Investigating the effects of uranium exposure on the DNA damage response and the inequitable exposure of indigenous populations

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ABSTRACT

Uranium, a widely sought resource to produce nuclear energy, is a radioactive element that has been mined in open areas around the United States. Human exposure to uranium poses serious health risks, like cancer, organ function loss, and whole bodily debilitation. However, Native Americans are and have been greater exposed to uranium due to mines' presence on reservations, and thus these risks incline towards such demographics. The National Institute of Health highlights this environmentally unjust exposure as a public health crisis, as uranium exposure damages critical repair pathways needed to repair DNA damage, which can lead to genomic instability and carcinogenesis. This paper attempts to create both an understanding of the molecular-scale damage uranium causes in the form of damaging non-homologous end-joining and homologous recombination pathways, while also covering a broader perspective of how indigenous peoples of America face a serious risk of such damage from their heightened exposure. First, the increased exposure risk in native communities is discussed by covering articles from government surveys, communications, and studies that gauge the danger of uranium concentrations in the environmental systems of Native nations. In the DNA repair portion, studies focusing on damage to key repair proteins in the DNA repair pathway, like the loss of zinc in its respective motif on the PARP-1 protein, and the increase of ROS causing oxidative stress in cells, pointing to the previously mentioned instability, are covered, and their methods and findings are presented.

Introduction

Uranium is a radioactive element that fuels human endeavors in energy but can still be found naturally. Its biggest contributions lie in creating nuclear energy, a new source of energy that many have and continue to look to as an alternative to the primary source, fossil fuels. Uranium is a radioactive element that is a silver-white metal; its isotope Uranium-238 is the most common naturally-occurring isotope of the primary three: uranium-238, uranium-235, and uranium-234.[1] Its weak radioactivity lends to background radiation, which can be observed and measured in the environment.[1] The decay of uranium leads to the creation of various decay products that are also radionuclides.[2] Unlike uranium-238 with a half-life of about 7 billion years, uranium-234 and uranium-235 have much shorter half-lives, increasing the frequency of the release of radiation in the form of alpha and gamma rays, extremely strong forms of such.[1,2]

In a societal context, uranium's primary use lies in producing nuclear energy. Because modern nuclear reactors require a greater concentration of uranium-235 than what naturally occurs, a process known as "enrichment" increases the amount of uranium-235 in a sample via three different processes: gaseous diffusion, gas centrifuge, and laser separation.[3] Uranium-235 produced from such enrichment processes is then used as fuel for nuclear power plants or nuclear reactors for armed ships and weapons.[4]

As uranium is naturally found in the form of ore, there exist several mining methods used to extract the resource. Open-pit mining involves the removal of surface rock in order to access the uranium-containing ore underneath it.[5] Underground mining involves creating a pit in which workers enter to extract ore manually.[5] However, underground mining poses a threat to local aquifers and other water supplies.[5] Finally, in-situ leaching is a mining process where chemicals, mainly carbonates and oxidants, are poured into groundwater, which passes over ore and subsequently dissolves the uranium into the solution.[6]

In all existing methods, uranium waste is inevitable. Milling processes have byproducts, or "tailings" and "raffinates," that are left behind in ponds or contained facilities.[6] In open-pit mining as well, the "waste rock" that was moved aside originally, as Scholle explains, is not only financially inefficient to utilize, but when exposed to the air, is threatening to the environment as well.[5] Exposure to such hazardous byproducts and waste rock occurs in the form of it being used as materials in buildings, dust in the air, or contaminated water supply from leaked waste ponds.[6] External or topical exposure to uranium does not affect the body's function as alpha particles cannot penetrate the

skin; however, ingestion or inhalation can lead to extreme health problems.[4] Internal uranium exposure heightens the risk of cancer, as ingestion can lead to bone and liver cancers, while inhalation leads to lung cancer.[4] While carcinogenesis from radioactive exposure is more cumulative, immediate chemical exposure poses damage to the kidneys and is linked to various renal diseases.[7]

On a molecular level, chemical uranium exposure has been found to suppress the repair pathways of double-strand breaks (DSBs) in DNA, leading to cell death and genomic instability.[8] Through ionizing radiation, the homologous recombination (HR) and the non-homologous end-joining (NHEJ) repair pathways are impeded due to the damage to critical repair proteins involved in the processes.[8] However, not only are the repair pathways damaged, but the introduction of uranyl ions correlates to a higher incidence of DSBs as well.[8] The consequent release of hydrogen peroxide introduces the loss of function to component proteins of DNA damage repair.[9]

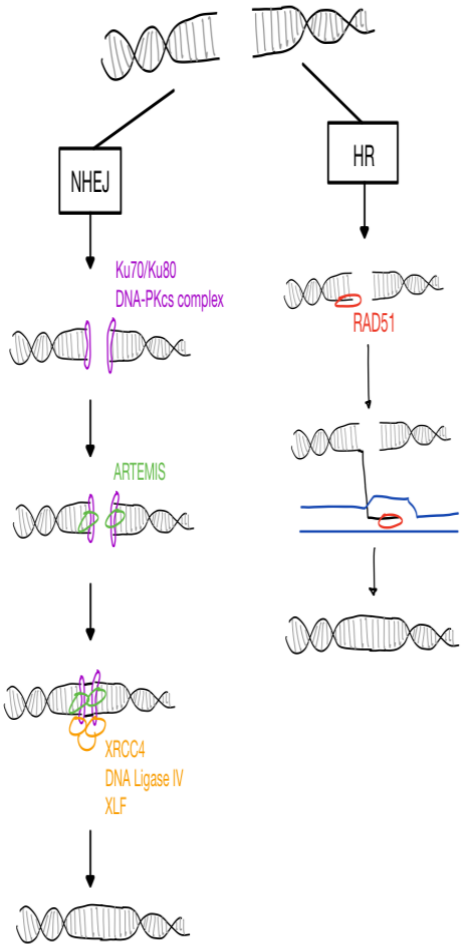

Figure 1. The NHEJ and HR pathways both end in thorough repair of DSBs but utilize different proteins and molecular structures.

A double-strand break (DSB) is extreme damage to a DNA strand, where both strands of dual-stranded DNA are split at a certain point, which consequents genomic instability and cell apoptosis.[10] Two key repair pathways used to repair DSB are non-homologous end-joining (NHEJ) and homologous recombination (HR), helping avoid the fatal result of DSB.[11] The NHEJ pathways offer much more flexibility as its component enzymes are multifunctional and highly versatile in the types of DSBs. In the presence of a DSB, the Ku heterodimer, consisting of the Ku70/80 subunits acts as a "toolkit protein" where other ligases, polymerases, and nucleases can be easily attached.[11] Next, ARTEMIS, an end processing protein, is recruited on either end in order to slice off any DNA overhangs in disrepair.[11,12] However, this makes NHEJ a highly mutagenic process as the broken strand undergoes the removal of a number of codons by the Artemis:DNA-PKcs nuclease complex.[11] After ARTEMIS has trimmed the ends appropriately, the DNA-PKcs complex recruits the X-ray cross-complementing protein 4 (XRCC4) and DNA Ligase IV, a flexible ligating protein, and the XRCC4-like factor (XLF), thus fixing the breakage.[12] In general, the NHEJ pathway is called on in the G1/G2 phases and is preferred in these stages because of its flexibility and speed.

The homologous recombination (HR) pathway occurs in the S phase and, unlike NHEJ, is considerably more accurate and less mutagenic. The HR pathway is conceptually divided into three main stages: pre-synapsis, synapsis, and post-synapsis.[13] Here, a single strand of DNA in the DSB is bounded by replication protein A (RPA).[13] Because RPA has high specificity and affinity towards ssDNA, the numerous RAD51 proteins that follow recognize the strand to surround.[13] Then the ssDNA tail is guided by the RAD51 proteins to invade a duplicate strand of DNA in a homologous chromosome; then, the copying of DNA from the chromosome to the donor DNA.[14] The replication of the DNA induces a double Holliday junction, which results in the exchange of the interacted sects of DNA between the ssDNA strand and the template chromosome.[14]

Ultimately, the inhibition of such repair pathways from the introduction of uranium has detrimental consequences on human health, as such double-strand breaks, if not caught and repaired by NHEJ and HR, can allow deletions, translocations, and other unintended effects on the DNA to go unnoticed. Ultimately, the inhibition of solutions leads to the growth of tumors, cell death, and overall genomic instability. As ionizing radiation is a known factor in the development of DSBs, the body will have to face a surge of DSBs from the radioactivity of uranium, atop of not having the solutions to prevent such invasive damage. This ultimately obscures the vitality of the person's health, as their defenses to cancer and organ damage are dissolved.

Although the entire U.S. population has some degree of exposure to uranium via the water supply, infrastructure, or other previously mentioned products, Native American communities have strikingly greater exposure to uranium due to contaminated water supplies, proximity to abandoned mines, and proximity to waste rock.[15] One of the co-authors of the paper "Uranium Exposure in American Indian Communities: Health, Policy, and the Way

Forward," a member of the Navajo people herself, discusses the severity and breadth of this health crisis: "Flint was the red flag for toxic metals in urban drinking water, but if you look in Indian Country, there are Flints everywhere."[15] Knowing that the effects of uranium exposure are critically threatening to the body due to carcinogenesis and other life-threatening diseases, this literature review attempts to garner to synthesize a multi-faceted understanding of the public health threat to indigenous communities by combining a compilation of previous analyses on health disparities surrounding uranium in Native American communities and a review of the molecular-level detriments of uranium in DNA damage repair. It is important, however, to clarify that there exists not much scholarly literature or study on the heightened exposure of uranium in indigenous communities, and thus further study is duly recommended. Nonetheless, this literary review ultimately aims to address the question: How does exposure to uranium in humans inhibit the function of the DNA damage response of cells, and how does this greater affect indigenous populations in the United States of America?

Discussion

Heightened exposure in indigenous communities

A recent study analyzed particulate matter on tree bark on Native American lands to assess uranium mine-induced pollution in the area.[16] In prior environmental studies of uranium pollution, airborne pollution of uranium is often neglected or ignored in the study; thus this study analyzes specifically that pathway of exposure.[16] Researchers studied the Midnite Mine, an open pit mine, which was opened on the Spokane Indian Reservation in 1954, and continued to operate until 1981.[16] With the 2.4 million tons of ore and 33 million tons of waste rock left behind, the investigations of airborne particulate matter pollution were conducted using 31 Pinus ponderosa trees as a biomonitor for such.[16] Mass spectrometry (ICP-MS) conducted on the tree bark revealed that uranium was the most abundant trace metal element present up to 232ppb.[16] This concentration, gauged to be a high level from pertinent indices, proves that the presence of open-pit raffinates and waste rock did leak uranium into adjacent ecosystems to a degree of concern.[16] High levels of concentration of uranium within the living system on native reservations are pointed directly to the presence of mines on reservations, where later illnesses among the population can be correlated.[16]

Another cross-sectional study across 11 years gauges the level of environmental injustice regarding community water systems on native land and their exposure to uranium.[17] Using the EPA's data on concentrations of uranium, along with other metals, from 2000 to 2011, researchers analyzed levels of uranium from community water systems (CWS) across the United States to detect any sociodemographic inequities that exist.[17] Findings suggest that levels of uranium were at some of their highest in CWSs that utilized groundwater, and in the Central Midwest, both factors being included in native reservations, along with Hispanic and semi-urban communities.[17] This indicates environmental injustices in the form of water-borne uranium exposure occur for native reservations, whose reservations' locations fall under using wells (as they use groundwater) and the Central Midwest.[17]

One study in 2020 focuses more specifically on the largest example of environmental injustice, the Navajo nation.[18] As a significant amount of Navajo land has been used for hard rock mining, uranium exposure, along with other potentially toxic metals like arsenic, poses a great risk whose ripples can already be detected in the health of the population.[18] Done hand-in-hand with the Navajo Tribal Utility Authority, water samples were collected between 2003 and 2018 from various sources in the Navajo nation for analysis.[18] Using inductively-conducted plasma-mass spectrometry, the levels of metal ions were recorded.[18] Out of the 21 elements analyzed across 294 samples, uranium was one of six elements that recorded the most number of levels that exceed the national regulatory limit, posing a serious public health concern for the communities.[18]

Adverse effects of uranium exposure on DNA repair

A 2016 study illuminated the cytotoxicity of uranium via its damage to zinc finger motifs on target DNA repair proteins.[9] Cooper *et al*. tested whether uranium further emphasizes DNA damage by introducing uranyl acetate (which contains 99% uranium-238) in tandem with hydrogen peroxide or solar-simulated UV radiation (ssUVR), both known to be cytotoxic.[9] The experiment used embryonic kidney cells and neonatal epidermal keratinocytes that were cultivated for 24 hours, followed by uranyl being introduced and incubated for 24 hours.[9] DNA damage was assessed by using markers and pyrimidine dimers acting as immunofluorescence.[9] After the introduction of uranyl acetate to cells, poly(ADP-ribose) polymerase-1, the protein that detects DNA damage and signals for repair, was activated by the introduction of hydrogen peroxide.[9] Afterward, cell viability was measured by PrestoBlue Reagent, and viability

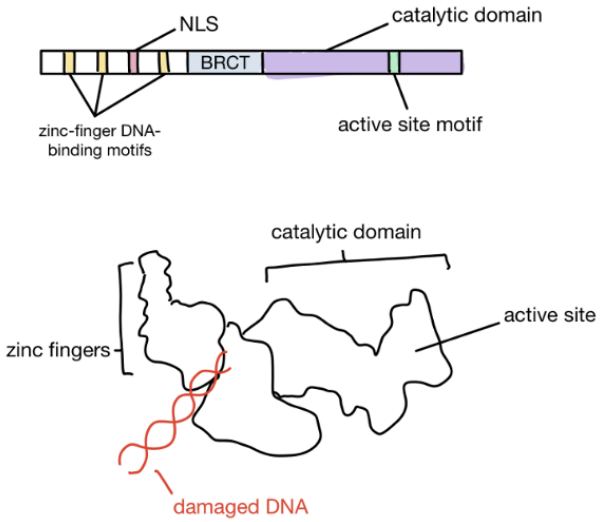

Figure 2. Organizational model of the PARP-1 protein. There exist three zinc-finger motifs that bind to DNA on the protein, and where such motifs are placed in reference to the damaged DNA entering is shown.

levels with UA suggest that UA does in fact impede DNA repair. Compared to cells treated with only hydrogen peroxide, cells that were also given UA presented more DNA damage, which researchers interpreted as the lessened capability of the repair pathways.[9] With ssUVR, staining levels in the control (no UA) were maintained after time unlike those with UA, whose stainings were 3.55-fold more than before UA's addition. Further analysis was conducted on how UA specifically attacks the zinc finger function of the DNA repair proteins, as the key detector protein PARP-1 is a zinc finger protein.[9] The C3H1 zinc motif is what PARP-1 uses to bind to damaged DNA and activate further enzymatic processes.[9] UA had a concentration-dependent inhibitory effect on PARP-1 activation, where its inhibitory concentration was 4.95 μM.[9] Upon further analysis, UA was found to cause concentration-dependent loss of zinc on the PARP-1 protein.[9] A 2007 study supplements the pattern of zinc loss on zinc finger proteins by uranium, as they reported that the DNA binding capacity of the C_2H_2 zinc finger protein specificity protein 1 (Sp1) was also hampered by UA.[19]

Another study conducted in 2021 contributes further to the study of how the introduction of uranium-238 directly binds to the PARP-1 protein in neonatal epidermal keratinocytes.[20] The researchers focused on the toxicological effects of uranium as a metal in understanding how it replaces zinc in the zinc finger motif of the protein.[20] Neonatal epidermal keratinocytes were treated with UA and zinc for 24 hours, then cells were lysed and the PARP-1 proteins of such were isolated via immunoprecipitation using primary antibodies at a 1:100 dilution ratio.[20] The isolated peptides were then given a double-stranded DNA probe sequence under the EpiQuik™ General Protein-DNA Binding Assay Kit (EpiGentek) to gauge the peptides' binding activity.[20] Results indicated that as a higher amount of UA was introduced, more PARP-1 proteins of that sample were bound to uranium instead of zinc, demonstrating a direct binding effect of uranium on the PARP-1 protein.[20] Evidential to the suppressive effects of uranium as replacing zinc on the peptide, zinc content was seen to have decreased as more UA was introduced in HEKn cells.[20] The loss of zinc is critically threatening to the maintenance of the zinc finger motif, whose structure is essential to recognizing and binding with DNA in the DNA damage response.[20] Additionally, while the binding to zinc on the motif maintains the peptide's tertiary structure, the binding to uranium loosens the tertiary structure of PARP-1.[20] A decreased ability to bind to the DNA probe sequence was recognized in the study as uranium presence heightened.[20]

Another study done by Jin *et al*. in 2017 focuses on the decreased efficacy of the homologous recombination pathway on DSB repair, although the uranyl ion was also observed to affect NHEJ as well.[8] Researchers introduced various concentrations of uranyl nitrate in samples of human bronchial epithelial cells, and as the uranyl nitrate concentration increased, the colony-forming ability of the samples decreased, indicating uranium's harm to cells' ability to sustain and proliferate.[8] Starting at a concentration of 20 μM, cell apoptosis occurred with greater frequency, and with further analysis, ROS was increased in a dose-dependent manner as uranyl nitrate became higher concentrated.[8] To assess whether uranyl nitrate contributed to increased DSBs in nuclear genomic DNA, phosphorylated histone H2AX, a biomarker for DSBs, was measured after the introduction of uranyl nitrate.[8] Researchers observed an increased amount of phosphorylated H2AX in the bronchial epithelial cells after any concentration of uranyl nitrate was added.[8] As further analysis into which mechanisms of the DNA repair pathways were impeded to bring about more DSBs, the study observed that critical repair proteins ATM, BRCA1, and EXO1 of the HR pathway were decreased when uranyl nitrate was introduced, while the DNA-PKcs and 53BP1 proteins, major components of the NHEJ repair pathway, were decreased at doses of 10 μM or higher (although doses of 5 μM of lower actually increased the number of such).[8] However, the expression of Ku70 and Ku80 proteins, which become part of the DNA-PK complex, was not affected by any used concentration of the uranyl compound.[8] Ultimately, the study concludes that uranium compounds are genotoxic in that they induce genomic instability by damaging the DSB repair pathways of NHEJ and HR.[8]

Conclusion

Findings accumulated from the aforementioned scholarly studies highlight how uranium exposure in the body poses cytotoxic harm by either denaturing or blocking the function of critical repair proteins in both the NHEJ and HR pathways, exemplified by the replacement of zinc with uranium on the PARP-1 protein.[9,19,20] Findings also demonstrate that not only does uranium impede existent DNA repair processes, but it actually causes new damage to occur, as observed by the higher number of DSBs detected by the histone biomarker H2AX.[8] Sociologically, environmental surveys that detail a heightened level of uranium in the tree bark of adjacent native land, a biomarker for pollution, and water quality results that show unhealthy levels of uranium in what America's native people drink regularly, reveals how the native populations of America are victims to such critical harm, that potentially results in cancer, to a greater degree than the rest of America.[16,17,18] Such a public health crisis not only requires further study to understand how DNA repair pathway damage has appeared in the form of certain diseases in indigenous nations, but with equal emphasis, focus on cleanup strategies that can remove waste rock, polluted waste ponds, and treat the groundwater to eliminate uranium exposure in native nations to some degree, which can lessen the chance for further damage down the line.

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References

1. *Radionuclide Basics: Uranium.* (2015, April 15). US EPA. https://www.epa.gov/radiation/radionuclide-basics-uranium

2. *Radioactivity and Radiation*. (n.d.). Depleted UF6 Management Information Network. Retrieved July 19, 2022, from https://web.evs.anl.gov/uranium/guide/ucompound/rad/index.cfm

3. *Uranium Enrichment*. (2020, December 2). NRC Web. https://www.nrc.gov/materials/fuel-cycle-fac/ur-enrichment.html#diffusion

4. *Radioisotope Brief: Uranium*. (2022, January 25). CDC Radiation Emergencies. https://www.cdc.gov/nceh/radiation/emergencies/isotopes/uranium.htm

5. Ulmer-Scholle, D. S. (n.d.). *Uranium: How is it Mined?* New Mexico Bureau of Geology & Mineral Resources. Retrieved July 20, 2022, from https://geoinfo.nmt.edu/resources/uranium/mining.html

6. *Radioactive Waste From Uranium Mining and Milling*. (2018, November 28). US EPA. https://www.epa.gov/radtown/radioactive-waste-uranium-mining-and-milling

7. *Uranium Health Effects*. (n.d.). Depleted UF6 Management Program Information Network; Argonne National Laboratory. Retrieved July 20, 2022, from https://web.evs.anl.gov/uranium/guide/ucompound/health/index.cfm

8. Jin, F., Ma, T., Guan, H., Yang, Z.-H., Liu, X.-D., Wang, Y., Jiang, Y.-G., & Zhou, P.-K. (2017). Inhibitory effect of uranyl nitrate on DNA double-strand break repair by depression of a set of proteins in the homologous recombination pathway. *Toxicology Research*, *6*(5). https://doi.org/10.1039/c7tx00125h

9. Cooper, K. L., Dashner, E. J., Tsosie, R., Cho, Y. M., Lewis, J., & Hudson, L. G. (2016). Inhibition of poly(ADP-ribose)polymerase-1 and DNA repair by uranium. *Toxicology and Applied Pharmacology*, *291*, 13–20.

https://doi.org/10.1016/j.taap.2015.11.017

10. Davis, A. J., & Chen, D. J. (2013). DNA double strand break repair via non-homologous end-joining. *Translational Cancer Research*, *2*(3). https://doi.org/10.3978/j.issn.2218-676X.2013.04.02

11. Lieber, M. R. (2010). The Mechanism of Double-Strand DNA Break Repair by the Nonhomologous DNA End Joining Pathway. *Annual Review of Biochemistry*, *79*. https://doi.org/10.1146/annurev.biochem.052308.093131

12. Ciccia, A., & Elledge, S. J. (2010). The DNA Damage Response: Making it safe to play with knives. *Molecular Cell*, *40*(2). https://doi.org/10.1016/j.molcel.2010.09.019

13. Li, & Heyer. (2008). Homologous recombination in DNA repair and DNA damage tolerance. *Cell Research*, *18*(1), 99–113. https://doi.org/10.1038/cr.2008.1

14. Sung, P., & Klein, H. (2006). Mechanism of homologous recombination: mediators and helicases take on regulatory functions. *Nature Reviews Molecular Cell Biology*, *7*(10), 739–750. https://doi.org/10.1038/nrm2008

15. Lyon-Colbert, A., Pino, M., Warne, D., Chischilly, A. M., & Redvers, N. (2021, March 26). *Uranium Exposure in American Indian Communities: Health, Policy, and the Way Forward*. Environmental Health Perspectives. https://ehp.niehs.nih.gov/doi/full/10.1289/EHP7537

16. Flett, L., McLeod, C. L., McCarty, J. L., Shaulis, B. J., Fain, J. J., & Krekeler, M. P. S. (2021). Monitoring uranium mine pollution on Native American lands: Insights from tree bark particulate matter on the Spokane Reservation, Washington, USA - PubMed. *Environmental Research*, *194*. https://doi.org/10.1016/j.envres.2020.110619

17. Ravalli, F., Yu, Y., Bostick, B. C., Chillrud, S. N., Schilling, K., Basu, A., Navas-Acien, A., & Nigra, A. E. (2022). Sociodemographic inequalities in uranium and other metals in community water systems across the USA, 2006-11: A cross-sectional study. *The Lancet. Planetary Health*, *6*(4), e320–e330. https://doi.org/10.1016/S2542-5196(22)00043-2

18. Ingram, J. C., Jones, L., Credo, J., & Rock, T. (2020). Uranium and arsenic unregulated water issues on Navajo lands. *Journal of Vacuum Science & Technology A: Vacuum, Surfaces, and Films*, *38*(3). https://doi.org/10.1116/1.5142283

19. Hartsock, W. J., Cohen, J. D., & Segal, D. J. (2007). Uranyl acetate as a direct inhibitor of DNA-binding proteins - PubMed. *Chemical Research in Toxicology*, *20*(5). https://doi.org/10.1021/tx600347k

20. Zhou, X., Xue, B., Medina, S., Burchiel, S. W., & Liu, K. J. (2021). Uranium directly interacts with the DNA repair protein poly (ADP-ribose) polymerase 1. *Toxicology and Applied Pharmacology*, *410*, 115360. https://doi.org/10.1016/j.taap.2020.115360