Aurintricarboxylic Acid Mitigates the Negative Effects of Uranium upon the Blood-Brain Barrier

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BACKGROUND

Uranium-235 (U) is a radioactive actinide released during nuclear detonation and serves as a fuel source in nuclear weapons. The risk of exposure to U is heightened for military servicemembers due to current geopolitical conflicts. Of significant concern are the potential short- and long-term neurological effects, as U has a biological half-life of approximately 15 days and can cross the blood-brain barrier (BBB). Currently, no FDA-approved treatment exists for internal U contamination. To investigate this, uranyl nitrate, an intermediate U compound, was administered alone or in combination with ionizing radiation (IR) to analyze the resulting insult and survival. Additionally, aurintricarboxylic acid (ATA) was tested as a potential treatment for U exposure. ATA's chemical structure has carboxylic and hydroxyl groups that can bind metals, making it an ideal candidate for decorporation studies. Our laboratory has previously shown that ATA binds U.

For these studies, our laboratory used human brain microvascular endothelial cells (hBMEC) and astrocytes to study the effects of U and ATA on the BBB.

METHODS

- ***In vitro* Model:** Human brain microvascular endothelial cells (hBMEC) were treated with uranyl nitrate and irradiated 1-2h post-exposure at 6Gy or 8Gy (dose rate: 0.513 Gy/min). U-exposed cells were treated with 60µM ATA 1-2h post-irradiation.
- **Cytotoxicity Assays:** Media was taken from experimental samples at 24h or 48h post-treatment and plated in replicate. Cytotoxicity was determined using CytoTox96© (Promega).
- **Oxidative Stress Determination:** Media was taken from experimental samples at 48h post-treatment and plated in replicate. Oxidative stress was determined by In Vitro ROS/RNS Assay (Cell Biolabs).
- **Cytokine Array:** Media was taken from experimental samples at 48h post-treatment. Cytokine markers were detected using a human inflammation array (RayBiotech).
- **Western Blot:** Cells were collected 48h post-treatment and lysed to isolate protein. Protein concentrations were determined by Micro BCA Protein Assay (ThermoFisher). Samples were loaded at 20µg/well on 4-15% polyacrylamide gels (Bio-Rad) and run. After run completion, gels were transferred to nitrocellulose membrane (Bio-Rad) and probed with antibodies of interest.
- **Transendothelial Electrical Resistance Measurements (TEER):** Cells were seeded in transparent membrane cell culture inserts (Falcon). After 24h, the inserts were treated with U and/or ATA and irradiated as described above. TEER was measured before treatment and/or irradiation and at 24h, 48h, and 72h.

RESULTS

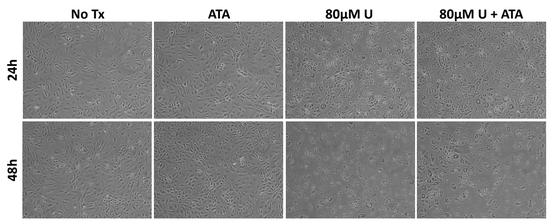


Figure 1. ATA mitigates U toxicity in hBMEC.
Inspection by microscopy at 10x magnification revealed U-treatment causes stress in the hBMEC monolayer by altering cell morphology. At 48h post-treatment, the monolayer was disrupted when exposed to U alone. ATA treatment helped mitigate the monolayer disruption, although its effect on cellular stress was negligible.

CELLULAR DAMAGE

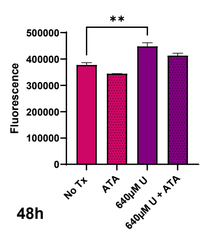


Figure 2. U increases oxidative stress.
hBMEC exposed to 640µM U showed increased reactive oxygen species (ROS) while ATA treatment appeared to decrease ROS.

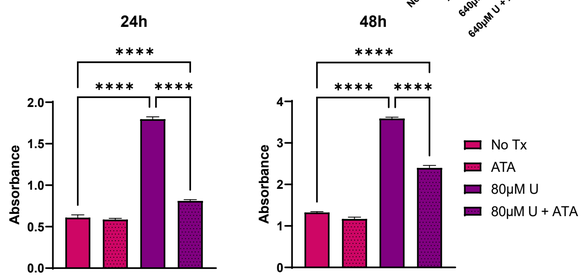
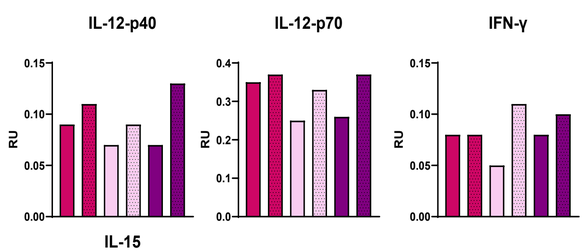


Figure 3. ATA decreases cytotoxicity caused by U in hBMEC.
hBMEC exposed to 80µM U showed increased cytotoxicity as measured by lactate dehydrogenase (LDH) release at both 24h and 48h, but ATA treatment mitigated these effects.

BIOMARKERS



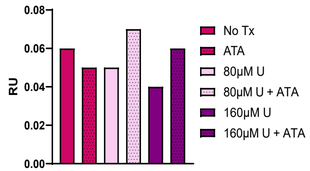


Figure 4. ATA modulates cytokine expression.
Media samples taken from hBMEC treated with U and/or ATA revealed that ATA affects human inflammation markers. When used to treat U-exposed hBMEC, ATA also improves cytokine expression to non-treatment levels.

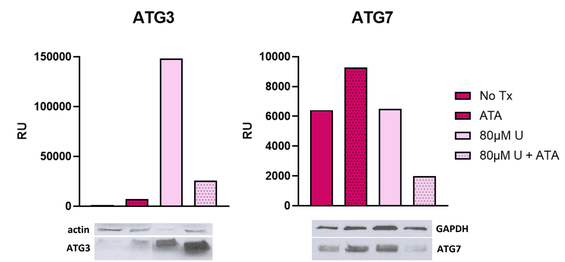


Figure 5. ATA reverts the cellular death pathway triggered by U in hBMEC.
ATG3 and ATG7 are major components of the autophagy pathway. U increases expression of ATG3 while treating U-exposed hBMEC with ATA significantly lowers it. Adding ATA to U-exposed hBMEC also lowers expression of ATG7.

BARRIER INTEGRITY

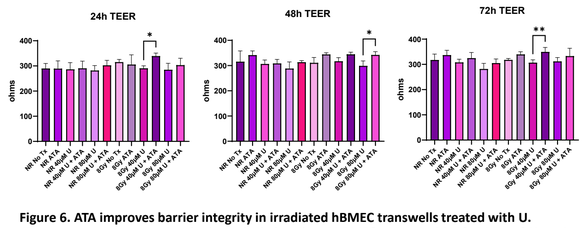


Figure 6. ATA improves barrier integrity in irradiated hBMEC transwells treated with U.
ATA treatment preserves barrier integrity in irradiated hBMEC treated with 40µM U at 24h and 72h post-exposure. At 48h post-exposure, ATA preserves barrier integrity in irradiated hBMEC treated with 80µM U.

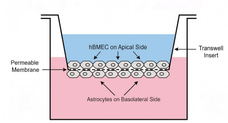


Figure 7. Diagram of Transwell Co-Culture.
A contacting transwell co-culture was used to model the BBB. hBMEC was plated on the apical side of the transwell insert and astrocytes on the basolateral side in their respective media.

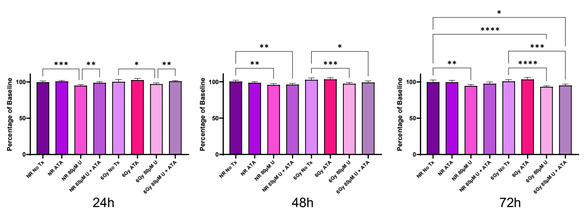


Figure 8. ATA improved barrier integrity in hBMEC/astrocyte transwell co-cultures at 24h.
U decreased barrier integrity compared to baseline at all timepoints in both non-irradiated and irradiated co-cultures. At 24h, ATA treatment showed maintained barrier integrity in both non-irradiated and irradiated co-cultures, but this protective effect was lost at 48h and 72h.

CONCLUSION

U is cytotoxic to hBMEC, a critical component of the BBB. It negatively impacts hBMEC barrier integrity, an effect exacerbated when combined with IR. ATA mitigates this toxicity, preserves barrier integrity, and modifies the cellular death pathway triggered by U. These findings suggest that ATA is a promising decorporation agent for U, supporting progression to *in vivo* studies.

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DISCLAIMER

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