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A highly reproducible mice model of chronic kidney disease: Evidences of behavioural abnormalities and blood-brain barrier disruption

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ABSTRACT

Aims: In the present study, a novel mice model of chronic kidney disease (CKD) was developed, and psycho-motor behavioural abnormalities, blood-brain barrier (BBB) integrity and brain histology were studied.
Main methods: Swiss albino female mice were given high adenine diet (0.3% w/w mixed with feed) for 4 weeks. Serum urea and creatinine levels and renal histological studies were performed to validate the model. Psycho-motor behavioural abnormalities and neurological severity were studied. BBB integrity was assessed using Evans blue extravasation method. Nissl staining was performed to see possible morphological aberrations in brain.
Key findings: There was a significant increase in serum urea and creatinine levels in mice given high adenine diet, and the mice had abnormal kidney morphology. Deposition of adenine and 2,8-dihydroxyadenine crystals, and increased collagen deposits in the renal tissues were found, which validate induction of CKD in the mice. Motor behavioural abnormalities, depression-like and anxiolytic behaviour and increase in neurological severity were prevalent in mice with CKD. Evans Blue dye extravasation was found to occur in the brain, which signifies disruption of BBB. However, Nissl staining did not reveal any morphological aberration in brain tissue.
Significance: The present study puts forward a highly reproducible mice model of CKD validated with serum parameters and renal histopathological changes. The mice showed psycho-motor behavioural abnormalities and BBB disruption. It is a convenient model to study the disease pathology, and understanding the associated disorders, and their therapeutic interventions.

1. Introduction

Chronic kidney disease (CKD) refers to a wide range of disease condition that affect the structure and function of the kidney, lasting for >-3 months, and leads to albuminuria and reduction in glomerular filtration rate (GFR) [7,40]. The major causes for the development of CKD are diabetes, hypertension, glomerulonephritis and interstitial nephritis [7,10]. There is an increase in the global prevalence of CKD, affecting >15% of the population in developed nations [14,17,27]. With reduction in the GFR there occurs retention of uremic toxins and electrolyte imbalance [40], which necessitates, renal replacement therapies in the form of dialysis and kidney transplantation [44]. Due to retention of uremic toxins and electrolyte imbalance, CKD leads to cardiovascular disease, stroke [48,49] and neurological complications (reviewed in Ref. [36]). In CKD patients, anxiety, depression, cognitive dysfunction, restless leg syndrome and reduced intelligence quotient are common diagnosis which hamper the quality of life [23,28,34,36,38,39,41,60,65]. In animal models, depression, anxiety and reduced exploratory and locomotor activities are reported [5,16,66].

One of the most widely used animal models of CKD is the surgical model [2,21,26,37,50]. However, owing to high mortality and need of sophisticated surgical methods, which alter the morphology of the animals as well, alternative models are sought. Yokozawa et al. [74] introduced rat model of CKD by administration of adenine with feed. Adenine and its metabolite (2,8-dihydroxyadenine) get deposited in renal tissues as crystals, as shown by Ikeda et al. [30] in rat model, and cause renal damages [5,58], which leads to elevation in serum urea, creatinine and renal histological damage [9,45,62].

In rats with adenine-induced CKD, cardio-vascular damages have been investigated [22], while Ali et al. [5] reported on psycho-motor behavioural abnormalities. However, no behavioural study has been undertaken yet in mice model, and female mice have not yet been used as adenine-induced CKD model, to the best of our knowledge. In mice model of acute renal injury, disruption of blood-brain barrier (BBB) has been reported [42]. However, any such study is lacking in adenine-induced CKD model. In the present study, we induced CKD in

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adult female Swiss albino mice by administering high adenine diet. In this novel model, we investigated for renal fibrosis, psycho-motor behavioural abnormalities, neurological severity, BBB disruption, and histopathological changes in discrete brain regions.

2. Material and methods

2.1. Animals

Adult Swiss albino female mice of weight between 25 and 27 g (10 weeks old) were obtained from Pasteur Institute, Shillong, Meghalaya (India). They were housed in standard conditions of 12 h light/dark cycle, 24 ± 2 °C temperature and 60 ± 5% humidity; and were given standard feed and water ad libitum. An acclimatization time of 10 days was given prior to start of the experiment. The experimental protocols met the National Guidelines and were approved by the Institutional Animal Ethics Committee.

2.2. Chemicals and others

Adenine, chloral hydrate, poly-L-Lysine, Sirius Red (Direct Red 80) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Evans Blue, Cresyl violet, Eosin, Haematoxylin and other chemicals were of analytical reagent grade (extra-pure), and were procured from SISCO Research Laboratories Pvt. Ltd. (Mumbai, India) and HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Urea and creatinine assay kits were obtained from Siemens Ltd. (India). Serum collection vials containing clot activator were obtained from BD Franklin Lakes, NJ, USA.

2.3. Experimental design

Animals were randomly divided into 2 groups ($n = 12$ each): first group served as control and was given standard powdered feed, and second group received high adenine diet for 4 weeks, following Ali et al. [3]. Fasting body weight of the mice was taken every alternative day. On 28th day, the mice were subjected to neurological severity scoring and motor behavioural studies (Akinesia, Catalepsy and Swim Test). Forced swim test and Light–Dark (LD) box test were performed on the subsequent day, following which the mice were sacrificed. Half of the mice from each group ($n = 6$ each) were anaesthetized by Chloral hydrate (350 mg/kg; i.p.), and blood was collected by cardiac puncture in serum collection vials to separate serum following centrifugation. The mice were then transcardially perfused with 50 mL ice-cold phosphate buffered saline (PBS; 0.1 M; pH 7.4) and 50 mL ice-cold 4% (w/v) paraformaldehyde (in 0.1 M PBS, pH 7.4). Kidneys and brains were dissected out and stored in the same fixative for 48 h, then transferred to 30% sucrose (w/v) solution pending histological studies. Rest of the mice of each group ($n = 6$) were used for BBB integrity study using spectrophotometric and spectrofluorimetric methods.

2.4. Induction and validation of CKD in mice

The CKD model was generated by giving high adenine diet (0.3% w/w mixed with powdered feed) for 4 weeks, following Ali et al. [3]. However, we used a higher dose of 0.3% instead of 0.2%.

2.4.1. Estimation of serum urea and creatinine level

Serum urea level between 80 and 100 mg/dL is considered a parameter to ascertain induction of CKD in mice model [32]. Urea was estimated from the serum following Berthelot Method [11] with minor modifications [24], using urea assay kit. Briefly, urea is hydrolysed by Urease enzyme to produce ammonia which reacts with a phenolic reagent to produce a green coloured complex, the intensity of which is directly proportional to the amount of urea present in serum. Absorbance was recorded at 570 nm using spectrophotometer (BioRad SmartSpec™ Plus, USA). Serum creatinine level was estimated following Alkaline Picrate method [13,67], using creatinine assay kit. Briefly, in an alkaline medium, picric acid reacts with creatinine to form an orange coloured complex with the alkaline picrate. Intensity of the colour formed is proportional to the amount of creatinine present in the sample. Absorbance was recorded at 520 nm using spectrophotometer.

2.4.2. Relative kidney weight

The mice were weighed just before sacrifice, after which the kidneys were dissected out, blotted and weighed. Relative kidney weight was expressed as % of body weight. Relative kidney weight = (combined kidney weight / body weight) × 100.

2.4.3. Renal histology

Mid-longitudinal, 5 μm thick sections of kidney were taken using Cryostat (0620E, Thermo Shandon, UK) on poly-L-lysine coated slides and stained using Haematoxylin-Eosin. Briefly, the sections were cleared in xylene, hydrated in decreasing alcoholic grades, stained with Haematoxylin, washed in running tap water, dehydrated in increasing alcoholic gradient, counterstained with Eosin, washed in absolute alcohol, cleared in xylene, mounted in DPX and photographed using Trinocular microscope (Eclipse Ci, Nikon, Japan) under bright-field illumination.

To see possible fibrosis and quantify the extent of collagen deposits in the renal tissue, Picro-sirius red staining was performed following Chua et al. [15]. Briefly, sections were taken on poly-L-lysine coated slides, cleared in xylene, hydrated, stained with Haematoxylin, washed in running water, stained with Picro-sirius red (1% Sirius red in saturated solution of picric acid) for 1 h, washed with acidified water (0.5% acetic acid), removed water by vigorous shaking, dehydrated in absolute alcohol, cleared in xylene, mounted in DPX and photographed under bright field illumination as well as polarized light (Eclipse LV100 POL, Nikon, Japan). The amount of fibrotic area with collagen deposits was estimated by counting the pixels using Adobe Photoshop software and was expressed as % of the total area. For images of bright field illumination, total red pixels were counted, and pixels of all the different colours: green, yellow, orange and red, were counted for the images of polarized light.

2.5. Behavioural studies

2.5.1. General behaviour

Weight of the mice was taken every alternative day, and general behaviour like water and food intake was monitored.

2.5.2. Motor behaviour

2.5.2.1. Akinesia. Akinesia is the latency (in seconds) of the animals in moving all the four limbs, and is tested for 180 s [12]. The animals were acclimatized to a wooden platform (40 cm × 40 cm × 30 cm) for 5 min, and then the latency was recorded. The platform was cleaned with ethanol after each test.

2.5.2.2. Catalepsy. Catalepsy is the inability of an animal to correct an externally imposed posture [46]. The animals were placed on a flat surface with both hind limbs on a wooden platform of 3 cm height. The time taken by the animals in moving both hind limbs to the flat surface was counted following Bhattacharjee et al. [12]. The platform was cleaned with ethanol after each test.

2.5.2.3. Swim test. Swimming ability test was performed following Haobam et al. [29] in plastic tubs (40 cm × 25 cm × 16 cm) with 12 cm high water, maintained at 27 ± 2 °C. Mice were released in water and their swimming activity was scored for 10 min, on a scale of 0–3: '3 — continuous swimming, 2 — swimming with occasional floating, 1 — more floating with occasional swimming with hind