Running title:

Topical arsenicals and transcriptional changes in the kidney

Corresponding author:

James F. George, PhD
Division of Cardiothoracic Surgery
Department of Surgery
University of Alabama at Birmingham
703 19th St. S.
Birmingham, AL 35294
Phone: 205-934-4261
Fax: 205-975-0085
Email:jgeorge@uab.edu

Co-corresponding author:

Kyle H. Moore, PhD
Division of Nephrology
Department of Medicine
University of Alabama at Birmingham
703 19th St. S. Birmingham, AL 35294
Email: kylemoore@uabmc.edu

Text pages: 22
Tables: 1
Figures: 4
References: 32
Abstract: 207
Introduction: 569
Discussion: 1022

Abbreviation list:
PAO, phenylarsine oxide; AKI, acute kidney injury; CKD, chronic kidney disease; BRD4, bromodomain-containing protein 4; snRNAseq, single nucleus RNA sequencing; NGAL, neutrophil gelatinase-associated lipocalin; NLB, nuclei lysis buffer; PCA, principal component analysis; DEG, differentially expressed gene, KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, gene Set Enrichment Analysis; PPAR, peroxisome proliferator-activated receptor; PI3-Akt, phosphoinositide 3-kinase-protein kinase B; AMPK, adenosine monophosphate-activated protein kinase; FoxO, forkhead box class O; ROS, reactive oxygen species

Recommended Section:
Special issue: Medical Countermeasures
Abstract

Arsenicals are deadly chemical warfare agents which primarily cause death through systemic capillary fluid leakage and hypovolemic shock. Arsenical exposure is also known to cause acute kidney injury, a condition that contributes to arsenical-associated death due to the necessity of the kidney in maintaining whole-body fluid homeostasis. Because of the global health risk that arsenicals pose, a nuanced understanding of how arsenical exposure can lead to kidney injury is needed. We utilized a non-targeted transcriptional approach to evaluate the effects of cutaneous exposure to phenylarsine oxide, a common arsenical, in a murine model. Here we identified an upregulation of metabolic pathways such as fatty acid oxidation, fatty acid biosynthesis, and PPAR-\(\alpha\) signaling in proximal tubule epithelial cell and endothelial cell clusters. We also revealed highly upregulated genes such as Zbtb16, Cyp4a14, and Pdk4, which are involved in metabolism and metabolic switching and may serve as future therapeutic targets. The ability of arsenicals to inhibit enzymes such as pyruvate dehydrogenase have been previously described in vitro. This along with our own data lead us to conclude that arsenical-induced acute kidney injury may be due to a metabolic impairment in proximal tubule and endothelial cells, and that ameliorating these metabolic effects may lead to the development of life-saving therapies.
Significance Statement

In this study, we demonstrate that cutaneous arsenical exposure leads to a transcriptional shift enhancing fatty acid metabolism in kidney cells, indicating that metabolic alterations might mechanistically link topical arsenical exposure to acute kidney injury. Targeting metabolic pathways may generate promising novel therapeutic approaches in combating arsenical-induced acute kidney injury.
Introduction:

Arsenicals are defined broadly as chemical compounds which contain arsenic and are classified as vesicants, a group of chemical blistering agents designed for chemical warfare. Among them is lewisite, which was first discovered and manufactured for use during World War I and was produced in large quantities during World War II (Pechura and Rall, 1993). Many countries manufactured large quantities of arsenicals such as lewisite and still maintain stockpiles (Li et al., 2016b). Exposure of skin to arsenicals causes severe burns and blisters. Depending on the dose, they can also cause death through pulmonary damage or hypovolemic shock due to systemic capillary fluid leakage (Cullumbine and Box, 1946). Antidotes to lewisite are scarce and most are heavy metal chelating agents. British anti-Lewisite (BAL, 2, 3, dimercaptopropanol) is one of the most effective antidotes, but requires intramuscular injections and has limited use due to its own toxic side effects (Webb and Van Heyningen, 1947; Zvirblis and Ellin, 1976; Stine et al., 1984). Because of the large stockpiles of arsenicals, imperfect therapeutic options, and our limited understanding of the mechanisms of arsenical-induced death, the possibility of chemical warfare or large-scale accidental exposure are looming health concerns. In fact, in the past two decades there have been incidences of accidental arsenical exposure to citizens of countries with known stockpiles, proving that arsenical exposure remains a threat (Ishii et al., 2004; Hanaoka et al., 2006). Therefore, research is needed to understand the cellular and molecular mechanisms by which arsenicals act in order to generate novel therapeutic options to combat this public health concern.

Phenylarsine oxide (PAO) is a membrane permeable arsenical, an analog of lewisite which reacts with proteins in a similar manner and elicits comparable effects in animal models of arsenical exposure (Srivastava et al., 2016). Unlike lewisite, PAO is procurable and approved for use in standard institutional facilities. Because of this, PAO has been utilized by our group and others to study the mechanistic pathology of arsenicals in murine models (Srivastava et al.,...
2016). PAO and lewisite both elicit systemic effects within a few hours due to their lipophilic nature and resultant rapid absorption through the skin (Li et al., 2016a). Of special note, lewisite and PAO induce acute kidney injury (AKI) in murine models of arsenical exposure similar to the AKI observed in human exposures (ATSDR; Jan et al., 2015; Srivastava et al., 2018). Survivors of arsenical exposure will potentially face future kidney-related complications, as the progression of AKI to chronic kidney disease (CKD) has been well characterized (Wald et al., 2009; Sato et al., 2020). Adding to this, it is estimated that one in seven US adults has CKD, which makes them more susceptible to secondary kidney injury, such as AKI (CDC, 2021). Because of these risks, targeted interventions to ameliorate arsenical-induced kidney injury are needed. Given the role of the kidney in maintaining body fluid homeostasis and the hypovolemic shock that can cause death after arsenical exposure, understanding the pathways and mechanisms involved in arsenical-induced AKI could offer new and lifesaving therapeutic options.

The current study sought to determine the kidney’s pathophysiological response to cutaneous PAO exposure on a mechanistic and cellular level. By utilizing a non-targeted transcriptomic approach and leveraging single nuclei RNA sequencing (snRNAseq), we revealed altered gene transcription in metabolic pathways which may be involved in arsenical-induced AKI after cutaneous PAO exposure. These candidate genes and pathways offer potential therapeutic targets for ameliorating arsenical-induced AKI.

**Materials and Methods:**

*Murine model of PAO-induced AKI*

Male C57BL/6J mice 9-10 weeks old were shaved and Nair was applied to the area to completely remove hair twenty-four hours prior to topical administration of PAO on a 2.56 cm²
skin area. PAO (Sigma-Aldrich, P3075) was dissolved in 30 μL of ethanol and administered at a
dose of 4 mg/kg, as previously described (Srivastava et al., 2018). Additional mice were treated
with an equal volume of ethanol as a control. Twelve mice were used in total: two six-hour
vehicle, three twenty-four-hour vehicle, three six-hour PAO, and four twenty-four hour PAO.
Mice were sacrificed six and twenty-four hours after exposure and kidneys were harvested for
snRNAseq. Briefly, mice were anesthetized with isoflurane, blood was collected via cardiac
puncture for serum NGAL analysis, mice were exsanguinated, and their kidneys were flushed
with 10 mL cold PBS before being excised, minced, and flash frozen in liquid nitrogen for
subsequent snRNAseq. Urine was also collected for urine NGAL analysis. All animal studies
were approved by the UAB institutional animal care and use committee.

Serum and Urine NGAL Analysis

Serum and urine NGAL measurements were made via sandwich ELISA (R&D, MLCN20)
per manufacturer protocol. Urine NGAL measurements were normalized to urine creatinine
levels, which were determined by the UAB O’Brien Center using liquid chromatography with
tandem mass spectrometry.

Single Nuclei RNA Sequencing

Previously frozen kidneys were placed on 60 mm dishes with 1 mL of nuclei lysis buffer
(NLB) (Nuclei EZ Lysis Buffer, Sigma-Aldrich; cOmplete ULTRA tablets, Roche; RNasin Plus,
Promega; Invitrogen) and minced finely with a razor blade. The homogenates were then
transferred into Dounce homogenizers and an additional 1 mL of NLB was added before
samples were ground 20-30 times with a pestle. Homogenates were then passed through 200-
μm strainers, transferred back into Dounce homogenizers, and ground an additional 10-15
times. An additional 2 mL of NLB were added and the homogenates were passed through 40-
µm strainers into 50 mL conical tubes and centrifuged at 500 x g for 5 minutes at 4°C. The
supernatants were discarded, and the pellets were resuspended in 4 mL of buffer and
transferred to new tubes. After incubating on ice for 5 minutes, the samples were centrifuged at
500 x g for 5 min at 4°C. The supernatants were discarded again, and the pellets were
resuspended in 2 mL of nuclei suspension buffer (1x PBS, 1% BSA, and 0.1% RNasin Plus)
and passed through 5-µm strainers into new tubes before being processed and sequenced
using 10x Genomics nuclei isolation kit (CA, USA) by the UAB Flow Cytometry and Single Cell
Core Facility. The constructed 3’ biased gene expression libraries were sequenced using an
Illumina Nextseq500 instrument targeting 20,000 reads/cell. The sequencing cycles consisted of
28bp for read 1, 91bp for read 2 and 10bp for i7 and i5 reads (10x Barcode reads).

Sequencing Data Analysis

Analyses of sequencing data were performed using packages in the R statistical
analysis environment (version 4.06). Analyses were primarily carried out using the following
packages: Seurat R package version 4.3.0 and its dependencies, Harmony R package version
0.1.1, and Escape R package version 1.6.0. Data were imported using the Read10x function
and Seurat objects were then created for each sample and cells with feature counts under 200
filtered out. Mitochondrial and ribosomal genes were filtered out and the data were then
normalized using the SCTransform function. Objects were labeled with unique group identifiers
based on their treatment (6hr Veh, 24hr Veh, 6hr PAO, 24hr PAO), merged into a single object,
and normalized using SCTransform again. Data were then integrated using RunHarmony.
Principal component analysis (PCA) was performed, and then cells were clustered with the
FindNeighbors and FindClusters functions with a resolution parameter of 0.11. Next,
dimensional reduction was carried through uniform manifold approximation and projection by
using the RunUMAP function. Clusters were identified as specific cell types through their expression of known unique transcripts attributed to kidney parenchymal and immune cells as follows: proximal convoluted tubule- *Slc5a2, Slc5a12*; proximal straight tubule- *Slc27a2, Slc7a13*; endothelial cell- *Fli, Pecam1*; loop of Henle (ascending limb)- *Umod, Slc12a1*; mesangial cell/fibroblast- *Cfh, Lama2, Pdgfrb*; distal convoluted tubule- *Slc12a3*; principal cell- *Aqp2, Scnn1b*; immune- *Ptprc*; loop of Henle (descending limb)- *Slc14a2, Proser2, Sorcs3*; intercalated cell- *Atp6v1g3, Slc4a9*; type A intercalated cell- *Slc4a1*; type B intercalated cell- *Slc26a4, Lsamp*; podocyte- *Nphs1, Ptpro*.

Differentially expressed genes (DEGs) of each cluster were determined using the FindAllMarkers function. Gene enrichment analysis, which evaluates the overrepresentation of gene pathways, was performed with WebGestaltR for pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Gene Set Enrichment Analysis (GSEA) was performed with Escape to analyze additional selected pathways. These pathways were analyzed by rank ordering of all genes followed by statistical analysis of the genes in each pathway in order to determine if their distribution was the same in each group by using a non-parametric Kruskal-Wallis rank sum test. This was followed by a pairwise Wilcoxon rank sum test with Benjamini & Hochberg “BH” adjustment to determine p-values for each comparison. Further comparisons of specific pathways were made through enrichment score analysis.

**Statistics**

Serum and urine NGAL measurements are presented as mean ± SEM and their significance was determined in GraphPad Prism 8 by a two-way ANOVA with a Sidak’s multiple comparisons test (for data with matched repeated measures) or a mixed-effects model with a Sidak’s multiple comparisons test (for data without matched repeated measures). The medians of the ranked sum analysis from GSEA are presented along with p-values from a Wilcoxon rank
sum test performed in RStudio. P-values below 2.225074e-308 in Rstudio are rounded down to zero, and so are presented as <2.225074e-308.

Results:

Biomarkers of AKI following cutaneous exposure to PAO

Serum NGAL was significantly increased in the PAO group at six hours compared to baseline (PAO 2275 ± 254 pg/mL vs Baseline 101 ± 10 pg/mL; p=0.0051) as well as at twenty-four hours compared to baseline (PAO 7276 ± 920 pg/mL vs Baseline 102 ± 3 pg/mL; p<0.0001). Urine NGAL (normalized to urine creatinine) was not significantly increased in the PAO group at six hours compared to baseline (PAO 186 ± 15 pg/mg vs Baseline 76 ± 7 pg/mg; p=0.157), but was significantly increased at twenty-four hours compared to baseline (PAO 8739 ± 2212 pg/mg vs Baseline 97 ± 8 pg/mg; p=0.0117) (Supplementary Figure 1A-D). No vehicle treated groups were significantly elevated at any time point compared to baseline values.

Identification of kidney parenchymal cells in snRNAseq data

Transcriptomes of twelve mice were used to generate our working data set (Figure 1A). After dimensional reduction with the top 30 principal components, 16 total cell clusters were identified, one of which was only present in and fully represented by one mouse sample and was therefore removed (Supplementary Figure 2A & B). Afterwards, 15 cell clusters remained which were all identified as kidney parenchymal cells or immune cells (Figure 1B). Clusters were identified as specific cell types based on their expression of known kidney and immune cell gene markers as follows: Cluster 0 is proximal tubule cells (PT1); cluster 1 is proximal tubule cells (PT2); cluster 2 is endothelial cells (EC1); cluster 3 is ascending loop of Henle cells (LoH(AL1)); cluster 4 is mesangial cells and/or fibroblasts (MC/FIB); cluster 5 is proximal tubule cells (PT3); cluster 6 is distal convoluted tubule cells (DCT); cluster 7 is principal cells (PC);
cluster 8 is immune cells (Immune), cluster 9 is descending loop of Henle cells (LoH(DL));
cluster 10 is ascending loop of Henle cells (LoH(AL2)); cluster 11 is type A intercalated cells
(ICA); cluster 12 is type B intercalated cells (ICB); cluster 13 is endothelial cells (EC2); cluster
14 is podocytes (POD) (Figure 1C).

Differentially expressed genes in individual cell clusters after cutaneous PAO exposure

Analysis of cell clusters across time points (6hr Veh vs 6hr PAO and 24hr Veh vs 24hr
PAO) revealed upregulated DEGs, of which the top 5 for each cluster are shown in Table 1. We
then concentrated our analysis on proximal tubule cell clusters and endothelial cell clusters, as
their abundance within the kidney and sensitivity to cellular injury is well defined. The DEGs of
all three proximal tubule clusters (PT1, PT2, PT3) six and twenty-four hours after PAO exposure
included Txnip, Hmgcs2, Dpyd, Cyp4a14, Cyp4a10, Pck1, Plin2, Zbtb16, Frmd4b, Pdk4,
Atp6v0a4, Glt1d1, Gldc, Fkbp5, Hsp90b1, Ghr, Slc13a1, Acnat1, and Malat1. Heatmaps of the
combined top 5 DEGs from all proximal tubule clusters mapped onto each individual proximal
tubule cell cluster revealed dynamic alterations in expression after PAO exposure (some genes
overlap between time points and clusters, and genes that are not included in heatmaps did not
display enough variability for Seurat to map them) (Supplementary Figure 3). The top 5
positive DEGs of both endothelial cell clusters included Zbtb16, Fkbp5, Plcb4, Arhgap26, Insr,
Pdk4, Frmd4b, Cyp4a14, Cyp4a10, Entpd1, Rai14, Gldc, and Map3k5. Heatmaps of these
DEGs mapped onto each endothelial cell cluster in Supplementary Figure 4 once again reveal
an alteration in the transcriptional landscape both six and twenty-four hours after cutaneous
PAO exposure. Among these and other cell types, notable genes that were upregulated in at
least three clusters and/or time points included Zbtb16, Fkbp5, Cyp4a14, Pck1, Frmd4b, Pdk4,
Gldc, Malat1, and Pde10a. Of these, Zbtb16, Cyp4a14, Pck1, Pdk4, and Gldc are all involved in
metabolism and/or energy deficit response, and their expression patterns between vehicle and
PAO treatment within all kidney cell clusters is displayed in Supplementary Figure 5.
Enriched pathways in proximal tubule cell and endothelial cell clusters after cutaneous PAO-exposure

Pathway analysis of cluster PT1 revealed enrichment of KEGG pathways involved in fatty acid degradation, peroxisome proliferator-activated receptor (PPAR) signaling, and phosphoinositide 3-kinase-protein kinase B (PI3K-Akt) signaling six and twenty-four hours after PAO exposure (Figure 2A). Additionally, at the six-hour time point PT1 was enriched in the glyoxylate and dicarboxylate metabolism and adenosine monophosphate-activated protein kinase (AMPK) signaling pathways. Cluster PT2 was enriched in KEGG pathways involved in fatty acid degradation, fatty acid metabolism, PPAR signaling, adipocytokine signaling, peroxisome, and forkhead box class O (FoxO) signaling six and twenty-four hours after PAO exposure (Figure 2B). Pathways for insulin resistance, glucagon signaling, AMPK signaling, and glycerophospholipid metabolism were also enriched in the PT2 cluster at six hours. At twenty-four hours, Cluster PT2 was also enriched in the butanoate metabolism pathway. Cluster PT3 was enriched in KEGG pathways involved in fatty acid degradation and PPAR signaling both six and twenty-four hours after PAO exposure (Figure 2C). At six hours, PT3 was also enriched in glycolysis/gluconeogenesis, peroxisome, adipocytokine signaling, glucagon signaling, FoxO signaling, AMPK signaling, and insulin resistance pathways. Pathway analysis of EC1 revealed enrichment of KEGG pathways involved in p53 signaling and PI3K-Akt signaling twenty-four hours after PAO treatment (Figure 2D). There were no KEGG enriched pathways identified in the EC1 cluster six hours after PAO treatment. The EC2 cluster was enriched in KEGG pathways involved with fatty acid degradation, PPAR signaling, peroxisome, fatty acid metabolism, FoxO signaling, AMPK signaling, glucagon signaling, and insulin resistance at both six and twenty-four hours after PAO exposure (Figure 2E). Additionally, at six hours KEGG pathways involved in tyrosine metabolism and tryptophan metabolism were
enriched and at twenty-four hours KEGG pathways involved in butanoate metabolism and adipocytokine signaling were enriched in cluster EC2.

**Enrichment of specific pathways in kidney parenchymal cells after cutaneous PAO exposure**

Analysis of specific pathways of interest revealed increased expression (as determined by rank order) in additional fatty acid metabolic pathways. Expression of the “Fatty acid β-oxidation” pathway was increased in PAO treated compared to vehicle treated groups in PT1 (6hr), PT2 (6hr & 24hr), and EC2 (6hr & 24hr) (Figure 3A). Expression of the “Fatty acid biosynthesis” pathway was also increased in PT1 (6hr), PT2 (6hr & 24hr), PT3 (6hr), and EC2 (6hr) (Figure 3B). Expression of the “Fatty acid ω-oxidation” pathway was increased in PT1 (6hr & 24hr), PT2 (6hr & 24hr), PT3 (6hr & 24hr), EC1 (6hr), and EC2 (6hr & 24hr) (Figure 3C). Expression of the “Fatty acid transporters” pathway was increased in PT1 (6hr & 24hr), PT2 (6hr & 24hr), PT3 (6hr & 24hr), EC1 (6hr & 24hr), and EC2 (6hr & 24hr) (Figure 3D). Expression of the “Mitochondrial long chain fatty acid β-oxidation” pathway was increased in PT1 (6hr), PT2 (6hr & 24hr), PT3 (6hr & 24hr), and EC2 (6hr & 24hr) (Figure 3E). Analysis of specific PPAR pathways also revealed increased expression of the “PPAR-α pathway” in PT1 (6hr), PT2 (6hr & 24hr), PT3 (6hr & 24hr), and EC2 (6hr & 24hr) (Figure 3F). Additionally, all of the pathways mentioned above were expressed at a higher level in the 6hr PAO group compared to the 24hr PAO group in PT1, PT2, PT3, EC1, and EC2 groups, with the exception of the “Fatty acid biosynthesis” pathway in the EC1 group, which was not significantly different between 6hr and 24hr PAO groups. Medians and p-values from the pairwise Wilcoxon rank sum test for all comparisons can be found in Supplementary File 1. Plotted enrichment scores for each cluster compared across time points can be found in Supplementary Figure 6.

**Discussion:**
In order to reveal potential single genes and pathways for therapeutic targets after cutaneous PAO exposure, we leveraged snRNAseq to analyze transcriptional changes of kidney parenchymal cells. PAO-induced AKI was confirmed by the elevation of serum and urine NGAL. We identified fourteen clusters as major kidney cell types and one cluster as immune cells. We focused our analysis primarily on proximal tubule epithelial cells and endothelial cells in the kidney. Proximal tubule epithelial cells are the most abundant kidney cell type and are sensitive to injury, making them likely targets of PAO-mediated kidney injury (Chevalier, 2016; Clark et al., 2019). Endothelial cells (particularly vascular endothelial cells) are also abundant within the kidney and appear to be the primary cell type by which arsenicals exert systematic effects, namely systemic capillary fluid leakage and intravascular fluid loss (Cullumbine and Box, 1946). Additionally, the kidney vasculature follows alongside the length of the nephron and could therefore be a conduit for PAO-mediated injury within compartments of the kidney.

Some of the most commonly upregulated DEGs after PAO exposure included Zbtb16, Cyp4a14, Pck1, Pdk4, Gldc. These five genes were of particular interest to us because they are known to directly affect or be directly affected by cellular metabolism and energy homeostasis. Zbtb16 encodes a transcription factor induced by glucocorticoid signaling and energy deficit, which is involved in regulating gluconeogenesis and negatively regulating insulin signaling (Krupková et al., 2018). Cyp4a14 encodes a cytochrome P450 enzyme that catalyzes the ω-hydroxylation of medium-chain fatty acids and arachidonic acid. Pck1 encodes the phosphoenolpyruvate carboxykinase enzyme, which regulates the gluconeogenic and glyceroneogenic pathways in the kidney (Yu et al., 2021). Pdk4 encodes pyruvate dehydrogenase kinase 4 which inactivates pyruvate dehydrogenase to switch cellular resource utilization from glucose metabolism to fatty acid metabolism and is under the control of FoxO1 transcription factor (Gopal et al., 2017). Finally, Gldc encodes the protein glycine dehydrogenase which is responsible for glycine degradation (Hasse et al., 2013). These genes
could be future therapeutic targets in combating the injurious effects of PAO on the kidney. They also support a notion that metabolic alterations are a major mechanism through which PAO triggers AKI.

The KEGG pathways that were enriched in proximal tubule cells and endothelial cells were almost exclusively related to metabolism, such as fatty acid metabolism (degradation and/or metabolism), PPAR signaling, and AMPK signaling. Because of the prevalence of pathways related to fatty acid metabolism, we sought to determine if additional, more specific, pathways were upregulated. In doing so, we determined that there was an increase in PPAR-\(\alpha\) signaling, which is involved in regulating fatty acid metabolism, and in pathways that enhance fatty acid utilization as an energy source. These findings are particularly important because pyruvate dehydrogenase complexes are extremely sensitive to the enzyme-inhibitory capacity of arsenicals and may mechanistically link cutaneous arsenical exposure to kidney injury. Lewisite inhibits pyruvate dehydrogenase through its arsenic atom binding to the two sulfur atoms of pyruvate dehydrogenase, which makes it very plausible that PAO's arsenic atom reacts in the same manner (Peters et al., 1946; Samikkannu et al., 2003). Proximal tubule epithelial cells are especially dense with mitochondria and rely heavily on oxidative phosphorylation for the metabolically demanding amount of ion exchange they perform. Because of their large metabolic needs, an inhibition of pyruvate dehydrogenase and the subsequent inability of carbohydrates to enter the tricarboxylic acid (TCA) cycle in proximal tubule epithelial cells could directly lead to cellular injury and death. The increase in PPAR-\(\alpha\) and other fatty acid metabolic pathways could be a compensatory response attempting to accelerate the generation of acetyl-CoA directly from fatty acid molecules. While proximal tubule epithelial cells rely primarily on fatty acids for their metabolic needs, completely inhibiting carbohydrates from entering the TCA cycle could still have severe effects on the cellular metabolic state, leading to cellular injury or death. Furthermore, during AKI recovering tubules shift to more glycolytic activity and less fatty
acid oxidation as they dedifferentiate and proliferate (Lan et al., 2016). As they redifferentiate, the metabolic profile is restored to primarily mitochondrial respiration, but if this process is impaired injury persists. It is becoming increasingly evident that the fate of the recovering proximal tubule cell is dependent on the precise timing and control of these metabolic processes (Lan et al., 2016; Cargill and Sims-Lucas, 2020). Aside from the direct effects of metabolic stress, a backup in pyruvate’s conversion to acetyl-CoA could also cause shunting to lactate dehydrogenase, resulting in excessive lactate and lactic acid levels which have been associated with multiple types of AKI, likely through excessive reactive oxygen species (ROS) generation (Luo et al., 2017; Wang et al., 2021; Yan et al., 2021; Gong et al., 2022). Figure 4 depicts these theoretical processes.

Interestingly, ongoing work has identified a role for bromodomain-containing protein 4 (BRD4) inhibition in mitigating the effects of arsenical-induced injury and novel small molecule therapeutics targeting BRD4 are currently being developed (Yatchang et al., 2022). While it is believed the primary mechanism of action of BRD4 inhibition is the attenuation of inflammation, a recent study suggests that it also affects metabolism (Barrow et al., 2016). In this study, BRD4 inhibition promoted oxidative phosphorylation and preserved mitochondrial energetics in the presence of Complex I defects, suggesting this class of therapeutics may be exerting their effects not only through reducing inflammation, but also through metabolic reprogramming (Barrow et al., 2016). Our results along with this study provide an additional explanation for how BRD4 inhibition may attenuate arsenical-induced kidney injury, and future studies will seek to validate these metabolic effects in PAO-induced AKI.

Considered together, these findings suggest that an altered metabolic state of kidney cells is a key factor in arsenical-induced kidney injury. Specifically, our data strongly indicate an interference in the glucose metabolic pathway along with a simultaneous upregulation of genes and pathways for fatty acid metabolism, among others. Examining the effect of therapeutic
targets on metabolism may be significant criteria to consider. Future studies will seek to validate these findings and to evaluate the metabolic state of kidney parenchymal cells after cutaneous PAO exposure in order to assess the potential for metabolically targeted therapeutics in arsenical-induced kidney injury.

Acknowledgments

The authors would like to thank Matthew D. Cheung for technical RStudio support.

Data Availability

All sequencing data has been made available online through Gene Expression Omnibus (GSE233859). All other data supporting the findings described in this manuscript are contained within the manuscript and accompanying supplemental data set.

Authorship Contributions

Participated in research design: Moore, Boitet, Traylor, Athar, Agarwal, George

Conducted experiments: Moore, Boitet, Traylor, Esman, Srivastava, Khan

Performed data analysis: Moore, Chandrashekar, Traylor, Esman, Erman

Wrote or contributed to the writing of the manuscript: Moore, Traylor, Erman, Agarwal, George
References


Li C, Srivastava RK, Weng Z, Crouch CR, Agarwal A, Elmets CA, Afaq F and Athar M (2016b) Molecular Mechanism Underlying Pathogenesis of Lewisite-Induced Cutaneous...


Footnotes

Financial Support: This work was supported by the National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases (F32 DK136187, T32 DK007545, P30 DK079337); the National Institute of Environmental Health Sciences (U54 ES030246); and the National Institute of General Medical Sciences (K12 GM088010).

No author has an actual or perceived conflict of interest with the contents of this manuscript.
Figure Legends:

Figure 1

**Single nuclei RNA sequencing cluster identification:** Validation of successfully integrated single nuclei RNA sequencing data of twelve mice into a combined data set, grouped according to the individual mice used in the study (A). Combined UMAP showing cluster identities of kidney parenchymal cell types and one immune cell population (B). A dot plot showing expression of known gene transcripts and the proportion of cells expressing them for the identification of kidney and immune cell types (C).

Figure 2

**KEGG enriched pathways in proximal tubule cell and endothelial cell clusters:** Enrichment analysis of the Kyoto Encyclopedia of Genes and Genomes database pathways at six and twenty-four hours in the PT1 cluster (A), PT2 cluster (B), PT3 cluster (C), EC1 cluster (D), and EC2 cluster (E). False discovery rate (FDR) was <0.05 for all pathways shown.

Figure 3

**Fatty acid metabolic pathways:** Violin plots of specific fatty acid metabolic pathways of interest expressed in rank order density of member genes for each gene set, sorted by cluster identity and time points (Blue = 6hr Veh, Green = 24hr Veh, Orange = 6hr PAO, Red = 24hr PAO). Boxplots show medians, lower quartile, and upper quartile distributions across all samples. Fatty acid β-oxidation (A), fatty acid biosynthesis (B), fatty acid ω-oxidation (C), fatty acid transporters (D), mitochondrial long chain fatty acid β-oxidation (E), and PPAR-α pathway (F). Significance is indicated by * (6hr PAO vs 6hr Veh p<0.05) and # (6hr PAO vs 6hr Veh p<0.05 and 24hr PAO vs 24hr Veh p<0.05)
Figure 4

Theoretical pathological process of PAO-induced AKI: A theoretical process by which phenylarsine oxide (PAO) may induce kidney injury through pyruvate dehydrogenase inhibition. Fatty acid metabolic process upregulation may be a compensatory response.
### Tables

**Table 1: Top positive differentially expressed genes, ranked by p-value**

<table>
<thead>
<tr>
<th>Cluster-Cell Type</th>
<th>6hr positive DEGs</th>
<th>24hr positive DEGs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-PT1</td>
<td>Txnip, Hmgs2, Dpyd, Cyp4a14, Cyp4a10</td>
<td>Gldc, Fkbp5, Cyp4a14, Hsp90b1, Pck1</td>
</tr>
<tr>
<td>1-PT2</td>
<td>Pck1, Txnip, Hmgs2, Dpyd, Plin2</td>
<td>Ghr, Gldc, Slc13a1, Zbtb16, Cyp4a14</td>
</tr>
<tr>
<td>5-PT3</td>
<td>Zbtb16, Frmd4b, Pdk4, Atp6v0a4, Glt1d1</td>
<td>Frmd4b, Acnat1, Malat1, Ghr, Zbtb16</td>
</tr>
<tr>
<td>2-EC1</td>
<td>Zbtb16, Fkbp5, Plcb4, Arhgap26, Insr</td>
<td>Zbtb16, Fkbp5, Entpd1, Rai14, Cyp4a14</td>
</tr>
<tr>
<td>13-EC2</td>
<td>Zbtb16, Pdk4, Frmd4b, Cyp4a14, Cyp4a10</td>
<td>Frmd4b, Zbtb16, Fkbp5, Gldc, Map3k5</td>
</tr>
<tr>
<td>3-LoH(AL1)</td>
<td>Fkbp5, Zbtb16, Pkp4, Pde10a, Zhx3</td>
<td>Malat1, Fkbp5, Zbtb16, Spp1, Sult1d1</td>
</tr>
<tr>
<td>10-LoH(AL2)</td>
<td>Zbtb16, Pdk4, Cyp4a14, Fkbp5, Frmd4b</td>
<td>Frmd4b, Fkbp5, Gldc, Cyp4a14, Zbtb16</td>
</tr>
<tr>
<td>4-MC/FIB</td>
<td>Zbtb16, Fkbp5, C7, Tacc1, Cp</td>
<td>Zbtb16, C7, Fkbp5, Cp, Clca3a1</td>
</tr>
<tr>
<td>6-DCT</td>
<td>Pde10a, Zbtb16, Neat1, Fkbp5, Malat1</td>
<td>Pde10a, Zbtb16, Kcnj16, Fkbp5, Cyp4a14</td>
</tr>
<tr>
<td>7-PC</td>
<td>Malat1, Zbtb16, Pde10a, Fkbp5, Cyp4a14</td>
<td>Malat1, Zbtb16, Fkbp5, Cyp4a14, Pde10a</td>
</tr>
<tr>
<td>8-Immune</td>
<td>Fkbp5, Ccnd3, Zbtb16, Cyp4a14, G6pc</td>
<td>Fkbp5, Cdk14, Tgfbi, Ccnd3, Picalm</td>
</tr>
<tr>
<td>9-LoH(DL)</td>
<td>Zbtb16, Fkbp5, Cyp4a14, Vmp1, Rbpms</td>
<td>Zbtb16, Fkbp5, Gclc, Hsp90aa1, Flt1</td>
</tr>
<tr>
<td>11-ICA</td>
<td>Zbtb16, Malat1, G6pc, Cyp4a14, Tshr</td>
<td>Abr, Cyp4a14, Slc13a1, Pck1, Dmxl1</td>
</tr>
<tr>
<td>12-ICB</td>
<td>Gm47795, Zbtb16, Pde10a, Abr, Gm47790</td>
<td>Gm47795, Zbtb16, Pde10a, Sh3rf1, Kcnq1ot1</td>
</tr>
<tr>
<td>14-POD</td>
<td>Fkbp5, Zbtb16, Malat1, Neat1, Klf9</td>
<td>Slc41a2, Plod2, Fkbp5, Srgap3, Arhgef26</td>
</tr>
</tbody>
</table>
Figure 1: This article has not been copyedited and formatted. The final version may differ from this version.

JPET Fast Forward. Published on September 12, 2023 as DOI: 10.1124/jpet.123.001742

Downloaded from jpet.aspetjournals.org at ASPET Journals on November 23, 2023
Figure 2
This article has not been copyedited and formatted. The final version may differ from this version.