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A high dose of short term exogenous D-galactose administration in young male rats produces symptoms simulating the natural aging process

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ABSTRACT

Aims: D-Galactose (D-gal) induced accelerated senescence has been used to develop an aging model for brain. Previously, long term administration of a wide range of doses has been used for this purpose. In the present study we investigate whether short term administration of a high dose of D-gal in rats induces significant signs and symptoms similar to natural aging.
Main methods: Young rats were injected intraperitoneally with D-gal at a dose of 300 mg/ml/kg for one week. Behavioral analysis for depression and anxiety like symptoms were monitored by forced swim test (FST) and light/dark transition (LDT) test. Assessment of memory was done using the Morris water maze (MWM), passive avoidance test (PAT) and elevated plus maze (EPM) test. Biochemical analysis was done for estimation of antioxidant enzymes and acetylcholinesterase. Determination of brain biogenic amines was performed by HPLC–EC.
Key findings: Short term administration of D-gal significantly altered behavioral, biochemical and neurochemical responses in rats. D-Gal injected rats exhibited depressogenic and anxiogenic behaviors while memory was also significantly impaired in these rats. Brain lipid peroxidation and superoxide dismutase activity were significantly increased while catalase and glutathione peroxidase decreased. Increased activity of acetylcholinesterase was also exhibited by D-gal injected rats while brain biogenic amines were significantly decreased. Food intake and growth rate were however comparable in both groups.
Significance: Together the behavioral, biochemical and neurochemical impairments following the high dose of D-gal suggest that symptoms similar to natural aging may be developed in rats in as early as one week.

Introduction

D-Galactose (D-gal) is classified as a monosaccharide. It is abundantly present in milk products and other non-dairy foodstuffs such as fruits and vegetables [1]. It is a reducing sugar, which at normal concentrations is metabolized into glucose but at higher doses is converted into aldose and hydroperoxide through the action of galactose oxidase resulting in the formation of superoxide anions and oxygen-derived free radicals [56]. It may also change the structure of protein and peptide by reacting with their free amine groups resulting in the accumulation of advanced glycation end (AGE) products through non-enzymatic glycation [12,56]. Studies have shown that these AGEs give rise to age-related disorders including cataract, renal failure, atherosclerosis, arthritis and oxidative damage in brain [52]. Free radical formation from oxidation of D-gal inhibits the defense capacity of cells and as a result increased lipid peroxidation (LPO) occurs which releases end products that react with proteins and phospholipids resulting in cellular injury and central nervous system impairment [28]. Through the formation of reactive oxygen species (ROS) and AGEs, D-gal leads to neurotoxic effects [54]. The excess production of ROS accelerates the breakdown of biomolecules leading to an increased concentration of malondialdehyde (MDA) levels in brain which is an important biomarker of oxidative damage [48]. Evidence shows that overload of D-gal results in behavioral and neurochemical alterations [54].

Previous studies demonstrated that D-gal intoxication caused cognitive dysfunction and neuropathological changes in the rat brain [35]. Studies also show that these pathological and physiological symptoms in D-gal intoxicated rats were similar to those observed in normal aged rats [55]. ROS has become a major field of research in age-related disorders because of their role in a number of neurological diseases [17,53]. Abnormal accumulation of ROS by excess D-gal could be responsible for neurotoxicity which may lead to memory impairment and other behavioral deficits [54,59]. Decline in cognitive functions is a well known fact in aging. The cholinergic system has been connected to age-related memory disorders [51]. It has also been stated that D-gal administration results in marked increase in acetylcholinesterase

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(AChE) activity and may lead to cognitive impairment [35]. Previously chronic administration using a range of doses from 50 to 500 mg/kg D-gal has been used to develop an animal model of aging [23]. However, deleterious effects of short-term D-gal overload have not been given much attention. Therefore, the aim of the present study was to investigate the neurotoxic effects of a high dose of D-gal for 7 days in young rats on biochemical, neurochemical and neurobehavioral parameters. Since oxidative stress is the main causative factor in the natural aging process, the study was further aimed to monitor if a short duration of administration of D-Gal overload induced changes that could simulate the natural aging process.

Materials and methods

Animals

Twelve locally bred young adult male Albino-Wistar rats having weight of 120-130 g, bought from the Dow University of Health Sciences, OJHA campus, Karachi, Pakistan, were used in the study. Rats were caged individually (to avoid effect of social interaction) with ad libitum access to standard rodent diet (cubes) [a control diet (4.47 kcal/g) containing fat, carbohydrate, and protein at a ratio of 1:2:1] [9] and tap water under a 12:12 h light/dark cycle (lights on at 7:00 am) at controlled room temperature (22 ± 2 °C). Prior to the experiments, animals were subjected to 1 week of acclimation period and to behavioral processes to nullify the psychological affliction of environment to reduce novelty and handling stress. All animal experiments were approved by the Institutional Ethics and Animal Care Committee and were done in strict accordance with National Institutes of Health's Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and the UK Animals (Scientific Procedures) Act 1986.

Reagents and chemicals

Hydrogen peroxide (H_2O_2) stock (35%) solution, thiobarbituric acid (TBA), trichloroacetic acid (TCA), nitro blue tetrazolium (NBT), and dithiobisnitrobenzoic acid (DTNB) were purchased from the British Drug House (BDH, Dorset, UK). Hydroxylamine hydrochloride ($H_3NO \cdot HCl$), acetylthiocholine (ATC), and all other analytical grade reagents and D-galactose were purchased from Sigma Chemical Co. (St. Louis, USA).

Experimental protocol

The rats (n = 12) were allocated into two groups: Group 1: control (n = 6), Group 2: test (n = 6). D-Gal was injected to the test group at a dose of 300 mg/ml/kg (intraperitoneally) for 7 days while controls received 0.9% saline. 24 h after the last injection rats were subjected to behavioral tests. Food intake and body weight were examined daily during the time period of D-gal administration to monitor the general health of the control and test rats. The behavioral parameters assessed in the current study included open field test (OFT) to observe locomotor activity, forced swim test (FST) to monitor depression-like behavior and light/dark transition (LDT) test to assess anxiety. Passive avoidance test (PAT), the Morris water maze (MWM) test and elevated plus maze (EPM) test were used to assess the memory performance of rats. All behavioral assessments were performed between 09:00 and 13:00 h. Behavioral tests were continued for 3 days after D-gal administration; OFT, FST, LDT and PAT were performed on the 8th day whereas MWM and EPM were carried out on the 9th and 10th days of injection respectively. Twenty-four hours later, rats were subjected to decapitation to collect their brains. Brains of animals were removed from the skull within 30 s after decapitation. All brain samples were immediately stored at a temperature of −70 °C for the biochemical and neurochemical assays. A balanced design was followed for all treatment and behavioral procedures in order to avoid the effect of order and time.

Behavioral analysis

Forced swim test (FST)

FST is a commonly used and pharmacologically recognized model for examining depression-like behavior of animals [45]. The depression-like behavior was reflected by a cessation of persistent escape-directed behavior (immobility) when the rats were placed in a water-filled inescapable chamber. In the test session rats were forced to swim in a water-filled glass tank, having a height of 56 m and a width of 20 cm for 5 min. The water (23 ± 2 °C) was filled to a height of 22 cm so that the animal was unable to touch the tank's bottom. The rat's swimming behavior was monitored for 300 s. The struggling time during which the rat struggles to escape from tank was recorded. The immobility time wherein the rat becomes immobile and makes no more efforts to escape [33] was obtained by determining the difference between the total time spent and the struggling time [300 s − struggling time].

Light/dark transition (LDT) test

Anxiety of rats was monitored with the help of LDT which consists of two compartments. Both compartments were of the same size (26 × 26 × 26 cm), with a door (12 cm × 12 cm) between the compartments. One compartment is made up of black plastic walls while the other compartment was made up of transparent plastic. First the rat was placed in the light compartment and the extent of anxiety was determined by monitoring the number of entries and time spent in the light box for a cutoff time of 5 min [32]. The activity in this box was determined at a lighted place (360 lx) using a 60 W white light bulb.

Passive avoidance test (PAT)

The PAT apparatus contains two compartments of equal size, one is an illuminated 'safe' and other is a dark 'punishable' one. Both of these compartments are linked with the help of a door which allows rats to freely cross from one compartment to another. These compartments contain a grid floor that consists of rods having a diameter of 5 mm and having a distance of 0.5 cm between the rods. Initially the animals were trained by placing them in the illuminated compartment, when rats entered the dark compartment with its four paws, a foot shock of 1.5 mA was delivered for 3 s to its paws via the rods of the grid floor. The moment the rat received an electric shock, it moved back to the safe 'illuminated' compartment. After 60 min of training, the test session was carried out in which rats were again placed in the safe 'illuminated' compartment and the time taken by the rat to enter the dark compartment was monitored (step-through latency) for 3 min (cutoff time) [31].

The Morris water maze (MWM) test

The MWM test is widely performed to assess the spatial memory performance of animals [42]. The apparatus consists of a circular pool having a diameter of 45 cm and a height of 37 cm. The tank was filled with water (23 ± 2 °C) having a depth of 12 cm. Both circular pool and platform are made of white painted metal. The escape platform has a flat metallic top with a surface diameter of 8 cm, and was placed 2 cm below the water level. The water within the pool was made opaque by adding powdered milk in order to make the platform invisible and secondly to track the rat's swim paths proficiently [27]. In our experiment, we have assessed the time taken by the rat to find the hidden platform (escape/retention latency) to determine working memory performance. This test consists of two sessions: training and testing. Spatial memory performance was assessed by recording the retention latency with a cutoff time of 2 min for every animal within both sessions. First, the training phase was carried out in which each rat was positioned into the water-filled circular pool in such a manner that its