchased and acclimatized for two weeks. The rats were divided into seven groups of seven each; NC group received normal saline, CAD group received 6 mg/kg of Cd-solution, CAD + PSG group received Cd-solution and prostigmine (5 mg/kg), CAD + PCA-10 and CAD + PCA-20 groups received Cd-solution and PCA (10 mg/kg and 20 mg/kg) respectively, PCA-10 and PCA-20 groups received 10 mg/kg and 20 mg/kg PCA each. Animals were administered normal saline, Cd and PCA daily by oral gavage for 21 days. After which the animals were sacrificed, the brain excised, homogenized and centrifuged. The activities of enzymes (Na^+/K^+-ATPase, cholinesterases, catalase, glutathione peroxidase, superoxide dismutase) and levels of oxidative stress markers (lipid peroxidation and reduced glutathione) linked to neurodegeneration were subsequently assessed.
Results: Significant ($p < 0.05$) alterations in the enzyme activities and levels of oxidative stress markers were observed in CAD group when compared to the NC group. However, the activities of the enzymes were reversed in CAD + PSG and CAD + PCA groups.
Conclusions: PCA may protect against cadmium-induced neurotoxicity by altering the activities of Na^+/K^+-ATPase, acetylcholinesterase, butyrylcholinesterase and endogenous antioxidant enzymes.

1. Introduction

Neurotoxicity occurs as a result of exposure to artificial or natural toxic substances (neurotoxic compounds) which alters the normal functioning of the nervous system in a way that oxidative injury or damage is inflicted on the nervous tissues [1]. This can on the long run disrupt or even destroy the vital cells responsible for the transmission and processing signals in the brain (neurons) and other regions of the nervous system leading to various neurological disorders [2]. Neurotoxicity can develop from exposure to heavy metals, radiation treatment, chemotherapy substances, drug therapies, certain foods and drug abuse as well as food additives and pesticides [3].

Cadmium (Cd) is a heavy metal and one of the most widely distributed toxic pollutants in the environment with a long biological half-life as a result of its low body excretion rate [4]. In view of this, over time, continuous vulnerability to Cd tend to lead to its accumulation thereby causing toxic effect in a variety of tissues [5]. The main source of exposure to cadmium is food such as grains and vegetables. Also, Cd can be taken or inhaled through the olfactory pathways or nasal mucosa into the central and peripheral neurons [6] which may alter the activities of the enzymes of the central nervous system such as cholinesterases. Cd induced toxicity is implicated in the production of reactive oxygen species (ROS) and exhaustion of antioxidants leading to oxidative stress in brain cells and alterations in the structural integrity of lipids [7] as well as neutralizing enzymes, which leads to the disturbances in

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brain metabolism [8]. The role of cadmium in neurological damage has been a target of research over the years and several researchers have shown that the effect of cadmium in neurological damage can be inhibited by some synthetic drugs, but this always comes with adverse side effects [9]. Also, some recent evidences have shown that plant phytochemicals such as polyphenols can inhibit the effects of cadmium neurotoxicity with little or no side effect [10].

Polyphenols are the mostly available antioxidants found in human diets. They are categorized in different classes as flavonoids, phenolic acids and lignans. Phenolic acids are naturally occurring compounds found in plants which are characterized with unique structural similarities like presence of carboxylic group as in protocatechuic, caffeic, *p*-coumaric, gallic, vanillic and ferulic acids, which exhibit antioxidant properties and contribute to the prevention of oxidative stress [11]. Recent findings by Oboh et al. [12] reported the anticholinesterase property of caffeic and chlorogenic acids (phenolic acids) in the management of Alzheimer's disease and oxidative stress. Protocatechuic acid (3,4-hydroxylbenzoic acid) is widely distributed in hundreds of plants as active phytoconstituent imparting various pharmacological potentials [13]. These effects may be attributed to its antioxidant activities, along with other possible mechanisms, such as interaction with several enzymes involved in the progression of some degenerative diseases.

Epidemiological and experimental evidences have affirmed that phenolic compounds may exert several biological effects such as antimicrobial, antihelminthic, antibacterial, anti-inflammatory, anti-diabetic, antioxidant and neuroprotective properties [14–17]. Although few studies have demonstrated hepatoprotective, antioxidative, nephroprotective, anti-cancer and neuroprotective potentials of PCA [18–21], however, the likely mechanisms of action of PCA in tissue and organ protection have not been fully addressed and investigated. Thus, this present study sought to investigate the possible neuroprotective mechanisms on cadmium-induced oxidative stress and neurotoxicity in rats' brain *in vivo* and antioxidant property of PCA as typified by its effect on antioxidant enzymes and lipid peroxidation.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals such as protocatechuic acid (PCA), cadmium, prostigmine, acetylthiocholine iodide, butyrylthiocholine iodide, adenosine triphosphate (ATP), ouabain, ammonium molybdate, thiobarbituric acid (TBA), dinitrophenylhydrazine (DNPH), sodium acetate, sodium dodecyl sulphate, phosphorus red, ascorbic acid, magnesium chloride, phosphate buffer of different molarity, adenosine monophosphate (AMP), sodium hydroxide, benzene, and 5,5′-dithio-bis(2-nitrobenzoic acid) were bought from Sigma-Aldrich, Chemie GmBH (Steinheim, Germany) while acetic acid was purchased from BDH Chemical Ltd., (Poole, England). The water used was glass distilled and all other chemicals and reagents used are of analytical grade.

2.2. Experimental animals and design

Forty-nine male Wistar rats of 160 g average weight were purchased from the Central Animal House, University of Ibadan, Ibadan, Oyo State Nigeria. The rats were acclimatized in stainless steel cages under controlled conditions of a 12 h light/dark cycle, 28 ° C temperature and 50% humidity for two weeks. The rats were allowed access to food and water ad libitum. There is average exposure to cadmium in the homes of some developing countries that derive their drinking water from ground, well and stream water located near industries that release cadmium as waste. Hence, we administered 6 mg/kg body weight of cadmium chloride orally to rats in this study to mimic average human cadmium exposure as previously reported [22–24]. The drug (prostigmine) and PCA used in this experiment were also administered orally.

2.3. Treatment groups

The animals were divided into seven groups of seven (7) rats each per steel cages and were treated for 21 days as follows:

NC- Wistar rats administered normal saline (Normal control).

CAD - Wistar rats administered 6 mg/kg body weight of cadmium only.

CAD + PSG- Wistar rats administered 6 mg/kg body weight of cadmium and treated with 5 mg/kg of prostigmine.

CAD + PCA-10 - Wistar rats administered 6 mg/kg body weight of cadmium and treated with 10 mg/kg body weight of PCA.

CAD + PCA-20 - Wistar rats administered 6 mg/kg body weight cadmium and treated with 20 mg/kg body weight of PCA.

PCA-10 - Wistar rats treated with 10 mg/kg body weight of PCA only.

PCA-20 - Wistar rats treated with 20 mg/kg body weight of PCA only.

2.4. Preparation of tissue homogenates

Rats decapitation were carried out via cervical dislocation while the cerebral tissue (whole brain) was rapidly dissected, weighed and placed in phosphate buffer pH 7.4 on ice. Subsequently, the tissue was rinsed with the phosphate buffer pH 7.4 and then homogenized with same buffer pH 7.4 (1:5 w/v), with about 10-up and down strokes at about 1200 rev/min in a Teflon-glass homogenizer (Pyrex Potter-Elvehjem model 7725T-45). The homogenate was centrifuged using microhematocrit centrifuge (LW Scientific M24) for 10 min at 3,000*g* to yield a pellet that was discarded and the supernatant was used for lipid peroxidation assay [25] as well as source of enzyme for the enzyme assays.

2.5. Experimental protocol

2.5.1. Na^+/K^+ –ATPase activity assay

The method described by Fiske and Subbarow. [26] was used to measure the activity of Na^+/K^+-ATPase in whole brain homogenate. Assay mixture consisted of 100 μL of Na^+, K^+ –ATPase substrate buffer (pH 7.4) (containing 120 mM Tris–HCl, 0.4 mM EDTA, 200 mM NaCl, 20 mM KCl, and 24 mM $MgCl_2$), 50 μL of supernatant (tissue homogenate) in the presence or absence of 50 μL of ouabain (1 mM), in a final volume of 200 μL. The reaction was initiated by the addition of 50 μL adenosine triphosphate (ATP). After incubating for 30 min at 37 °C, the reaction was terminated by the addition of 50 μL of 10% (w/v) trichloroacetic acid (TCA). The inorganic phosphate (Pi) released was quantified using a reaction mixture that contained 250 μL of ammonium molybdate (100 mM), 200 μL of reaction mixture from first grid, 500 μL of distilled water and 50 μL of ascorbic acid (8%). The absorbance was read at 620 nm using a UV/Visible spectrophotometer (Jenway Model No. 6315).

2.5.2. Acetylcholinesterase (AChE) assay

AChE activity investigation was carried out in a reaction mixture containing 50 μL of tissue homogenate, 50 μL of 5, 5′-dithiobis-(2-nitrobenzoic) acid (DTNB), 1175 μL of 0.1 M phosphate-buffered solution, pH 8.0). After incubation for 20 min at 25 °C, 25 μL of acetylthiocholine iodide solution was added as the substrate. The AChE activity was determined as changes in absorbance reading at 412 nm for 3 min at 25 °C [27,28] using a UV/Visible spectrophotometer (Jenway Model No. 6315).