Effects of PARP inhibitors, SGLT2 inhibitors, and Colchicine on Doxorubicin-induced Premature Senescence Induced-Phenotype

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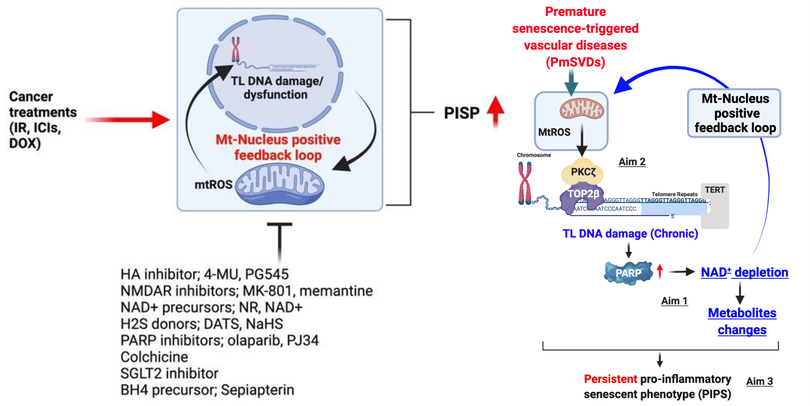
Introduction

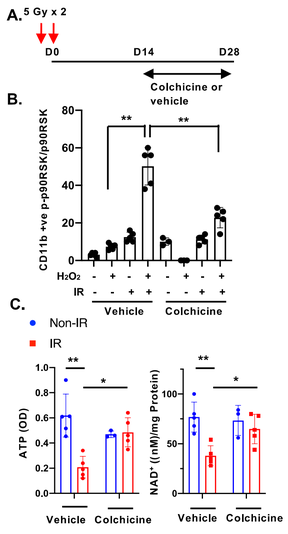
Post-radiation atherosclerosis and coronary artery disease (CAD), present as an increasing disease burden in cancer survivors who have received radiation therapy. This disease is categorized as a premature senescence-triggered vascular disease (PmSVD)[1].Endothelial dysfunction in PmSVD has been thoroughly studied, but the lack of available treatments to prevent cardiovascular complications should be remedied[2, 3].

Extracellular signal-regulated kinase 5 (ERK5/BMK1/MAPK7), has been shown to inhibit shear stress-induced anti-inflammatory effects in endothelial cells[4]. p90RSK activation is associated with endothelial cell inflammation and atherosclerosis. PCK-ζ is a redox-sensitive kinase that directly associates with TOP2β and inhibits its activity[5]. TOP2β protects against telomeric dysfunction. Additionally, Poly(ADP-ribose)/PARP synthetase activation-induced NAD+ depletion increases the Mt-Nucleus positive feedback loop.

The point of our experiment was to clarify whether premature senescence-induced phenotype (PISP) in post-chemotherapy cells could be inhibited by eliminating sources of mitochondrial damage with metabolite inhibitors (PARP inhibitor/Olaparib, SGLT2 inhibitor, and colchicine.)



Late phase of colchicine treatment inhibited monocyte priming, ATP/NAD+ depletion and inflammation after IR in vivo.

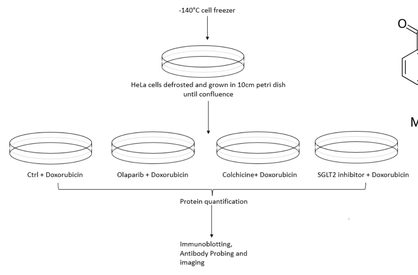


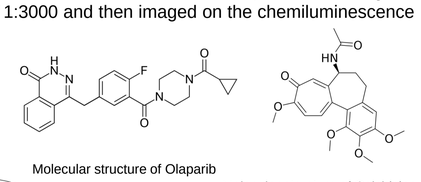
C57Bl6 mice were exposed to localized IR (5Gy x2) in the neck and thoracic area (5 Gy). A low dose of colchicine treatment was started after 2 weeks of IR, and continued for 2 weeks. Bone Marrow Derived Macrophages were isolated from the femurs and tibias of mice, which were not exposed to ionizing radiation. (B) p90RSK in circulating monocytes leads to prolonged inflammation and atherosclerosis. Activation leads to radiation-induced inflammation by establishing Mt-nucleus positive feedback loops containing mitochondrial reactive oxygen species production, telomere damage and NAD+ depletion. p90RSK activity in CD11b+ (mouse monocyte) cells was detected by flow cytometry. The data was obtained from total 5-6 mice. (C) ATP and NAD+ levels were detected from BMDMs isolated from mice treated as shown in (A). Significant long-lasting decrease of ATP and NAD+ levels in cultured BMDMs isolated from tibia and femurs, which were not directly exposed to ionizing radiation, even after 30 days of radiation.

Materials/Methods

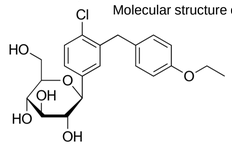
Cell Culture: Hela cells were cultivated until 80~100% confluency from frozen stocks in DMEM, penicillin-streptomycin, and 10% FBS. The cells were then passaged into 6-cm plates equally, and cultured until 90~100% confluence. The cells were pre-treated with Olaparib (10μM), Colchicine (10 nM), or the SGLT2 inhibitor dapagliflozin (10μM). 1 hour after pre-treatment, the cells were treated with Doxorubicin at 10 μM for 24 hours, and were then lysed with RIPA+ buffer with phosphatase inhibitors to collect protein. The protein lysates were spun down at 7000 RPM for 10 minutes at 4°C to isolate the protein lysate. The lysates were then transferred to a different Eppendorf tube and stored at -80°C until quantification.

Western Blotting: Protein quantified using the BCA Assay and BSA standards. The samples were loaded at 30ug/well and then run on a 10% SDS-PAGE gel. The blot was then transferred at 90V for 1 hour to a nitrocellulose membrane, blocked in 5% powdered milk in TBST, and then probed with antibodies to p-p90RSK, p-PKCζ, and p-ERK4-S496 at a concentration of 1:1000, 1:3000, and 1:1000 respectively. The blots were then washed for 30 minutes with TBST, incubated in goat-produced anti-mouse IgH, at a concentration of 1:3000 and then imaged on the chemiluminescence machine.




Molecular structure of Olaparib

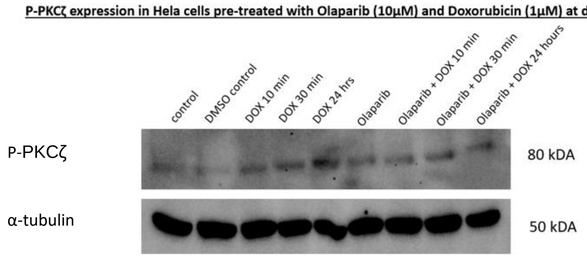
Molecular structure of Colchicine


Molecular structure of dapagliflozin

Histology: LDLR -/- mice were fed a high-fat diet for 2 weeks, exposed to localized IR (5gy) in the neck and thoracic area twice. Mice were then fed a normal diet until their weight recovered, and HFD was restarted for ~2 weeks. Thoracic aorta coarctation (TAC) was then performed at ~4 weeks later, echocardiography was performed and samples were harvested from mice after euthanization. Hematoxylin + eosin staining 4 weeks after TAC at different distances from the aortic valve to the tip of the heart were performed, as well as quantification of stenosis grades. Fractional shortening was also measured.

Results

P-PKCζ expression in Hela cells pre-treated with Olaparib (10μM) and Doxorubicin (1μM) at different time points



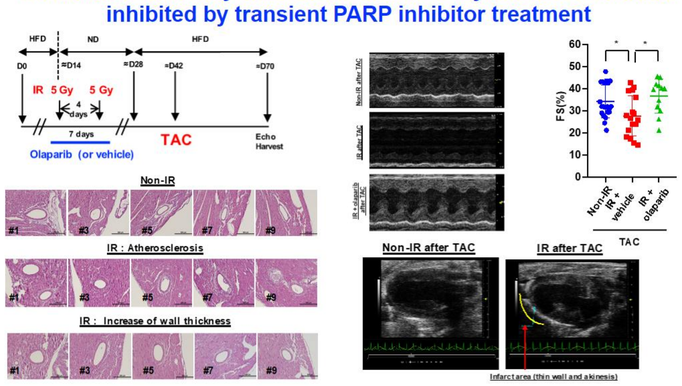
We are looking at the role of p-PKCζ in cancer cells. We wanted to see whether Olaparib (PARP inhibitor) would inhibit phosphorylation of PKCζ or increase it. After 24 hours of treatment with Doxorubicin after 1 hour of inhibitor treatment, we observed an increase PKCζ phosphorylation in Hela cells treated with doxorubicin, indicating the presence of chemotherapeutic mtDNA damage. The band of Hela cells treated with doxorubicin for 10 and 30 minutes shows slightly more phosphorylation at 30 min vs 10 min. The same trend is true in Hela cells treated with Doxorubicin after 10 and 30 minutes. However, Olaparib induction for 1 hour and doxorubicin treatment for 24 hours shows significant reduction in P-PKCζ, indicating that Olaparib inhibits phosphorylation of PKCζ. These results indicate that by Olaparib may be able to act prophylactically against chemotherapy-induced cardiotoxicity.

Discussion

Seen inhibition of phosphorylation of PKCζ in HeLa cells treated with doxorubicin after 1 hour of stimulation with Olaparib. This indicates that Olaparib has therapeutic potential in reducing post-chemotherapeutic atherosclerosis. This experiment must be repeated 2 more times and re-run with other inhibitors (SGLT2, Colchicine).

Role of Colchicine, SGLT2 inhibitors and Olaparib in inhibiting PISP

IR-induced coronary atherosclerosis and myocardial infarction inhibited by transient PARP inhibitor treatment

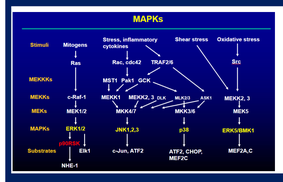


We have shown that PARP inhibitors can reduce IR-induced coronary atherosclerosis and myocardial infarction. Additionally, SGLT2 inhibitors such as dapagliflozin have been shown to improve cardiovascular outcomes such as heart failure, mortality, and metabolic outcomes in patients with type 2 diabetes (T2DM) at high cardiovascular risk [6, 7, 8]. This drug has also been shown to prevent high-fat, high-sucrose-induced pathological cardiac remodeling, improved contractile reserve and increased expression of oxidative phosphorylation and fatty acid metabolism independent of diabetes status in mice [9]. SGLT2 inhibitors have also been shown to attenuate inflammation and promote fat utilization as well as insulin resistance in obese mice by polarizing M2 macrophages[10]. Colchicine has anti-inflammatory and anti-coagulant properties, and the COLCOT (Colchicine CV outcome trial study), Colchicine reduced the risk of ischemic CV events[11].

Directions for future study/comments

We will test other inhibitors (HA inhibitors such as 4-MU, PG545, NMDAR inhibitors such as MK-801, memantine, NAD+ precursors such as NR, MAD+, H2S donors such as DATS, NaHS, BH4 precursors such as Sepiapterin) to see if they are protective against mitochondrial reactive oxygen species and telomeric DNA damage and dysfunction triggered by cancer treatments such as irradiation, cardiotoxic drugs such as doxorubicin, and immune checkpoint inhibitors to potentially find prophylactic treatments for post-chemotherapeutic atherosclerotic disease. These inhibitors may be able to inhibit the Mt-nucleus positive feedback loop and protect against mitochondrial damage.

Summary



Certain treatments for cancer (IR, ICI, and doxorubicin) cause comorbidities such as PmSVD. We want to try and find medications that will protect against these complications, such as agents that will protect against mitochondrial and inflammatory damage due to ROS.

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Acknowledgements

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