Mercury Exposure in Diabetic Rats

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ABSTRACT

Diabetes is a significant health problem and comorbidity throughout the world that can lead to renal injury and insufficiency. Exposure to environmental toxicants such as mercury (Hg) may also lead to renal injury and insufficiency. Indeed, exposure to Hg has been shown to exacerbate chronic kidney disease (CKD). However, the impact of Hg exposure on renal function in diabetic patients is unclear. The goal of the present study is to test the hypothesis that exposing diabetic animals to Hg will enhance renal injury. To test this hypothesis, we used diabetic and control Wistar rats. Diabetic rats (n = 10) were fed a high-fat diet for 10 days followed by an injection with streptozotocin (65 mg/kg). Control rats (n = 10) were fed a normal diet for 10 days followed by an injection of buffer. Rats with blood glucose levels above 150 mg/dL were considered diabetic. Five rats from each group were administered Hg (5 mg/kg) intravenously and the remaining five rats were administered saline. Rats were sacrificed 24 h after injection, and we measured biomarkers of renal function. Diabetic rats had decreased renal function, evidenced by increased serum creatinine levels and KIM-1 expression. Additionally, the expression of Klotho, SIRT1, and ATG13 was decreased, suggesting impairment in some cellular homeostatic processes. Histological analyses revealed pathological changes in diabetic rat kidneys. The current study provides data related to the mechanisms of renal injury in diabetic patients and shows that, under the current exposure conditions, exposure to Hg does not enhance renal injury in diabetic patients.

SIGNIFICANCE

As of 2018, the total number of people living with diabetes in the United States was 34.2 million, which was 10.5% of the country's population.[1] There were 26.9 million people diagnosed with diabetes.[1] However, a significant number of individuals living with diabetes at the time were undiagnosed, which represented 7.3 million people.[1] Diabetes is also a major health concern in rural Georgia, and in one rural Georgia county (Jones County), a total of 22.7% of its residents were diagnosed with diabetes in 2017.[2] Decreased renal function results from diabetes because hyperglycemia contributes to an increase in type IV collagen and fibronectin, reactive oxygen species, and glomerular filtration rate, along with other detrimental effects.[3]

METHODS

Young and old Wistar rats were fed either a high-fat diet or a normal, control diet for 10 days. Following this, the rats were injected intraperitoneally with either 65 mg/kg streptozotocin or buffer. The next day, blood glucose levels were measured, and the rats were injected intravenously with either 5 mg/kg methylmercury chloride (MeHg) or saline.

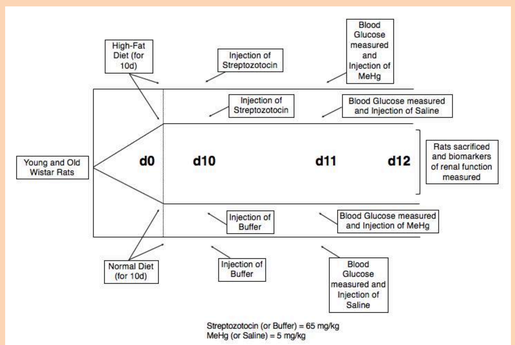

Figure 1. Diagram of Methods

ACKNOWLEDGEMENTS

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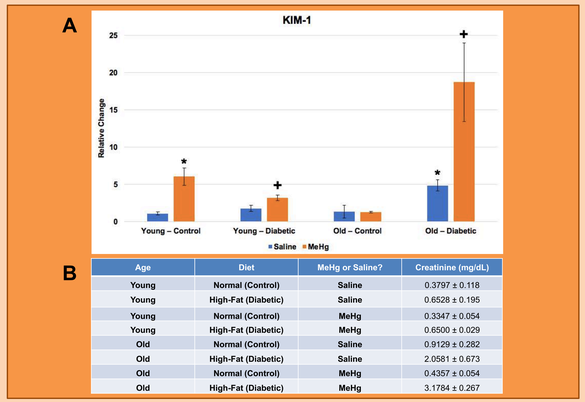

B

Age	Diet	MeHg or Saline?	Creatinine (mg/dL)
Young	Normal (Control)	Saline	0.3797 ± 0.118
Young	High-Fat (Diabetic)	Saline	0.6528 ± 0.195
Young	Normal (Control)	MeHg	0.3347 ± 0.054
Young	High-Fat (Diabetic)	MeHg	0.6500 ± 0.029
Old	Normal (Control)	Saline	0.9129 ± 0.282
Old	High-Fat (Diabetic)	Saline	2.0581 ± 0.673
Old	Normal (Control)	MeHg	0.4357 ± 0.054
Old	High-Fat (Diabetic)	MeHg	3.1784 ± 0.267

Figure 2. Decreased Renal Function in Older Diabetic Rats Exposed to MeHg

Panel A displays the expression of Kidney injury molecule-1 (KIM-1), which is a marker of renal injury. Old diabetic rats who were administered MeHg had a greater expression of KIM-1, which indicates increased renal injury (* indicates $p<0.05$ from control saline; + indicates $p<0.05$ from corresponding control). Panel B shows a table of the creatinine levels (mg/dL) for each group of rats. The increased creatinine levels in old diabetic rats administered MeHg indicates that this group of rats have decreased renal function.

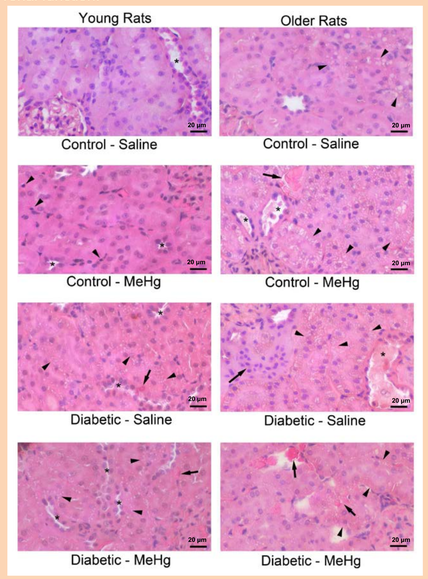

Figure 3. Hematoxylin and Eosin (H&E) Staining of Kidneys

For the young rats, Control-Saline rats have a normal glomerulus and tubules (* indicates a distal tubule; all others are proximal tubules). Control-MeHg rats have normal tubules, but inflammatory cells have appeared (arrowheads). Diabetic-Saline rats are suffering from cellular injury and hemorrhaging (arrow) as well as small cytoplasmic vacuoles (arrowheads). Diabetic-MeHg rats display similar injuries in proximal tubules, but distal tubules are not affected. For the older rats, Control-Saline rats have small cytoplasmic vacuoles (arrowheads). Control-MeHg rats have several vacuoles and a large hemorrhage (arrow). Diabetic-Saline rats have proteinaceous tubular casts and cellular debris (*) as well a dilated and swollen tubule (arrow). Diabetic-MeHg rats display cellular injury (arrowheads) and hemorrhages (arrows).

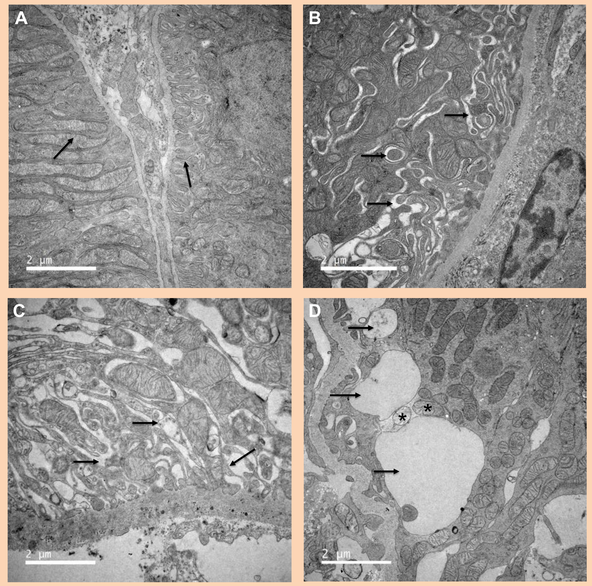

Figure 4. Renal Tubular Cells of Rats Administered Saline

Panel A indicates young control rats with the appearance of a normal basolateral membrane and infoldings (indicated by arrows) that seem to be functioning properly. Panel B indicates young diabetic rats with a disturbed basolateral membrane and visible gaps between infoldings (arrows). Panel C indicates old control rats with a thick basolateral membrane and multiple gaps between infoldings (arrows). Panel D indicates old diabetic rats that have lost their basolateral infoldings and vacuoles have formed (arrows), and their mitochondria have become deformed (*).

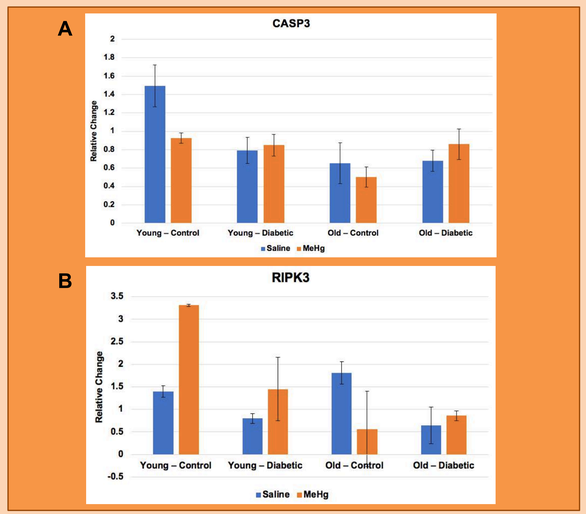

Figure 5. Biomarkers of Apoptosis and Necroptosis

Panel A displays the expression of Caspase 3 (CASP3), which is a marker of apoptosis. Panel B shows the expression of Receptor interacting protein kinase-3 (RIPK3), which is a marker of necroptosis. There are no significant differences in the expression of CASP3 and RIPK3 among the eight groups of rats.

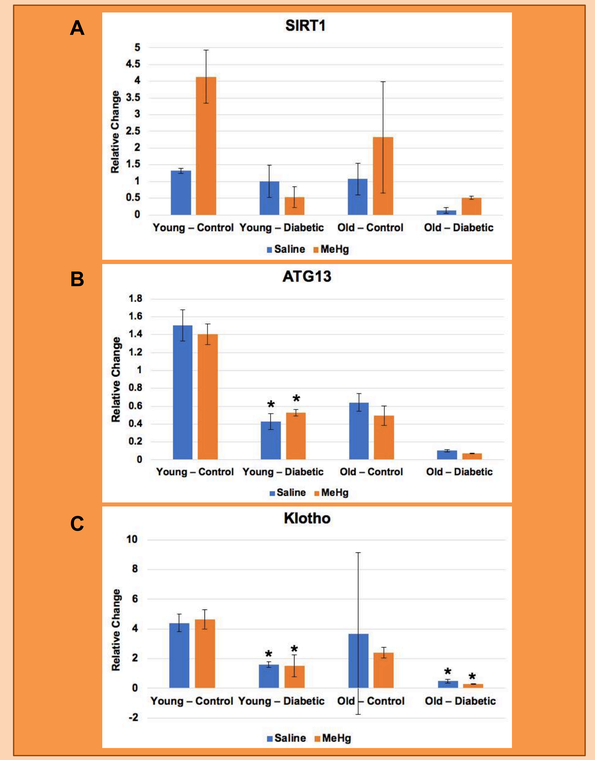

Figure 6. Decreased Expression of Three Different Primers in Diabetic Rats

Panel A displays the expression of Sirtuin-1 (SIRT1). SIRT1 decreases in cells with insulin resistance, and the results support this as SIRT1 expression is reduced in diabetic rats. Panel B shows the expression Autophagy-related protein 13 (ATG13), which is a marker of autophagy. Young diabetic rats had a decreased expression of ATG13 (* indicates $p<0.05$ from control saline), and the same trend is seen in older diabetic rats, but there is no significance. Panel C includes the expression of Klotho, which is known to decrease when there is oxidative stress and inflammation. Both old and young diabetic rats had significantly lower expressions of Klotho.

CONCLUSIONS

- Although the results from this study do not show that there is a significant difference in renal injury when diabetic rats are exposed to specified amount of MeHg (5 mg/kg), it is possible that MeHg could enhance renal injury to a larger degree if mercury exposure conditions are increased.
- The data from this present study can be applied to other heavy metal toxicants besides MeHg and other chronic diseases besides diabetes. This current study infers that heavy metal toxicants may have the potential to exacerbate renal injury in patients with chronic diseases.
- Diabetes is a significant health issue in rural Georgia. Therefore, both the results from this study and future research that builds upon this study can be used to educate diabetic patients in rural Georgia on how their exposure to mercury, or other heavy metal toxicants, can further diminish their renal function.

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