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Evaluation of abrin induced nephrotoxicity by using novel renal injury markers

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ABSTRACT

Abrin is a potent plant toxin analogous to ricin that is derived from the seeds of *Abrus precatorius* plant. It belongs to the family of type II ribosome-inactivating proteins and causes cell death by irreversibly inactivating ribosomes through site-specific depurination. In this study we examined the in vivo nephrotoxicity potential of abrin toxin in terms of oxidative stress, inflammation, histopathological changes and biomarkers of kidney injury. Animals were exposed to 0.5 and 1.0 LD50 dose of abrin by intraperitoneal route and observed for 1, 3, and 7 day post-toxin exposure. Depletion of reduced glutathione and increased lipid peroxidation levels were observed in abrin treated mice. In addition, abrin also induced inflammation in the kidneys as observed through expression of MMP-9 and MMP-9/NGAL complex in abrin treated groups by using zymography method. Nephrotoxicity was also evaluated by western blot analysis of kidney injury biomarkers including Clusterin, Cystatin C and NGAL, and their results indicate severity of kidney injury in abrin treated groups. Kidney histology confirmed inflammatory changes due to abrin. The data generated in the present study clearly prove the nephrotoxicity potential of abrin.

1. Introduction

Abrin and ricin are among the most toxic plant proteins (Roxas-Duncan and Smith, 2012). Both proteins are derived from the seeds of the plants *Abrus precatorius* and *Ricinus communis* respectively. Abrin shows significant similarities to ricin both at the sequence and structural levels; however abrin is a more potent toxin than ricin (Surendranath and Karande, 2008). Both toxins belong to the family of type II Ribosome Inactivating Proteins (RIPs). Toxins from the RIP family irreversibly inactivate protein synthesis through an enzymatic mechanism. The reported human fatal dose of abrin is 0.1–1 μg/kg of body weight, with a molecular mass of approximately 60–65 KDa (Wooten et al., 2014). Abrin is a protein synthesis inhibitor and induces apoptosis in cells (Mishra and Karande, 2014). Abrin is a heterodimer consisting of two disulfide-linked polypeptides, known as the A-chain and B-chain. The active A-chain moiety has an enzymatic function. The toxicity of A-chain is due to its RNA N-glycosidase activity by which it brings about depurination of adenine at 4324 position in the 28S ribosomal RNA. The end result of this activity is complete inhibition of protein synthesis leading to cell death (Olsnes, 2004). The B chain has galactose-binding capability and shows lectin type activity, helping the toxin to internalize inside the cell. At cellular level both abrin and ricin cause apoptosis and subsequently at higher doses, severe necrosis in the organs of poisoned animals and in cultured cells (Griffiths et al., 1987; Narayanan et al., 2004). Earlier, we investigated oxidative stress mediated DNA damage and cell death by using ricin and abrin *in vitro* (Rao et al., 2005; Bhaskar et al., 2008).

Accidental or intentional exposure to abrin has been frequently reported (Sahni et al., 2007; Sahoo et al., 2008). Many of these poisoning cases showed unusual symptoms like central nervous system toxicity and renal failure (Subrahmanyan et al., 2008).

Nephrotoxicity is an important target of toxicological studies due to its crucial role in drug excretion and detoxification. A number of studies have described neuroinflammation and damage caused by nephrotoxicity (Burn and Bates, 1998; Liu et al., 2008; Brouns and De Deyn, 2004). Various nephrotoxicants produce different types of toxicity syndromes, including acute renal failure, chronic renal failure, renal tubular defects, nephrotic syndromes and hypertension. Tubular kidney cells are particularly vulnerable to toxin mediated acute kidney injury due to their disproportionate exposure to circulating chemicals and transport processes that result in high intracellular concentration. Due to abundant presence of polyunsaturated fatty acids in the composition of renal

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lipids, kidneys are very susceptible to oxidative stress (Ozbek, 2012). RIPs like trichosanthin and shiga toxin have been reported to cause kidney injury via tubular damage (Tang et al., 1997; Porubsky et al., 2014). Ricin induced nephrotoxicity is characterized by altered serum biomarkers of kidney function, increased lipid peroxidation and decreased activity of antioxidant enzymes, leading to oxidative stress induced renal toxicity (Kumar et al., 2003).

Oxidative stress, which occurs when there are excessive free radical production or low antioxidant levels, has been reported in chronic kidney disease conditions. These free radicals can damage proteins, lipids, carbohydrates and nucleic acids. Oxidative stress leads to tissue damage through different mechanisms, including lipid peroxidation, DNA damage and protein modification. These mechanisms have been implicated in the pathogenesis of several systemic diseases including kidney disease (Ozbek, 2012). Glutathione serves to protect cells from oxidative stress. Glutathione, normally present in high amounts in tubular cells, can neutralize ROS. Reduced kidney cellular glutathione levels and depletion of other antioxidants lead to oxidative stress in cells (Abraham et al., 2013). Inflammation is now believed to play a major role in the pathophysiology of acute kidney injury. Leukocytes and renal tubular cells release many cytokines into the injured kidney and are important components of both the initiation and extension of inflammation during acute kidney injury (Akcay et al., 2009). TNF-α mediated kidney injury has also been reported to follow exposure to shiga toxin, a known RIP of bacterial origin (Lentz et al., 2010).

Oxidative stress may increase the production of TNF-α and other cytokines by activation of NFκB (Manna et al., 1998). TNF-α is a proinflammatory marker produced by immune cells like macrophages and lymphocytes; however, further studies revealed that it is also produced by endothelial and epithelial cells (Ramseyer and Garvin, 2013). Recruitment of immune cells into the kidney, which releases cytokines, may cause inflammation and cell death (Zhu et al., 2009). TNF-α is a strong inducer of matrix metalloproteinase, MMP-9 (Bahar-Shany et al., 2010). Zhou et al. (2009) described TNF-α induced expression of MMP-9 mediated by activation of p21-activated-kinase-1 and JNK pathway. Matrix metalloproteinases (MMPs) are enzymes responsible for degradation of the extracellular matrix (ECM) and are involved in the pathogenesis of ischemia-re-perfusion injury. MMP-9 is gelatinase and cleaves to denatured collagens (gelatins) and laminin, as well as certain chemokines. In the kidney, MMP's are synthesized by intrinsic glomerular cells and tubular epithelial cells. ECM degrading ability of MMPs play an important role in the progression of nephropathies. Altered MMP expression, like ability of MMP-2 to induce or sustain an inflammatory mesangial cell phenotype, has been observed in a large number of kidney diseases (Marti, 2000).

Some of the prominent biomarkers of kidney injury include NGAL (Neutrophil Gelatinase Associated Lipocalin), Cystatin C and Clusterin. (Fassett et al., 2011). NGAL, a ubiquitous 25 KDa protein, covalently bound to gelatinase from human neutrophils, is a marker of tubular injury (Cowland and Borregaard, 1997; Dharnidharka et al., 2002). Serum and urine NGAL levels predict acute kidney injury in different clinical conditions (Mishra et al., 2005). Cystatin C is produced in all nucleated cells. This protein is a marker of kidney injury from drug toxicity and many chronic diseases. It is a marker of glomerular filtration rate and also indicates proximal tubule injury. Clusterin is a multifunctional glycoprotein with roles in metabolism and transport of lipids. Reduced clusterin expression results in an increase in cell death or renal tissue injury. The mechanism behind clusterin's kidney protection is unclear. Up regulation of clusterin expression may cause anti-apoptosis and inactivation of complements which may partly contribute in protection of kidney injury (Zhou et al., 2010).

The renal response to poisons is dynamic, and the kidney adapts to maintain homeostasis during the cascade of repair and recovery that follows the primary insult (Bach, 1989). There are reports on abrin-induced neurotoxicity (Bhasker et al., 2014) and hepatotoxicity, but little information is available about the nephrotoxicity potential of abrin. Hence, the objectives of this study were to determine the following:

a) The mechanism of acute kidney injury after abrin exposure in a mouse model by using different kidney specific markers and histopathology.
b) The role of inflammation, proximal tubular damage, oxidative stress, and vascular injury on the pathogenesis of abrin-induced nephrotoxicity.

2. Materials and methods

2.1. Animals

The study used BALB/c mice randomly bred in the Institute's animal facility, weighing between 23 and 27 g. The animals were maintained according to standard conditions of temperature and humidity (25 ± 2 °C, relative humidity 40–60%), and the animals were fed a standard pellet diet (Ashirwad Brand, Chandigarh, India). Food and water were given ad libitum. The animals were handled according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals). The study was approved by Institutional Animal Ethics Committee, a statutory committee constituted by CPCSEA, Animal Welfare Cell, Ministry of Environment, Forests and Climate Change, Government of India.

2.2. Chemicals

Abrin was isolated from *A. precatorius* seeds as described elsewhere (Kumar et al., 2008). The purified abrin was lyophilized and stored at −80 °C and reconstituted as and when required by PBS. Reduced glutathione (GSH) was from Across (Belgium). O-phthaldialdehyde (OPT) was from Fluka (USA). All other chemicals were of extra pure grade and obtained from Sigma Chemical Co. (St. Louis, USA) unless otherwise mentioned.

2.3. Experimental design

The mice were divided into six groups: Control, 0.5LD50 1, 3 and 7 day, and 1LD50 1 and 3 day. Because not all animals survived to 7 days in the 1LD50 dose group, data for 7 day is not included in the data analysis. All treated groups including control consisted of six animals each. Four animals were used for biochemical assays and two for performing histopathology. 0.5LD50 (0.88 μg/kg) and 1LD50 (1.76 μg/kg) dose of abrin toxin was administered intraperitoneally in mice. Control mice received an equal volume PBS by same route. Mice were handled in accordance with the standard guide for the care and use of laboratory animals. At the end of experimental period, animals were sacrificed by cervical dislocation under deep anesthesia. Following induction of anesthesia, the mice were subjected to a midline abdominal incision and both left and right kidney tissues were harvested.

2.4. Biochemical assays

GSH level was measured spectrofluorimetrically using the method of Hissin and Hilf (1976) and expressed as μmloes of GSH/g tissue. Malondialdehyde (MDA) content was evaluated using the