Characterization of Nano-Sized Silica in Agricultural Exposures and their Role in CKDu Pathogenesis
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11/4/2021
Chronic Kidney Disease of An Unknown Etiology (CKDu)

There are multiple reported epidemics of chronic kidney disease that are affecting workers in coastal agricultural regions. Many such regions are heavily affected by adverse environmental conditions.

Largest affected areas:
- India (Uddanam nephropathy)
- Central America (Mesoamerican nephropathy)
- Sri Lanka (Sri Lankan nephropathy)
Agricultural Workers at Risk of CKDu

- Young, seemingly healthy agricultural workers develop end stage kidney disease.
- Often these workers have little to no access to PPE or quality medical care.
- Many symptoms (proteinuria, elevation in serum creatinine) present asymptomatically. Must be diagnosed histologically.

This worker is covered in soot and particulate matter without any mask or face covering.

Agricultural Workers at Risk of CKDu

- Due to the environmental stresses in centers of CKDu outbreaks, some causes are posited to be heat stress, dehydration, and excess exertion.
- By rescuing each of these effects, kidney damage persists in these individuals.
- There is another factor.
- Pesticide or heavy metal exposure may be a driver of CKDu as well.

This worker is covered in soot and particulate matter without any mask or face covering.

Exposures to Agricultural Workers

Sugarcane stalks are burnt to facilitate the harvest and fertilize the fields.

Sugarcane stalks are ~81% amorphous silica.

Sugarcane cutters disproportionately develop CKDu.

Amorphous mesoporous silica nanoparticles (SiNP) are present in Sugarcane Ash (SA).

Amorphous SiNP from SA is a major exposure to agricultural workers.

Rovani, et al. ACS Omega 2018, 3, 3, 2618–262
Link Between Disease and Exposure

- Brightfield microscopy shows signature chronic interstitial nephritis observed in CKDu
- Darkfield microscopy shows deposition of an unknown inorganic material in tubular regions of kidney

Is this unknown material silica?

Does SiNP accumulation in the kidney correlate with incidence of CKDu?
Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

- ICP-MS is used to assay elemental concentration.
- ICP-MS measures individual atoms and elements compared to molecules in conventional MS.
- ICP-MS is very sensitive, robust, and doesn’t require a separation technique.
# Single Particle ICP-MS

<table>
<thead>
<tr>
<th>Sample</th>
<th>Plasma</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Sample" /></td>
<td><img src="image" alt="Plasma" /></td>
<td><img src="image" alt="Result" /></td>
</tr>
<tr>
<td>Sample containing dissolved metals</td>
<td>Constant stream of charged ions</td>
<td>Constant signal</td>
</tr>
<tr>
<td><img src="image" alt="Sample" /></td>
<td><img src="image" alt="Plasma" /></td>
<td><img src="image" alt="Result" /></td>
</tr>
<tr>
<td>Sample containing metal NPs</td>
<td>Pulses of charged ions</td>
<td>Individual pulses</td>
</tr>
</tbody>
</table>

**Bulk Analysis**

**Single Particle Analysis**

Environ. Toxicol. Chem. 31, 2012
D.M. Mitrano et al.
## SP-ICP-MS Method

### Parameter Table

<table>
<thead>
<tr>
<th>Parameter</th>
<th>^28Si SP-NH\textsubscript{3} DRC</th>
</tr>
</thead>
<tbody>
<tr>
<td>R\textsuperscript{2}</td>
<td>0.9971</td>
</tr>
<tr>
<td>BEC</td>
<td>0 ppb</td>
</tr>
<tr>
<td>LLOD</td>
<td>0.011 ppb</td>
</tr>
<tr>
<td>LLOQ</td>
<td>0.032 ppb</td>
</tr>
<tr>
<td>LLOD\textsubscript{d}</td>
<td>185.4 nm</td>
</tr>
<tr>
<td>Interday Variability (Sizing)</td>
<td>2.47%</td>
</tr>
<tr>
<td>Interday Variability (Quant.)</td>
<td>6.79%</td>
</tr>
</tbody>
</table>

### Graph

- **Pristine 300 nm**
- **5 ppb Injection**

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*Skaggs School of Pharmacy and Pharmaceutical Sciences*

*Artizan Medical Campus*
Sizing of Nanoparticles

1. Sugarcane Ash
2. Aqua Regia Digestion
3. SP-ICP-MS, DLS, TEM Analysis
Sizing of Nanoparticles

<table>
<thead>
<tr>
<th></th>
<th>DLS (nm +/- SD)</th>
<th>SP-ICP-MS (nm +/- SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugarcane Ash Digested in Aqua Regia</td>
<td>180.0 nm +/- 29.2 nm</td>
<td>221.9 nm +/- 62.1 nm</td>
</tr>
</tbody>
</table>

Representative SiNP Population in Sugarcane Ash

Average Size: 221.9 nm

SiNP from Sugarcane Ash

Are these SiNPs present biologically?
El Salvador is one such nation strongly afflicted by CKDu.

The main exported crop in rural regions here is sugarcane.

Here we’re focusing on the specific relationship between sugarcane ash exposure and Mesoamerican nephropathy.
CKDu Positive Tissue Sample

0.054 Particles/μm³ tissue
Most Frequent Size: 193 nm

CKDu Negative Tissue Sample

0.006 Particles/μm³ tissue
Most Frequent Size: Below LLOD_d

Rep resentative SiNP Population in CKDu Biopsies

*Note that the regression for the negative biopsy doesn't fit a proper function
Representative SiNP Population in Ash Against Biopsy

Diameter (nm)

Frequency

Sugarcane Ash

CKDu Biopsy

SiNP from Sugarcane Ash

200 nm
Binning Relevant Particles

- Sugarcane ash has a particular median and mode particle size detected by SP-ICP-MS.

Looking at those particles in CKDu biopsies helps avoid bias from signals below LLOD_d and large sized outlier particles.

Biopsies with elevated particle levels (>0.01 particles/μm^3) at this size are considered reaching an "elevated threshold".
*CKD n = 18, CKDu n = 8
Summary of Patient Studies

- SiNPs of the same representative population as sugarcane ash are found in kidney biopsies of CKDu positive patients.
- Fewer SiNPs which do not correspond to sugarcane ash SiNPs are present in CKDu negative biopsies.
- If a threshold of elevated SiNP content is detected in kidney biopsies, there are increased odds that the patient was diagnosed with CKDu.
- There is a trend of higher average SiNP content in CKDu patient biopsies when compared to control CKD patient biopsies.

How does elevated SiNP content in the kidney lead to a CKDu-like phenotype?
**In vitro studies**

- We used HK-2 cells which are an immortalized human proximal convoluted tubule cell line.
- This cell line retains many functions of well differentiated primary PCT cells.
- One of the more robust, established, and cited cell types for *in vitro* kidney functional studies in literature.
Hypothesis of Cellular Effects by SiNPs

- In many fibrotic kidney diseases, SNAIL1 activation leads to epithelial mesenchymal transition (EMT).
- EMT leads to renal tubule cells gaining myofibroblast activity leading to a profibrotic phenotype (i.e., a CKDu-like phenotype).
- Presence of excess ROS can lead to EMT through SNAIL1 induction.
- SiNPs are well characterized to catalyze ROS generation.

Simon-Tillaux and Hertig., Nephrology Dialysis Transplantation, February 2017,
Hypothesis of Cellular Effects by SiNPs

- ROS generation is mediated by SiNPs through externalized silanol groups which catalyze the homolytic cleavage of $H_2O_2$ into $OH^-$. 

Sugarcane Ash Derived Silica Nanoparticles (SAD SiNPs)

Rovani, et al. ACS Omega 2018, 3, 3, 2618–262
ROS Generation is Induced by SiNP Treatment

Doses are 25 μg/mL for 1 hour (SAD SiNP dose is 2.5 μg/mL)

****p <0.0001 when compared to the de-silicated ash group
*p <0.05 when compared to the de-silicated group
Epithelial Mesenchymal Transition is Enhanced by the Presence of SiNPs in Sugarcane Ash

**p <0.01 when compared to the de-silicated ash group**

†Doses are 25 μg/mL for 48 hrs
Epithelial Mesenchymal Transition is Enhanced by the Presence of SiNPs in Sugarcane Ash

**Results**

- Doses are 25 μg/mL for 24 hrs (SAD SiNPs are 2.5 μg/mL)
- ***p < 0.0001 when compared to NT group, †p < 0.05 when compared to De-silicated ash group**
Summary of *in vitro* work

- Human PCT cell treatment with SiNPs induce ROS generation following 1 hour of treatment.
- EMT occurs in human PCT cells shown by vimentin staining as a result of SiNP treatment after 48 hours.
- Sugarcane ash with silica nanoparticles causes EMT in a greater amount than sugarcane ash without silica nanoparticles.
- Sugarcane ash derived silica nanoparticles induce ROS generation and EMT in human PCT cells.
Conclusions and Future Directions

- SiNPs appear to be present in greater amounts in CKDu patient kidney biopsies. Detecting the presence of SiNPs in patients may be utilized as a method of diagnosing CKDu.

- Because SiNPs induce a cellular change to a profibrotic CKDu-like phenotype through ROS mediated EMT, targeting the Snail1 pathway may be an opportunity for therapeutic intervention for CKDu.
Thank you!

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NIH NIDDK
$R^2 = 0.9971$
The entire respirable fraction of particles is introduced systemically.

The worker has been in the field for some time (i.e. $C_{ss}$ has been reached).

Renal eGFR is approximately average among a healthy individual and represents the entire particle containing volume.

Mixing and dispersion systemically is equal across the entire volume filtered (i.e. worker can be modeled with a CSTR model).

Thus, by using values from literature, doses of SiNP are 0.25, 2.5, and 25 μg/mL will be used for cell studies.
CSTR at $C_{ss}$ can be modeled as:
\[
dM/dt = Q_{in}C_{in} - Q_{out}C_{out} + kV
\]
@ $C_{ss}$, $dM/dt = 0$
Assuming silica doesn’t undergo any other transformation except for transportation to the kidneys, “k” can be modeled as a first order equation:
\[
k = 0.693/T_{1/2}
\]
\[
0 = Q_{in}C_{in} - Q_{out}C_{out} + kV
\]
So now we just need to plug in values from literature

Assuming 2 situations: burning and harvesting
Ambient respirable fraction concentration during burn: 1803 µg/m$^3$
Ambient respirable fraction concentration during harvest: 123 µg/m$^3$
Breathing rate of an outdoor laborer during work: 1.4 m$^3$/hr
2%-10% total particles of a similar size and charge to Si in Ash end up accumulating in kidneys
Half-life of SiNPs of a similar size in vivo: 4.97 hours
CSTR at $C_{ss}$ can be modeled as:

$$\frac{dM}{dt} = Q_{in}C_{in} - Q_{out}C_{out} + kV$$

@ $C_{ss}$, $\frac{dM}{dt} = 0$

$$k = \frac{0.693}{T_{1/2}}$$

$$0 = Q_{in}C_{in} - Q_{out}C_{out} + kV$$

In the case of burning:

$$0 = ((1803)(1.4) - (0.02)(1803)(\text{Dose to Kidney}) + (0.693/4.97)(6.3))$$

Concentration dose to kidney $\sim 25 \mu g/day$

Cell studies will be carried out at a high dose of 25 $\mu g/mL$

And a low dose of 2.5 $\mu g/mL$
Overlayed Biodistribution Functions

Ash Biodistribution

Serum
Urine
Spleen

Liver
Lung
Kidney