Science of the Total Environment 645 (2018) 297–317

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Silica - A trace geogenic element with emerging nephrotoxic potential

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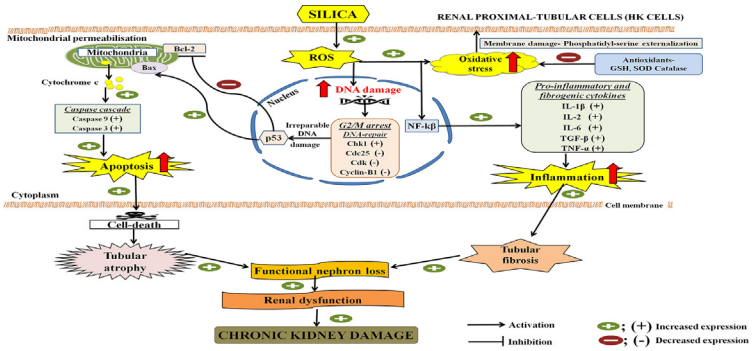
HIGHLIGHTS

- Cellular and molecular mechanisms of silica induced renal toxicity(CKD) described.
- Toxicity pathway studied in nephrotoxin susceptible renal proximal-tubular HK cells.
- Oxidative stress and DNA damage are key players of silica induced nephrotoxicity.
- Nephrotoxicity exhibited by induction of prolonged tubular-cell apoptosis and inflammation.
- Incessant apoptosis and inflammation manifests in tubular atrophy/fibrosis, hallmarks of CKD.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 January 2018
Received in revised form 14 June 2018
Accepted 6 July 2018
Available online xxxx

Keywords:
Silica
CKD
Oxidative-stress
DNA-injuries
Inflammation
Mitochondria mediated apoptosis-pathway

ABSTRACT

Silica is a trace-geogenic compound with limited-bioavailability. It inflicts health-perils like pulmonary-silicosis and chronic kidney disease (CKD), when available via anthropogenic-disturbances. Amidst silica-imposed pathologies, pulmonary toxicological-mechanisms are well-described, ignoring the renal-pathophysiological mechanisms. Hence, the present-study aimed to elucidate cellular-cum-molecular toxicological-mechanisms underlying silica-induced renal-pathology in-vitro.

Various toxicity-assessments were used to study effects of silica on the physiological-functions of HK-cells (human-kidney proximal-tubular cells - the toxin's prime target) on chronic (1–7 days) sub-toxic (80 mg/L) and toxic (100–120 mg/L) dosing.

Results depicted that silica triggered dose-cum-time dependent cytotoxicity/cell-death (MTT-assay) that significantly increased on long-term dosing with ≥100 mg/L silica; establishing the nephrotoxic-potential of this dose. Contrarily, insignificant cell-death on sub-toxic (80 mg/L) dosing was attributed to rapid intracellular toxin-clearance at lower-doses preventing toxic-effects. The proximal-tubular (HK-cells) cytotoxicity was found to be primarily mediated by silica-triggered incessant oxidative-stress (elevated ROS). This enhanced ROS inflicted severe inflammation and subsequent fibrosis, evident from increased pro-inflammatory-cum-fibrogenic cytokines generation (IL-1β, IL-2, IL-6, TNF-α and TGF-β). Simultaneously, ROS induced persistent DNA-damage

Abbreviations: Apaf-1, Apoptotic protease-activating factor-1; A498, Renal proximal tubular carcinoma; BCA, Bicinchoninic acid; Bax, Bcl-2 associated X protein; Bcl-2, B-cell lymphoma 2; BSA, Bovine Serum Albumin; Cdc25, Cell-division control protein 25(Dual-Specificity Phosphatase); Cdk1, Cyclin dependent kinase 1; Chk1, Checkpoint kinase 1; Chk2, Checkpoint kinase 2; CKD, Chronic Kidney Disease; CKDu, Chronic Kidney Disease of unknown etiology; DCFH-DA, 2′-7′-Dichlorodihydrofluorescein Diacetate; DEVD-pNAa, *N*-Acetyl-Asp-Glu-Val-Asp-p-Nitroanilide/Caspase-3 Substrate; DMEM, Dulbecco's Modified Eagle's Medium; FITC, Fluorescein Isothiocyanate; GSH, Glutathione; HK, Human Kidney cells/proximal tubular cells; IETD, pNA-*N*-Acetyl-Ile-Glu-Thr-Asp-p-Nitroanilide/caspase-8 substrate; IL-1β, Interleukin 1 beta; IL-2, Interleukin 2; IL-6, Interleukin 6; JC-1, 5,5′,6,6′-Tetrachloro-1,1′,3,3′-Tetraethylbenzimidazolylcarbocyanide Iodine; LEHD, pNA-*N*-Acety-Leu-Glu-His-Asp-p-Nitroanilide/caspase-9 substrate; LDH, Lactate Dehydrogenase; LPO, Lipid Peroxidation; MDA, Malondialdehyde; MMP, Mitochondrial Membrane Potential; MOMP, Mitochondrial Outer Membrane Permeabilisation; MTT, 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide; NAD, Nicotinamide Adenine Dinucleotide; NADH, Reduced form of Nicotinamide Adenine Dinucleotide; Na_2EDTA, Disodium Ethylenediaminetetraacetate; NLRP3, NLR Pyrin Domain-Containing Protein; PBS, Phosphate Buffered Saline; PI, Propidium Iodide; PMSF, Phenylmethane Sulfonyl Fluoride; SOD, Superoxide Dismutase; TBARS, Thiobarbituric Acid Reactive Substance; TGF-β, Transforming Growth Factor beta; TMB, 3,3′,5,5′-Tetramethylbenzidine; TNF-α, Tumor Necrosis Factor alpha.

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https://doi.org/10.1016/j.scitotenv.2018.07.075

(Comet-assay) that stimulated G2/M arrest for p53-mediated damage-repair, aided by checkpoint-promoter (Chk1) activation and mitotic-inducers (i.e. Cdc-25, Cdk1, cyclinB1) inhibition. However, DNA-injuries surpassed the cellular-repair, which provoked the p53-gene to induce mitochondrial-mediated apoptotic cell-death via activation of Bax, cytochrome-c and caspase-cascade (9/3). This persistent apoptotic cell-death and simultaneous incessant inflammation culminated in the development of tubular-atrophy and fibrosis, the major pathological-manifestations of CKD.

These findings provided novel-insights into the pathological-mechanisms (cellular and molecular) of silica-induced CKD, inflicted on chronic toxic-dosing (≥100 mg/L).Thereby, encouraging the development of therapeutic-strategies (e.g. anti-oxidant treatment) for specific molecular-targets (e.g. ROS) to retard silica-induced CKD-progression, for reduction in the global-CKD burden.

1. Introduction

Chronic Kidney Disease contributes majorly to the global burden of the health-care system in developing countries due to its inevitable association with the traditional causal-factors viz. diabetes and hypertension (Hsu and Powe, 2017). The associated mortality and predominance have heightened over the last few decades by the emergence of a swiftly progressing form of CKD, unlinked to these common risk-factors. Hence, this type of CKD is rechristened as Chronic Kidney Disease of unknown-etiology (CKDu). CKDu has hit several developing countries like Mexico, Central America, Sri-Lanka, Tunisia and India (Gifford et al., 2017). The disease is asymptomatic, irreversible and targets middle-aged adults owing to its slow-progression that causes fatality due to scarcity of renal-replacement options in these affected areas (Stanifer et al., 2016). The principal histo-pathological diagnosis of CKDu is Chronic Tubulo-interstitial Nephritis (CTN), characterized by proximal tubulo-interstitial damage encompassing interstitial-fibrosis and tubular-atrophy. This tubular-damage is majorly manifested on long-term exposure to environmental-nephrotoxins like heavy-metals and pesticides. This pathological resemblance between CTN and CKDu suggests the involvement of environmental renal-toxins in CKDu development (Jayasumana et al., 2016).

One such environmental toxicant that is recently emerging as a nephrotoxin with a plausible role in CKDu development is silica (Mascarenhas et al., 2017; Millerick-May et al., 2015). Silica abundantly constitutes the earth's crust occurring in rock, sand and soil. Despite its innate abundance, it lacks bioavailability under ideal environmental conditions thereby classifying it as a trace geogenic-compound (Cornelis and Delvaux, 2016). The silica bioavailability to human-beings can be rapidly enhanced on anthropogenic disturbances and contamination of its major exposure-routes viz. air and groundwater by various human-activities. Human-activities that gravely contaminate these exposure-routes include mining/sand-drilling (that enriches the air with silica-dust from the earth's crust) and acid-mine drainage (that leaches silica from the intrinsic silica-rich aquifer's bedrock into the groundwater) (Mascarenhas et al., 2017; OSHA, 2016). These anthropogenic invasions eventually result in prolonged exposure to excessive silica via chronic inhalation or ingestion of the contaminated air and groundwater (from bore-wells), which can ultimately inflict various pathological conditions like pulmonary-disorders (i.e. silicosis, bronchitis) and Chronic Kidney Disease(CKD).Therefore, endowing silica with the "emerging contaminant" reputation (Pollard, 2016; Vupputuri et al., 2012).

Epidemiological studies have primarily focused on the induction of respiratory diseases on silica exposure (Kawasaki, 2015; Sen et al., 2016) neglecting the plausibility of developing extra-pulmonary toxic-effects. However, limited studies have recently shifted attention to the risk-assessment of CKD development in conjunction with silica exposure (Mohner et al., 2017; Sponholtz et al., 2016). For instance, data-analysis of five cohort studies by Sponholtz (2016) stated that the silica-exposed subjects displayed a 55.1% risk of developing CKD and 21.8% risk of fatality consequential of chronic renal failure. Moreover, the clinical presentation and histo-pathological alterations reported from these studies and few in-vivo silica-nephrotoxicity analyses in animal-models (Dobbie and Smith, 1982; Markovic and Arambasic, 1971) were typical of chronic tubulo-interstitial nephritis; thus bearing similarities with heavy-metal induced CKD-manifestations (Sabath and Robles-Osori, 2012).

Hence reported evidence has prompted us to hypothesize that silica on gaining entry into the blood-stream [via anthropogenically contaminated exposure routes (air/groundwater)], could inflict renal-toxicity by damaging the kidney's nephrotoxin-susceptible compartment viz. proximal-tubule owing to its role in toxin-clearance (Ahmed et al., 2013). These elicited tubular injuries could gradually intensify on prolonged exposure to elevated silica dosages that can eventually exaggerate into chronic tubulo-interstitial nephritis, which contributes to CKDu development (Nakagawa et al., 2015). This hypothesis was backed by our previous investigation of the CKDu cases in an Indian sub-district that established a causal link between silica exposure routed through groundwater contamination and CKDu in this region. Additionally, the study also highlighted the potential of silica in inducing human renal proximal-tubular cellular death on prolonged exposure to high-doses (≥100 mg/L) (Mascarenhas et al., 2017). However, the toxicity mechanistic aspects remain to be elucidated. Although, preliminary silica induced nephrotoxicity analyses conducted in guinea pigs (in-vivo) highlighted the observed renal histo-pathological alterations to be typical of chronic tubulo-interstitial nephritis (Dobbie and Smith, 1982; Markovic and Arambasic, 1971); in-vitro *or* in-vivo studies explaining the cellular- and molecular-mechanisms of silica-induced renal-toxicity were lacking.

In the current study, we aimed to extract the mechanistic information underlying silica inflicted renal toxicity using the kidney's nephrotoxin clearing cells viz. normal human renal proximal-tubular cells (HK-cells) as an in-vitro model (Li et al., 2017). For this, the proximal-tubular cytotoxicity outcomes following prolonged exposure of HK-cells to increasing silica doses were analyzed via a panel of assessments comprising of cell-viability (Adan et al., 2016), mitochondrial-integrity (Verdugo et al., 2016), oxidative-damage (Gamboa et al., 2016), cell-cycle arrest (Halasi et al. 2013), inflammatory responses (Ernandez and Mayadas, 2016), genomic-damage (Glei et al., 2016) and apoptotic-pathway regulation (Verdugo et al., 2016). Furthermore, this investigation additionally aimed to rule out any anti-tumorigenic potential of silica by analysis of the toxicity inflicted (if any) on the carcinoma-equivalent of the proximal-tubular cells viz. A498 cells. As per our knowledge, this is the first-ever report that aimed to understand the cellular and molecular toxicological mechanisms of silica induced nephrotoxicity as a function of dose and time in normal human proximal-tubular cells(HK-cells). Moreover, this study further aimed to gather causal evidence for the role of silica in CKDu manifestation.

2. Material and methods

2.1. Chemicals and reagents

Cell-culture media viz. Dulbecco's modified eagle's medium (DMEM), phosphate buffered saline (PBS, 10×), 0.25% trypsin (1×),