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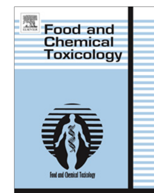

The effect of activated charcoal on adenine-induced chronic renal failure in rats

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ABSTRACT

Activated charcoal (AC) is a sorbent that has been shown to remove urinary toxins like urea and indoxyl sulfate. Here, the influence of AC on kidney function of rats with experimental chronic renal failure (CRF) is investigated. CRF was induced in rats by feeding adenine (0.75%) for four weeks. As an intervention, AC was added to the feed at concentrations of 10%, 15% or 20%. Adenine treatment impaired kidney function: it lowered creatinine clearance and increased plasma concentrations of creatinine, urea, neutrophil gelatinase-associated lipocalin and vanin-1. Furthermore, it raised plasma concentrations of the uremic toxins indoxyl sulfate, phosphate and uric acid. Renal morphology was severely damaged and histopathological markers of inflammation and fibrosis were especially increased. In renal homogenates, antioxidant indices, including superoxide dismutase and catalase activity, total antioxidant capacity and reduced glutathione were adversely affected. Most of these changes were significantly ameliorated by dietary administration of AC at a concentration of 20%, while effects induced by lower doses of dietary AC on adenine nephrotoxicity were not statistically significant. The results suggest that charcoal is a useful sorbent agent in dietary adenine-induced CRF in rats and that its usability as a nephroprotective agent in human kidney disease should be studied.

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1. Introduction

Administration of sorbents, i.e. compounds that bind other chemicals onto their outer surfaces, and within their interstices (Ash, 2009) has been suggested as either an alternative or supplementary treatment for patients with chronic kidney disease (CKD). They have been shown to remove waste products such as urea, indoxyl sulfate (IS) and other urinary toxins, and augment the dialysis process (Schulman, 2012; Winchester and Ronco, 2010; Yamamoto et al., 2011). One of these sorbents is charcoal (Cooney, 1995; Olson, 2010; Vaziri et al., 2013), which is produced by heating pulverized carbonaceous substances to temperatures of 600–900 °C, followed by "activation" using either steam or hot air to erode the internal surfaces of the product and thereby increase its adsorptive surface area. Typical surface areas for activated charcoals are about 800–1200 m^2/g. Thus, a 50-g dose of activated charcoal has an adsorptive surface area equivalent to about seven football fields or 5183 m^2, and "Superactivated" charcoals may have a surface area of 2800–3500 m^2/g and can adsorb greater quantities of drugs (Olson, 2010). Charcoal, in various forms, administered with low protein diets has been reported to control effectively some uremic symptoms in patients with different stages of renal disease, and this is achieved through the binding of urea and other urinary toxins to charcoal, and its excretion with feces, creating a concentration gradient for continued diffusion of these toxins (Ash, 2009). Scavenging of urinary toxins by charcoals has also been proposed (Fujii et al., 2009). The beneficial effect was reported especially in elderly patients with end-stage renal disease (ESRD) (Musso et al., 2010). Recently, Schulman et al. reported that the use of activated charcoal (AC) and other alternative agents, which are capable of blocking the actions of profibrotic cytokines,

Abbreviations: AC, activated charcoal; CAT, catalase; CKD, chronic kidney disease; CRF, chronic renal failure; DCF, dichlorodihydrofluoresceine; DHA, 2,8-dihydroxyadenine; ESRD, end-stage renal disease; GPx, glutathione peroxidase; GSI, glomerular sclerosis index; IS, indoxyl sulfate; MSI, mesangiolysis index; NGAL, neutrophil gelatinase-associated lipocalin; PAS, periodic acid-Schiff stain; SOD, superoxide dismutase; TAC, total antioxidant capacity; TGF-β, transforming growth factor-beta.

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such as transforming growth factor-beta (TGF-β), can either halt or prevent the development of CKD in early stages (Schulman, 2012).

The adenine-induced chronic renal failure (CRF) rat model, first reported by Yokozawa et al. (1986), produces metabolic abnormalities resembling CKD in humans, which may include azotemia, accumulation of uremic toxins, metabolic imbalances of amino acids and electrolytes, and hormonal imbalances (Yokozawa et al., 1986). Pathologically, renal tissue of adenine-fed rats show lesions in proximal and distal tubules, as well as in glomeruli (Ali et al., 2013a). In mammalian metabolism, excess adenine becomes a significant substrate for xanthine dehydrogenase (CAS serial number: EC 1.2.3.2), which can oxidize adenine to 2,8-dihydroxyadenine (DHA) via an 8-hydroxyadenine intermediate (Wyngaarden and Dunn, 1957). However, the very low solubility of DHA leads to its precipitation in kidney tubules (de Vries and Sperling, 1977; Yokozawa et al., 1986). The complex inflammatory phenomena associated with this model depend, at least in part, on NF-κB activation (Okabe et al., 2013).

Experimentally, several drugs and natural products have been used to ameliorate the effects of adenine-induced CRF. These include gum acacia (Ali et al., 2010), fucoidan derivatives from *Laminaria japonica* (Wang et al., 2012), ergone (ergosta-4, 6, 8(14), 22-tetraen-3-one) (Zhao et al., 2012) and lanthanum carbonate (Damment et al., 2011). As far as we are aware, there is no published report on the effect of dietary charcoal on adenine-induced CRF, and this is the subject of the present investigation. Since AC derivatives have been shown to be beneficial in CKD, mechanistic studies in CKD animal models are warranted.

2. Materials and methods

2.1. Animals

This project was reviewed and approved by the Animal Research Ethics Committee of Sultan Qaboos University. All procedures involving animals and their care were conducted in conformity with international laws and policies (EEC Council directives 86/609, OJL 358, 1 December, 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publications No. 85-23, revised 1996).

Forty-eight male Wistar rats, initially weighing 150–155 g, were obtained from the Sultan Qaboos University Animal House and were randomly housed in groups of six under standard temperature (22 ± 2 °C), humidity (50–60%) and light conditions (artificial light from 06:00 to 18:00 h). The rats had seven days to acclimatize to the new surroundings before being treated and tested. They had free access to water and a standard powder diet containing 0.85% phosphorus, 1.12% calcium, 0.35% magnesium, 25.3% crude protein and 2.5 IU/g vitamin D3 (Oman Flour Mills, Muscat, Oman).

2.2. Experimental design

After an acclimatization period, rats were randomly divided into eight equal groups of six rats each and treated as follows: The first group continued to receive the standard diet without treatment until the end of the study (control group). The second group was switched to a diet containing adenine (0.75%, w/w, in feed) for four weeks. The dose of adenine was chosen from our previous works (Ali et al., 2010, 2013b) based on the original method by Yokozawa et al. (1986). The third, fifth and seventh group was given standard diet mixed with charcoal at concentrations of 5%, 15% and 20%, w/w, for four weeks, respectively. The fourth, sixth and eighth group was given adenine in the feed together with charcoal, as in the third, fifth and seventh group, respectively.

The rats were weighed before the beginning of the treatment and weekly during the treatment period. At the end of the treatment period, rats were placed individually in metabolic cages to collect the urine voided during 24 h. Thereafter, the rats were anesthetized with an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (5 mg/kg), and blood (about 4 mL) collected from the anterior vena cava was placed into heparinized tubes. The blood and urine were centrifuged at 900*g* and 4 °C for 15 min. The plasma obtained, together with the urine specimens, were stored frozen at −80 °C pending analysis. The animals were killed by an overdose of anesthesia, and kidneys were removed, blotted on a filter paper and weighed. Part of the left kidney was placed in formalin pending histological analysis, the rest of the left kidney and the right one were wrapped in aluminum foil and stored at −80 °C to await biochemical analysis within seven days. For biochemical analysis, frozen renal tissues were thawed and homogenized in ice-cold Tris buffer (pH 7.4) to give a 10%, w/v, homogenate. The latter was centrifuged at 1500*g* at 4 °C for 15 min, and the supernatant obtained was used to measure several anti-oxidant indices.

2.3. Biochemical indices of renal function

The plasma concentration of IS was measured by an HPLC method, as previously described (Al Za'abi et al., 2013), plasma phosphorus and uric acid were analyzed using a Beckman Coulter Automated Clinical Chemistry Analyzer, synchron CX5. Plasma creatinine, urea and urinary creatinine concentrations were measured spectrophotometrically using commercial kits, as described before (Ali et al., 2011). Neutrophil gelatinase-associated lipocalin (NGAL) concentration was measured in plasma by an ELISA method using kits obtained from Bioporto Diagnostics (Gentofte, Denmark). Urine osmolality was measured by the freezing point depression method (−70 °C) using an osmometer (Roebling, Berlin, Germany). Vanin-1, a urinary biomarker of renal damage (Hosohata et al., 2011) was measured by an ELISA method using kits from USCN Life Sciences (Wuhan, China). The supernatants of renal homogenates were separated into two aliquots and were used for the measurement of the following parameters, using assay kits according to the manufactures' instructions: glutathione (GSH) concentration with GSH/GSSG assay kit (Biovision, Mountain View, CA, USA), total antioxidant capacity (TAC) (Randox Laboratories Crumlin, UK), glutathione peroxidase (GPx) (Oxis International, Inc., Foster City, CA, USA), catalase (CAT) (Cayman Chemical Co., Ann Arbor, MI, USA), and superoxide dismutase (SOD) (Cell Technology Inc., Mountain view, CA, USA).

The protein content of the supernatant was measured by the method of Lowry et al. (1951), using bovine serum albumin as a standard and protein contents were expressed as mg/mL of sample. Myeloperoxidase activity was determined in the supernatant fraction. Briefly, a sample of the renal homogenate was added to a medium containing potassium phosphate buffer (50 mM, pH 6.0), hexadecyltrimethylammonium bromide (0.5%) and *N*, *N*, N^0, N^0-tetramethylbenzidine (1.5 mM). The kinetic analysis of myeloperoxidase was started after the addition of hydrogen peroxide (0.01%), and the color reaction was measured at 655 nm at 37 °C (Correia et al., 2012). The dichlorofluoresceine fluorescence assay was used to measure cellular peroxide production and other reactive species (Perez-Severiano et al., 2004): aliquots of the supernatant of the kidney homogenates were added to a medium containing Tris–HCl buffer (0.01 mM, pH 7.4) and dichlorofluoresceine diacetate (7 μM). After the addition of dichlorofluoresceine diacetate, the medium was incubated in the dark for 1 h until the fluorescence measurement (excitation at 488 nm and emission at 525 nm, with both slit widths at 1.5 nm). Oxidized dichlorofluoresceine (DCF) was determined using a standard curve of oxidized dichlorofluoresceine and results were expressed as μmol of oxidized DCF/mg protein (Sarithakumari et al., 2013).

2.4. Renal histopathology

The kidneys were fixed in 10% neutral-buffered formalin, dehydrated in increasing concentrations of ethanol, cleared with xylene and embedded in paraffin. Two micrometer sections were prepared from kidney paraffin blocks and stained with hematoxylin and eosin, periodic acid-Schiff stain (PAS) and Sirius red stain. In renal tissues, the glomerular sclerosis index (GSI) and the mesangiolysis index (MSI) were assessed. For quantification of glomerular matrix expansion in PAS-stained kidney sections, scores of 0–4 were used, based on (Raij et al., 1984). A score of 0 indicated normal glomerulus, a score of 1 indicated mesangial expansion or sclerosis involving up to 25% of the glomerular tuft, a score of 2 indicated sclerosis 25–50%, a score of 3 indicated sclerosis 50–75% and/or segmental extracapillary fibrosis or proliferation, and a score of 4 indicated global sclerosis (>75%) or global extracapillary fibrosis or proliferation, or complete collapse of the glomerular tuft. In total, 50 glomeruli per animal were evaluated. The MSI was determined in PAS-stained paraffin sections and graded in 50 glomeruli per animal using the following scoring system: score 0: no changes of capillaries, score 1: capillary dilatation <25% of the capillary convolute, score 2: capillary dilatation >25% of the capillary convolute or capillary aneurysms <50% of the capillary convolute, score 3: capillary aneurysms comprising 50–75% of the capillary convolute, score 4: capillary aneurysms comprising >75% of the capillary convolute (Dimmler et al., 2003).

Fibrosis was separately evaluated on Sirius red-stained slides and inflammation on hematoxylin and eosin-stained slides within 40 (fibrosis) or 80–100 (inflammation) visual fields using a semi-quantitative scoring ranging from 0 to 4 (grade **0**: 0% fibrosis/inflammation, grade **1**: <25% fibrosis/inflammation, grade **2**: 25–50% fibrosis/inflammation, grade **3**: 50–75% fibrosis/inflammation, grade **4**: >75% fibrosis/inflammation), as described before (Ali et al., 2013a). All microscopic scoring of the kidney sections was carried out in a blinded fashion.

2.5. Chemicals

Adenine and activated charcoal were obtained from Sigma (St. Louis, MO, USA) and were prepared freshly every day. Kits for measuring the various analytes were obtained from commercial sources mentioned above. The rest of the chemicals were of the highest purity grade available.

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