



A Review Potential Radioprotective Mechanism of Molecular Hydrogen via Regulation of Cellular Signaling Pathways

Md. Sadikuj JAMAN^a, Md. MANIRUZZAMAN^{a,b}, Md. Rokibul Hasan BHUIYAN^c

^a*Department of Cellular and Molecular Anatomy, Hamamatsu University School of Medicine, 1-20-1 Handayama, Chuo-ku, Hamamatsu 431-3192, Shizuoka, Japan*

^b*Department of Pharmacy, School of Science & Technology, Varendra University, Rajshahi-6204, Bangladesh*

^c*Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh*

*Corresponding author: sadik09bio.ru19@gmail.com

Abstract

Molecular hydrogen is a biologically significant molecule with diverse applications in the treatment and prevention of diseases in both human and animals. Exposure to radiation from medical procedures such as CT scans, X-rays and radiotherapy can disrupt normal cellular functions and cause abnormal cellular behavior. These radiation-induced effects are linked to multiple signaling pathways that contribute to cellular damage. Molecular hydrogen has emerged as a promising therapeutic agent capable of mitigating radiation related injuries and supporting the recovery of affected cellular pathways. A thorough literature search was carried out using databases including Google Scholar, PubMed, Scopus and ScienceDirect to obtain detailed information on radiation related cellular signaling pathways and the possible radioprotective roles of molecular hydrogen. We identified several cellular signaling pathways associated with radiation exposure. Among them are the cell cycle checkpoint pathway, DNA repair pathways, EGFR/tyrosine kinase mediated pro survival signaling, Rac1-mediated signaling, ERK1/2 signaling, PI3k/AKT signaling, NF-kB signaling, autophagy signaling, HIF-1 α signaling and YAP signaling pathways. Molecular hydrogen can be administered through various delivery methods and supports the recovery of many of these pathways. It also exhibits multiple beneficial effects in both humans and animals including antioxidant, anti-apoptotic, anti-inflammatory and gene regulatory properties. Molecular Hydrogen has emerged as one of the most promising radioprotective agents given that radiation exposure is a major contributor to carcinogenesis and cellular damage. The objective of this review is to elucidate the cellular signaling pathways influenced by radiation and to examine the potential radioprotective mechanisms of molecular hydrogen in mitigating radiation induced biological effects.

Keywords: Molecular hydrogen, radioactivity, antioxidant, cell signaling pathway, radioprotectant.

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Introduction

Molecular hydrogen (MH) a Nobel biological molecule is presently being studied extensively in vitro cell and animal investigations before being progressively used in clinical settings. (Jaman & Sayeed, 2018) Molecular formula for MH at normal temperature, MH is colorless, odorless, tasteless, nonmetallic and harmless gas. Because it is flammable and can burn in air at quantities ranging from 4% to 75% by volume. Its autoignition temperature about 500°C at 4% below concentration. MH is not as harmful in day to day living and its byproduct of several forms of anaerobic metabolism in a variety of bacteria, typically through processes that are catalyzed by enzymes called hydrogenases that contain iron or nickel. (Jaman & Sayeed, 2018) MH further processed by enzymes to supply electrons to the electrons transport chain which serves as an energy source. The reversible redox reaction between MH and its constituent two protons and two electrons are catalyzed by these enzymes. However, the water splitting reaction which breaks down into protons electrons and oxygen happens in all photosynthetic species. Certain species such as cyanobacteria and the alga *Chlamydomonas reinhardtii* have developed a second stage in dark processes wherein specialized hydrogenases in cyanobacteria or chloroplasts convert protons and electrons to produce H₂ gas. (Jaman et al., 2017) In addition to this, due to human cells lack hydrogenase genes MH was thought to act as an inert gas. (Bhuiyan et al., 2022) Consequently it was thought that MH did not operate in our cells at body temperature where MH really did not appear to react with any biological substances, including (O₂) when catalysts were not present. In fact, MH gas employed to measure local blood flow because of its properties. (Department of Biochemistry and Molecular Biology, University of Rajshahi-6205 et al., 2023) MH plays other roles in the majority of model animal tissues such as anti-inflammatory, antiapoptotic and antiallergic tomography (CT) and X-ray exams over the past ten years has resulted in proportional increases in patients' exposure to ionizing radiation increasing public awareness of its harmful consequences. (Jaman et al., 2024) Increased use of medical imaging is a significant contributor to radiation exposure and radiation-related disease even though the radiation dose associated with individual scans has significantly decreased. Leukemia and solid malignancies have been found to be more common in long term studies of second world war atomic bomb survivors in Japan or those who had substantial radiation exposure. Though undoubtedly less spectacular than an atomic explosion, imaging related radiation may present serious radiation related dangers according to the linear no threshold concept. It is well recognized that younger patients are more at risk from radiation exposure. (Regassa et al., 2024) The higher incidence of leukemia and solid tumors among young atomic bomb survivors as opposed to those who experienced the same radiation dose later in life serves as evidence of this fact that related to leukemia, breast cancer, thyroid cancer and other solid tumors in a dose dependent manner. The immediate and quantifiable harmful effects of ionizing radiation on cells include the production of single stranded DNA breaks (SSBs) double stranded DNA breaks (DSBs) and increase in reactive oxygen species (ROS). Figure 1 Provides a summary of this explanation. Numerous writers have suggested modulating the cellular damage linked to radiation exposure by employing a range of medications. For instance, it is hypothesized that substances that increase glutathione or antioxidants or molecular hydrogen itself may be able to lessen DNA damage, hence lowering the risk of cancer after radiation exposure.

(Jaman et al., 2023) Radioprotective chemicals are not commonly given to patients before or after medical despite several studies showing potential advantages for a range of drugs. In contrast, ionizing radiation finds extensive usage in cancer treatment, medical diagnostics and other industrial settings. Carcinogenesis, genetic alterations and the triggering of cellular death are recognized risks linked to ionizing radiation exposure in humans. Radiation exposure can harm cells directly as well as producing reactive oxygen species such as hydrogen peroxide, lipid hydroperoxide, superoxide, hydride and peroxy nitrite while ionizing radiation is absorbed by the tiny molecules mostly water that surround cellular bio-macromolecules, reactive oxygen species (ROS) are created. Proteins and DNA between cells are reacted with by this ROS. (Department of Biochemistry and Molecular Biology, University of Rajshahi-6205 et al., 2023) Oppositely, researchers reported using MH for medical purposes. They found that mice with UV induced squamous cell cancer showed a significant regression when they inhaled hyperbaric MH. But except from a few research MH hasn't been thoroughly investigated for use in medicine. In a rat model of cerebral infarction inhaling H₂ gas reduced ischemia-reperfusion damage. The chapter demonstrated that MH is a molecular hydrogen or antioxidant that specifically lowers highly oxidative reactive oxygen species (ROS) and reactive nitrogen species (RNS) including peroxy nitrite (ONOO-) and -OH. Correspondingly H₂ does not react with other ROS like hydrogen peroxide (H₂O₂) and superoxide anions (O₂⁻). However, groundbreaking study on H₂'s antioxidant properties, researchers found that rat liver damage from chemical oxidants was lessened when they consumed neutral H₂ rich water made via water electrolytes. Research on the medical uses of MH has expanded globally as a result of these studies. (Sharp, 1970) We recently shown that despite being inert MH is the sole antioxidant molecule with mitochondrial permeability and the capacity to diminish -OH scavengers may find use in medicine. There have been reports of MH's effectiveness against a number of illnesses and disease models. (Xue et al., 2014) it is crucial to employ a safer and more efficient radioprotective agent in clinical settings. Through many medications have undergone various evaluations. For example, numerous natural and synthetic substances have been studied for their radioprotective properties. Studies on animal have shown that cytokines like granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin (IL-1) and IL-12 as well as natural substances like vitamin C, vitamin D, vitamin E, melatonin, succinate, alpha lipoic acid and N-acetyl cysteine (NAC) have radioprotective properties. Although several medications are still being evaluated and many of them are far from being the best radioprotective medicines. Moreover, only radioprotective agent authorized for clinical use by the US FDA is amifostine (WR2721) which was created as a radioprotective agent with free radical scavenging capabilities such as -OH. However, due to dose dependent adverse effects such as hypotension, nausea and vomiting. Although this medicine has not been commonly regarded as a helpful radioprotective agent of choice. Therefore, the statement that there are no therapeutically applicable radioprotective agent of choice. Therefore, the statement that there are no therapeutically applicable radioprotective medicines with high efficacy and little adverse effects is not hyperbole. (Oharazawa et al., 2010) Furthermore, MH may be a clinically reliable radioprotective drug as it has been found to exhibit radioprotective effects in numerous animal experiments and to have no adverse effects in clinical trials. Regarding its radioprotective properties, MH rich water enhanced the quality of life (QOL) of patients with liver cancer undergoing radiation therapy in clinical studies. During the end stage of cancer patients undergoing IMRT, we have reported that inhaling MH gas decreased bone marrow damage without sacrificing the anticancer effects. The aim of this review to address the radioprotective effects of MH and explores its mechanisms in intracellular responses such as anti-inflammatory, anti-apoptotic and gene expression regulation or related signaling pathways, in addition to its role as an antioxidant and talk about molecular Hydrogen's potential as a new clinical useful radioprotective agent (N. Zhang et al., 2018).

Methodology

A through chapter of the literature search in PubMed, Web of Science, MEDLINE, Embase and Scopus, Science direct, google scholar and SCISEARCH databases was carried out using following keywords: ((molecular hydrogen, radioactivity, radioprotective effect, relation with cancer and radioactivity, signaling pathway associated with radioprotective effect, molecular hydrogen activity on human and animal cell, Molecular Hydrogen source, Activity of molecular hydrogen in different organ) About 16 molecular hydrogen, 20 research's radio activity 25 findings signaling associated with radioactivity and molecular hydrogen as effective agents have been listed. Many duplicate studies and non-English studies were eliminated from a total of 756 studies. The remaining titles and abstracts were checked and studies not relevant to the topics were excluded along with papers for which no full text could be accessed. The titles and abstracts of the remaining papers were examined and studies that were unrelated to the subject and publications for which there was no full text available were both eliminated. In total 62 studies matched our inclusion criteria and their findings are described in this chapter. The majority of them focused on molecular hydrogen activity on radioactive cell and radioprotective activity of molecular hydrogen on different cell signaling pathways which is strongly supported for leukemia, cancer, solid cancer patients happened by radioactivity and their treatment.

Results and Discussion

1. Discussion of radioactivity on biological system including human and animal cell study.

For long times, ionizing radiation has been closely linked to a number of harmful biological impacts. A wealth of information regarding the biological impacts of radiation has been produced and recorded since World War II. Ionizing radiation exposure can cause a variety of risks such as carcinogenesis, cellular death induction and genetic alterations. (Saifujjaman et al., 2018) Hematopoietic system is one of the organs most vulnerable to radiation. Cellular damage is caused by radiation while Skin, reproductive system and gastrointestinal (GI) system can be either direct or indirect. (Moulder & Cohen, 2005) When radiation energy is absorbed by vital target molecules such as proteins, DNA and other related compounds where it can directly harm those molecules. For instance, radiation energy can directly affect DNA increasing the likelihood that atoms within the DNA molecule will become ionized. (Ward, 1994) Since the DNA helix is only around 2 nm in diameter, we might imagine this process as a direct hit of the radiation on the DNA. Naturally, this is a very uncommon occurrence because of the minuscule size of the target. (Hozyen et al., 2019) According to research, for direct action to be successful radiation induced ionization must take place extremely close to the DNA molecule- within a few nanometers. However, impair a cells capacity for survival and reproduction in the extremely rare event that a DNA molecule or perhaps another biological component is directly damaged. (Jeggo & Löbrich, 2006) Radiation affects water and other organic molecules in indirect damage which leads to the production of free radicals. Because of their high reactivity these free radicals have the ability to structurally alter DNA molecules. Compared to direct harm, this type of damage occurs far more frequently. (Giardi et al., 2013) According to reports, a therapeutic dose of 2Gy/fraction of IR can result 3000 DNA damages per cell. Since water makes up the majority of cells and tissues, the majority of radiation energy will be absorbed by cellular water. After being exposed to ionizing radiation water undergoes a complicated set of chemical reactions known as water radiolysis. (McBride & Schaeue, 2020) These chemical reactions start with ionization and excitation process caused by radiation $\text{H}_2\text{O} + e^-$ (fast electrons) and H_2O^* (electronically excited molecules). $\text{H}_2\text{O} + \text{H}_2\text{O}^*$ are very unstable they decompose within 10-13 seconds and form -OH and H free radicals. Hydroxyl radical is a very reactive oxidizing agent because it contains an unpaired electron. It can interact with important target molecules to produce additional radicals and disperse a short distance. In the meantime, the ejected secondary electrons can mix with water molecules to create hydrogen atoms and hydroxyl ions. Alternatively, those electrons may lose energy through a series of reactions with the medium until they reach thermal energies in around 10-11 seconds. Those thermalized electrons can have dielectric interactions with nearby molecules,

which solvate them to form eqq- , i.e eqq- is a free electron surrounded by a layer of orientated water dipoles forming a solvent cavity. It can interact with a proton to produce a hydrogen atom. In oxygenated solutions, eqq- interacts with O_2 and forms O_2^- , which is also a strong oxidizing agent and the precursor of hydrogen peroxide: $\text{eqq-} + \text{O}_2 \rightarrow \text{O}_2^-$. The major water radicals eqq- , OH- and H are extremely reactive with DNA, lipids, cell molecules and other subcellular components. These water radicals are intimately associated with severe site-specific oxidative damage, impairment of cell function and even apoptosis. Particularly during or quickly (within 5 milliseconds) after irradiation researchers have been speculating about the predominant involvement of covalent changes generated by free radicals in the biological consequences of ionizing radiation. (Crompton, 1998) Hydroxyl radicals are the most reactive of the free radicals and the source of 60-70% of the cellular damage caused by ionizing radiation. (Hutchinson, 1985a) Thus one basis for the development of radioprotectants is the scavenging of hydroxyl radicals. This case happens both in animal and human cell.

2. Radioactivity study in vitro model with ionization method.

Researchers investigated in vitro if MH may lessen -OH caused by IR in live cells. A549 cells were exposed to radiation for this purpose and the cells were evaluated for green fluorescence produced by oxidized HPF in PBS. (Kato et al., 2021) Irradiated cells displayed elevated HPF signals in their cytoplasmic areas when placed in control N_2 -rich PBS. The study discovered that MH can significantly lower HPF signals when cell membranes have adequate permeability. Based on these findings, they proposed that MH can efficiently lower the cellular ROS produced by irradiation in living lung epithelial cells. In contrast, MH dramatically reduced amount of 8-OH-dG a biomarker for oxidative DNA damage and 4-HNE an end product of lipid peroxides in irradiated cells. They discovered via western blotting that pretreatment with MH dramatically decreased the amounts of apoptosis-related proteins including caspase3 and Bax. In A549 cells in 24 hours after radiation. Moreover, they proved that MH could successfully preserve cell viability. (Kalman et al., 2017) A study was created and examined in vitro to see whether hydrogen enriched PBS might be utilized as a successful radioprotectant. Following treatment with a corresponding volume of hydrogen enriched PBS, Intestinal crypt HIEC cells and human lymphocyte AHH-1 cells were exposed to corresponding doses of γ -ray radiation (Zhao et al., 2014) In comparison to cells treated with irradiation alone at all tested doses (up to 8Gy) they discovered that pre-treating HIEC cells with 0.1-0.4 mmol/L MH prior to γ -ray irradiation significantly increased cell survival. Simultaneously, pretreatment with MH significantly decreased HIEC cells LDH leakage. This result was consistent with cell viability analysis. Results from the HIEC cells pretreated with hydrogen enriched PBS demonstrated the protective impact of hydrogen, as the number of apoptotic cells decreased to 26.1% from 49.3% in the control group. Additionally, the hydrogen enriched PBS pretreatment groups level of enzymatic antioxidants (SOD) and non-enzymatic antioxidants (GSH) rose for AHH-1 cells that restore antioxidant state. (Kuo et al., 2024) These in vitro results demonstrated that MH therapy can scavenge ROS and guard against apoptotic damage associated with oxidative stress caused by infrared radiation. Restoring antioxidant status may also lessen the damage that radiation causes to cells by preventing free radicals from attacking macromolecules like DNA and membrane lipids. (H. Liu et al., 2023).

3. Brief study of all signaling pathways associated with radioactivity and ionization process in human/animal cell study and possible explanation of potential radioprotective with molecular hydrogen.

3.1. Radioactive effect on signaling pathways associated with cell cycle check point.

SSBs, DSBs, base and sugar damage and DNA-protein crosslinks are among the various types of DNA damage caused by radiation. (Park et al., 2017) Since, mis repaired and unrepaired DSBs cause genomic instability and cell death are mostly type of DNA damage. (Chaudhry, 2007) After

identifying DNA damage three check point G1/S/G2 block the advancement of cell cycle at the G1/S border, Intra S border, G2/M border. Apoptosis, senescence, autophagy cell death or necrosis may be triggered to eradicate the damage cells if cell cycle checkpoints are malfunctioning or DNA damage cannot be repaired. Thus, DNA repair in cancer cells is facilitated by a properly functioning cell cycle checkpoint increase cell survival to radioactivity. (Warmerdam & Kanaar, 2010) Radiation induce DNA damage, ATM, ATR kinase mediated signaling pathways are crucial for activating the cell cycle checkpoint response and DNA repair shown in (Figure 2). Rapid activation of ATM and ATR upon detection of DNA damage triggers activation of downstream pathways such as P53, DNA-PK, Chk1 and Chk2. (Serrano et al., 2013) chk1/2 kinases are activated and cell division control protein Cdc25 phosphatase is phosphorylated. Cdc25 to be subcellularly sequestered by 14-3-3, degraded and/or inhibited. Otherwise Cdk1/Cyclin B activity is activated to support the G2/M transition of cell cycle. (Molinari, 2000) Additionally, P53 tumor suppressor can be directly phosphorylated and activated by ATM, ATR and Chk1 and Chk2 kinases in response to radioactivity. (Siddiqui et al., 2005) As a result, ATM, ATR, Chk1 and Chk2 activate p53, p21 protein is markedly elevated. This halting progression inhibits activities of Cdk1/Cyclin A/B, Cdk4/Cyclin D and Cdk6/cyclin D complexes. (Salvador et al., 2013) Cdk kinases are necessary for advancement of cell cycle. Cdk4/6 in conjunction with cyclin D is necessary for G1/S transition of cell cycle whereas Cdk1 in conjunction with cyclin B is necessary for G2/M transition. (Stark & Taylor, 2004) p53 and p21 bind to and inhibit directly responsible for protecting G1 checkpoint. (Kawabe, 2004) The majority of cancer cells exhibit G1 checkpoint defects as a result of frequent mutations in G1 checkpoints regulators p53, cyclin D etc. Nonetheless the majority of cancer cells have a functional G2 checkpoint is driven by p53 independent process. Therefore, cancer cells that have G1 checkpoint deficiency may become more sensitive to radiation if their G2 checkpoint is abrogated. (Saldivar et al., 2018) Wee1 and Myt1 kinases inhibitory induced G2 checkpoint activation. (Uckun et al., 1992) Due to Cdk1-Y15 is located in the ATP-binding domain of cdk1, phosphorylation of this site inhibits Cdk1 function by preventing ATP from binding to Cdk1. Cdc25 dual specificity phosphatase catalyzes the dephosphorylation of Cdk1-Y15 which turn promotes Cdk1 activity. (Minemoto et al., 2001) When radiation causes G2/M cell cycle arrest Cdk1-Y15 phosphorylation is preserved. (Y. Wang et al., 2004) Few cofactors attract ATM, ATM and DNA-PK three important activators of DNA repair to sites of DNA damage. MRE11-RAD50-NBS1 complex includes NBS1 for ATM. (Baretic et al., 2019) ATRIP for ATR and Ku80 for DNA-PKcs (Schumann et al., 2007) Additionally cofactors needed for the assembly of DNA repair machinery at the locations of DNA damage will be recruited as a result of initiation stage. Biochemically, ATM initiates both NHEJ and DNA-PK initiates DSB repair through NHEJ. (Lu et al., 2005) DDR mechanisms also crosstalk and have functional redundancy. In the end, radiation induced activation of the three pathways leads to Cdk activity suppression which causes cell cycle arrest, giving time for DNA repair and cell survival.

3.2. DNA Repair Pathways in Radioactivity

Cancer cells quickly activate ATM, ATR and DNA-PK all of which belong to the phosphoinositide 3 kinase related kinase family in response to the DNA damage caused by IR. These kinases activate DNA repair by transducing DNA damage signaling and coordinating the building of DNA repair apparatuses at the damaged locations (Figure 2). NHEJ or HR are primarily responsible for repairing radiation-induced DSBs. (Piechocki et al., 2007) [28] Ku 70/80 heterodimer must first be recruited to each end of the damaged DNA in order for NHEJ to start. The complex that is created then causes DNA-PKcs to be recruited which causes the two ends of the DNA to be juxtaposed. To process the final ligation, the DNA ligase complex is further recruited by the Ku70/Ku80/DNA-PKcs complex. Unlike NHEJ, HR accurately and faithfully repairs DSBs by utilizing sequence information found in the intact sister chromatid. NHEJ can therefore operate throughout the cell cycle because it doesn't need a DNA template for the repair. Otherwise, HR mostly functions during S and G2 stages, following DNA replication when DNA template becomes available. (Groelly et al., 2023) SSBs are

also produced by radiation and are mostly brought on by base oxidation by ROS/RNS. Cell uses bases excision mending process to fix this kind of damage. DNA polymerase and DNA ligase will work together to fill in nicks created by DNA glycosylase mediated incision and apurinic endonuclease 1 (APE1) mediated incision, respectively remove the single and multiple damaged bases. (Braithwaite et al., 1999) Ultimately, cells are able to survive and restart cell cycle when damaged DNA generated by radiation is successfully repaired. Senescence, autophagy, necrosis or apoptosis while will occur if damaged DNA cannot be repaired (in Figure 3).

3.3. Epidermal Growth Factor Receptor (EGFR) Tyrosine Kinases Mediate Prosurvival Signaling Pathways in Response to Radioactivity

Cell external is, transmembrane region and intracellular region are all present in the transmembrane proteins that make up the EGFR/ErbB/HER family of RTKs (in Figure 3). EGFR/HER1, ErbB2/HER2, ErbB3/HER3 and ErbB4/HER4 are four members of family (in Figure 3). Tyrosine kinase domain and phosphorylation regulatory tail are found in the intracellular area whereas the ligand binding and dimerization domains are found in the extracellular region. ErbB3/HER3 has very little enzymatic activity while ErbB2/HER2 does not bind to any ligand. Because of this ligand can only attach to EGFR/HER or ErbB4/HER4 which promotes homo or hetero dimerization of RTK receptors and subsequent transphosphorylation of receptors c-terminal regulatory tail (Figure 3). (Brunello, 1995) Then phosphorylated tyrosine residues create docking sites for downstream adaptors and signal transduces which activates downstream signaling pathways like JAK/STAT, phospholipase C- α /protein kinase C, PI3K/AKT signaling. (Kunte et al., 2020) Among these RAS/RAF/MEK/ERK and PI3K/AKT singling are important for enhancing cell survival after radiation (Figure 3). (Jit et al., 2021) According to reports, radiation causes phosphorylation of EGFR, HER1 that is activated. (Schmidt-Ullrich et al., 1997) Our research using human breast cancer cells shows that radiation triggers phosphorylation of HER2, HER3, HER 4 in addition to HER1. (Piechocki et al., 2007) Suppression of PTPs that dephosphorylated HER/RTKs may be the mechanism behind this impact of radiation. However, it has not been determined. This idea is supported by the fact that ROS/RNS can effectively deactivate receptor PTPs by oxidizing their enzymatic active sites such as those include cysteine residue. (Kemble & Sun, 2009) It has been demonstrated that radiation causes ROS/RNS generation through mechanism that is dependent on mitochondria. Therefore, PTPs may be inhibited radiation induced ROS/RNS which would then activate HER/RTKs. (Halliwell, 1999) Inducing prosurvival AKT and ERK1/2 signaling pathways (Figure 3) and facilitating the cell cycle checkpoint response to promote DNA repair are the two likely mechanisms by which ErbB/HER RTKs contributed to the survival of cancer cells in response to radiation. HER2 activation is required for the induction of G2/M checkpoint in breast cancer after radiation while studies have shown that EGFR/HER1 plays a role in promoting DNA-PK activation which is necessary for starting DSB repair by NHEJ (Goldberg & Kloor, 2006).

3.4. Ras-Related C3 Botulinum Toxin Substrate 1 (Rac1) Mediated Signaling Pathways in Radioactivity

Rac1 is a member of Rho family of GTPases which are essential for cell migration, polarity and cytoskeleton rearrangement. (Marei & Malliri, 2017) Rac1 is inert and activate in its GTP-bound from just like all other GTPases. Rac1's GEFs enhance conversion of GDP to GTP and its GAPs encourage the hydrolysis of GTP. Upon activation Rac1 transduces numerous downstream signaling pathways. (Kempska et al., 2023) Rac1 can trigger activation of ERK1/2 driven signaling that supports cell survival and proliferation by means of its downstream effector PAK1/2 kinases which phosphorylate/activate Raf1 and MEK1 kinases. (Coles & Shaw, 2002) Similarly, response to different stimuli, Rac1 can activate the AKT signaling pathway through PI3K which is important for cell survival (Singleton et al., 2005) After being exposed to radiation AKT and ERK1/2 both

signaling increase survival. (Gao et al., 2018) According to our research, Rac1 helps breast and pancreatic cancer cells survive when exposed to infrared radiation. According to our findings radiation causes a rapid activation of Rac1 activity which is necessary for radiation induced ATM/ATR signaling that result in activation of G2 checkpoints and cell survival. Our research further demonstrates that elevation of Bcl-xL anti-apoptotic protein expression in RT resistant cells and resistance of breast cancer cells to therapeutic protocol of hyperfractionated radiation treatment depend on Rac1 activity. (Fukuda et al., 2005) Studies conducted by others consistently demonstrate that a lack of Rac1 function reduces the responsiveness of cells to radiation and UV radiation of DNA repair, cell cycle checkpoint response and cell survival. (Deshmukh et al., 2017).

3.5. Radio-Activated ERK1/2 Signaling Pathway

Evidence points to at least four processes that contribute to biological event of ERK1/2 signaling activation in response to radiation which is frequently seen in cancer cells. ErbB/HER receptors are activated by radiation in the first method. As previously mentioned, we and others have shown that ErbB/HER RTKs play a crucial role in stimulation of ERK1/2 signaling in response to radiation in breast and lung cancer cells (in Figure 3). (Piechocki et al., 2007) Furthermore, EGFR - ligand synthesis is triggered by Ras activation via ErbB/HER receptors creating an autocrine feedback loop that can further strengthen the Ras/Raf/MEK/ERK signaling cascade. (Song et al., 2007) In line with result, radiation induced ERK1/2 signaling activation is reversed by ectopic production of Ras-N17 dominant negative mutant which suppresses native Ras GTPase. (De Luca et al., 2012). BRCA1 tumor suppressor is involved in the second mechanism. Radiation induced activation of ERK1/2 signaling breast cancer cells requires expression of BRCA1 protein and protein stability of BRCA in the irradiated breast cancer cells is supported by ERK1/2 activity. Inhibition of BRCA1 and ERK1/2 breast cancer cells eliminates G2/M cell cycle arrest and causes a concurrent induction of apoptosis; these findings point to a positive feedback loop regulation between ERK1/2 signaling BRCA1 protein stability in response to radiation which may be crucial in maintaining G2/M cell cycle checkpoint response after radiation. (Mirzoeva et al., 2009) ATM kinase is involved in the third mechanism. It has been demonstrated that blocking ATM partially prevents ERK1/2 signaling from being activated after radiation. Similarly blocking ERK1/2 reduces ATM phosphorylation and recruitment of ATM to DNA damage foci. (Goodarzi et al., 2004) Illustrates the radiation responses second positive feedback loop, this time including ERK1/2 and ATM signaling. Signaling between Rac1 and GTPase is involved in the fourth mechanism. As were previously mentioned radiation causes a quick activation of Rac1 which in turn triggers Raf/MEK/ERK signaling pathway through its downstream effector PAK1/2 kinases. (Kempska et al., 2023) Activation of several transcription factors by ERK1/2 signaling raises expression of genes that code anti-apoptotic proteins. (Munshi & Ramesh, 2013) P90rsk a substrate of ERK1/2 induces two most well-known anti apoptotic transcription factors that are activated by ERK1/2 in response radiation. CREB, C/EBP- β , Bcl -xL, Mcl-1 and c-FLICE are among anti apoptotic proteins that are induced by activated CREB and C/EBP- β . (Rodeck et al., 1997) Additionally, ERK1/2 directly suppresses a number of pro apoptotic proteins such as caspase 9, Bim and Bad. (Choi et al., 2004) In response of radiation, it has been demonstrated that ERK1/2 activation facilitates DNA repair. It has shown that ERK1/2 signaling plays a crucial role in activating the G2/M DNA damage checkpoint. This includes ERK1/2 activity in activating ATR and BRCA1 two important regulators of G2 checkpoint response and DNA repair. (Palayoor et al., 1995) Moreover, transcriptional up-regulation of genes involved DNA repairs including X-ray repair cross-complementing group 1 (XRCC), XPC and ERCC has also been connected to radiation induced ERK1/2 signaling. (W. Li & Melton, 2012) Additionally, ERK1/2 stimulates PARP-1 which is necessary for SSB repair and DNA-PK for NHEJ mediated DSB repair. Furthermore, ATM dependent HR for DSB repair is positively regulated by ERK1/2 signaling. (Motta et al., 2015) Therefore, by encouraging G2/M checkpoint activation and DNA repair, ERK1/2 signaling also has a beneficial function in cancer cell survival after radiation. These findings are supported by other

studies that show constitutive activation of Ras boosts cancer cells radio resistance while inhibiting MEK or ERK causes cancer cells to become more radiosensitive. (Mirzayans et al., 1995) Multiple pathways work together to activate ERK1/2 signaling by radiation. Otherwise, ERK1/2 activation increases cell survival by preventing the induction of apoptosis and encouraging DNA repair.

3.6. The PI3K/AKT Signaling Promotes Cell Survival in Response to Radioactivity

In order to prevent apoptosis induction which depends on AKT's ability to directly inhibit a number of pro-apoptotic proteins while upregulating a number of anti-apoptotic pathways PI3K/AKT signaling is essential (Figure 4). (Simonyan et al., 2023) AKT can directly block important pro-apoptotic proteins Bad, Bax and Bim all of which belong to Bcl-2 family by phosphorylating them (Figure 4). (Kloet & Burgering, 2011) In addition to AKT phosphorylates the transcription factor FOXO3a which causes it to be retained in the cytoplasm and then degraded by proteasomes. In contrast AKT enhances the expression of pro-apoptotic proteins Bim and Noxa to encourage apoptosis. (Aoki et al., 2004) Additionally, a number of anti-apoptotic pathways such as NF- κ B, XIAP and mTOR pathways are promoted by PI3K/AKT signaling (in Figure 4). First PI3K/AKT signaling releases the NF- κ B transcription factor from the binding inhibitor I κ B which activates it. This allows NF- κ B to move into the nuclei and trigger the production of several anti-apoptotic genes, particularly Bcl-2 and Bcl-xL. (Le Page et al., 2005) Second AKT activates and phosphorylates XIAP which then attaches and deactivates caspases 3, 7 and 9 which are necessary for the activation of apoptosis. (Asselin et al., 2001) Third, mTOR kinase is phosphorylated and activated by AKT while Mcl-1 anti-apoptotic protein is phosphorylated and activated by mTOR kinase. (Cerma et al., 2023) Fourth, the catalytic component of DNA-PK which drives the NHEJ repair of DSB and enhances cell survival in response to radiation is directly phosphorylated and activated AKT. (X. Liu et al., 2021) It has been demonstrated that AKT adversely regulates the production of apoptosis by hypoxia a situation frequently brought on by radiation therapy. (Banfalvi, 2017) It has been demonstrated that GSK3 activates the mitochondria dependent death signaling pathway which is a key factor in hypoxia induced apoptosis. (Fu et al., 2014) Nevertheless, AKT can inhibit GSK3 by inhibitory phosphorylation at Ser 9 which activates glucose transport and glycolysis and prevent hypoxia induced death. (Cao et al., 2022) In cancer, PI3K/AKT signaling activation after radiation has been commonly observed. (Szumiel, 1998) Phosphorylation of carboxyl-terminal regulatory tail of ErbB3/HER3 creates six docking sites for the binding of p85 adaptor subunit of PI3K (Figure 3) which phosphorylates PIP2 to generate PIP3 which in turn attracts and activates PDK1 making it the most likely mechanism involving activation of ErbB/HER receptor by radiation as previously discussed (Figure 3). (Jung et al., 2018) AKT is partially activated by PDK1's phosphorylation of AKT-Thr308 which sets the stage for PDK2's subsequent phosphorylation of AKT-Ser473 which fully activates AKT activity. Furthermore, by triggering synthesis of EGFR ligands which in turn trigger ErbB/HER signaling Ras activation by ErbB/HER receptors or mutations can also favorably regulate radiation induced PI3K/AKT signaling. (Toulany et al., 2008) The idea that PI3K/AKT signaling's pro-survival function will increase cancer cells radioresistance has been validated by a number of invitro and in vivo investigations. According to this research, many cancer cell types radiosensitivity can be boosted by pharmacological or biological inhibitors that block PI3K/AKT signaling. This is followed by decreased DNA repair and higher induction of apoptosis. (Bassullu et al., 2012) However, certain cell models, PI3K/AKT signaling impact on radiosensitivity. (B.-F. Wang et al., 2013) According to this research, impact of PI3K/AKT signaling on cancer cells radiosensitivity is most likely cell type specific.

3.7. NF- κ B Signaling Pathway Promotes Radio resistance

A transcription factor that is crucial in controlling the inflammatory response to a variety of stimuli such as radiation and chemotherapy medications is NF- κ B a heterodimer made up of p50 and

RelA (Figure 5). Inhibitory κ B proteins generally inhibit NF- κ B by sequestering it in cytoplasm. Activated I κ Ks phosphorylate I κ B after stimulation which causes β TrCP to promote its breakdown. The sequestered NF- κ B is released as a result and it moves into nuclei to trigger production of its target genes which aid in survival and growth. (Shen & Tergaonkar, 2009) Furthermore, NF- κ B pathway can be further enhanced by radiation induced ATM and ROS. (J. Yang, 2003) Members of Bcl-2 family, Bcl-2, Bcl-xL and Mcl-1 are the best validated antiapoptotic gene targets of NF- κ B. Moreover, radiation stimulates NF- κ B to produce genes relevant to the cell cycle including cyclin D1 which is also linked to radio resistance. (Mokim Ahmed & Li, 2008) As a result cancer cells radio resistance has been connected to NF- κ B signaling hyperactivity.

3.8. Autophagy Signaling Pathway that Activated by Radioactivity Leads to Either Cell Survival or Cell Death

A conserved cellular defense system against a variety of stress stimuli including oxidation, food deprivation, ER stress and DNA damage, autophagy is a highly regulated process of lysosome-mediated breakdown. (J. Li et al., 2021) Since autophagy induction is inhibited by AKT and MAPK and promoted by AMPK and p53 when it is inhibited mTOR kinase is essential for controlling autophagy induction. (Ito et al., 2005) Inhibition of mTOR kinase and activation of ULK kinase which then combines with and activates ATG13 and FIP200 initiate the activation of autophagy signaling. ATG14, VPS24, Beclin1 and p150 are the additional autophagy related proteins that PI3K-III forms a complex with in meantime. Following their recruitment to a double layer membrane structure, PI3KIII and ULK complexes form phagophores which eventually fuse with lysosomes and begin destruction of protein cargos. Autophagy is generally believed to support cell survival by preserving energy balance. To get rid of severely injured cells autophagy can potentially cause cell death. (Poljšak & Milisav, 2012) Radiation generated ROS/RNS have been shown to induce DSB/SSB DNA damage, oxidative stress and mitochondrial dysfunction in cells, all of which can set off an autophagy response that results in either cell death or survival. The latter promotes radio resistance by shielding the irradiated cancer cells. (X.-Y. Pei et al., 2004) The outcomes of the autophagy response to radiation can potentially be influenced by a number of circumstances. These consist of kinds of cells the extent of damage and nutritional circumstances (Huang et al., 2009).

3.9. HIF-1 α Signaling Pathway Facilitates Radio-Protective Mechanisms in Tumor Cells

According to recent research HIF-1 a transcription factor that acts as a master regulator of cellular responses to hypoxia has a significant role in promoting tumor cells radio resistance in both hypoxia and normoxic environments. (Manolescu et al., 2009) One hypoxia-inducible subunit HIF1 α , HIF-2 α , HIF-3 α and HIF1 β and HIF-2 α whose protein sequences have 48% similarity contribute to tumor resistance to radiation therapy. (Minassian et al., 2019) HIF-2 α is mostly found in vascular endothelium, lung and heart tissues and placenta whereas HIF-1 α is widely expressed in nearly all cell types and tissues. (Pi et al., 2015) Under normoxia VHL E3 ubiquitin ligase mediates proteasomal degradation of HIF-1 α by hydroxylated ODDD domain of HIF-1 α at pro402 and Pro564 residues using α -ketoglutarate and O₂-dependent prolyl-4-hydroxylases (Figure 6). Transcriptional adapter/histone acetyltransferase proteins p300 and CBP are then recruited to a transcription complex that activates transcription of HIF-1 targeted genes after HIF-1 α stabilizes and translocates into nuclei to form a complex with HIF-1 β under hypoxia. (Bragan $\tilde{c}$ a et al., 2003) STAT3, NF- κ B nuclear factor κ B, microRNAs/long noncoding RNAs, c-Myc, angiotensin II and signaling involving stress or mitogen activated kinases, PI3K and mTOR can all influence HIF1 α expression in addition to VHL primary regulation. (Meijer et al., 2012) Due to rapid growth of tumor volume and relatively delayed growth of blood vessels that supply oxygen, tumor microenvironment is generally more hypoxic than surrounding normal tissue. However, majority of cells are impaired in mitochondrial oxidative energy metabolism and instead use glycolysis as primary energy metabolic pathway, a phenomenon known

as Warburg effect. Consequently, ROS generated by radiation in cancer cells can be effectively absorbed by antioxidants (NADPH and glutathione) generated by glycolysis, stabilizing HIF-1 α and its radioprotective function in tumor cells under normoxia. (Nakashima et al., 2017) Activation of several radioprotective mechanisms such as angiogenesis, autophagy, apoptosis inhibition, promoting stemness of cancer cells and altering energy metabolism pathways, HIF-1 can increase radio resistance of tumor cells (Figure 6) Increasing anaerobic glycolysis and upregulating VEGF expression, HIF-1 α stimulates angiogenesis. Boosting expression of beclin and LC3-II two essential elements of autophagic pathway and improving miR-210/Bcl-2 and Akt/mTOR/P70S6K pathways, HIF-1 α has been demonstrated to promote autophagy induction in hypoxic cells. HIF-1 α has been demonstrated to increase cancer cell survival in response to radiation. This function HIF-1 α 's capacity to reduce ROS generation and boost microRNA-210 expression which alters its mRNA targets to support DNA repair autophagy induction and apoptosis inhibition. (Meijer et al., 2012) Cancer stem cells are known to be radioresistant and HIF-1 α induced miR-210 via a positive feedback loop, further stabilizes HIF-1 α and amplifies its beneficial effect on radio resistance of hypoxic tumors. Numerous genes necessary for maintaining stemness of CSCs are induced to be transcriptionally active by HIF-1 α . These genes include those that promote EMT, Phenotype (e.g. TAZ, Snail, Twist, Slug, Zeb-1/2) aid in survival and promote self-renewal (e.g. hTERT, ABC-Ts, Notch), HIF-1 α is essential for hypoxia- activated metabolism regulators and enzymes including Glut-1 which leads Warburg effect. This occur when ATP-generating pathway switches from more effective mitochondrial oxidative phosphorylation to the less effective glycolysis. (Geng et al., 2022) As a result, this change causes a significant reduction in ROS generation and an intracellular buildup of reduced GSH which can both significantly lower radiosensitivity of hypoxic tumor cells and cause radio sensitization of tumor cells because HIF-1 α collectively regulates several radioprotective processes in hypoxic tumors.

3.10. YAP Signaling Pathway Promotes Radio resistance of Tumors

TEAD family of transcription factors is YAP. Many genes necessary for carcinogenesis and the spread of majority of solid tumors are activated by YAP.(L. Li et al., 2014) Hippo tumor suppressor pathway inhibits YAP when it is activated, YAP is phosphorylated at several locations which causes 14-3-3 to retain it in cytoplasm and β TrCP-SCF ubiquitin ligase complex to promote its proteasomal destruction. (X. Yang & Xu, 2011) (Figure 7). Intrinsic radio resistance of a number of cancer types including brain, breast, lung and pancreatic cancers has been positively correlated with YAP activation. (Schroeder & Halder, 2012) Previous research indicates that YAP function is generally involved in transactivation of gene expressions necessary for cell survival (surviving Bcl-2/Bcl-XL etc) DNA repair (p53 etc), proliferation (EGFR/HER, Axl, cell cycles genes, MAPK etc) and cancer stem cells (SOX2, CTGF, Cyr61 etc). However, precise mechanisms underlying this YAP effects are still unclear. (Zhou et al., 2024) Moreover, promoting DNA repair, preventing apoptosis and protecting cancer stem cells, these YAP promoted prosurvival pathways are expected to simultaneously decrease radiation induced cytotoxicity and increase radio resistance of cancer cells.

4. Background of Molecular Hydrogen Turn into Medicine Industry

H₂ is chemically stable gas that is colorless, odorless and tasteless. The human digestive tract typically produces and releases between 35 and 321 milliliters of H₂ everyday through bacterial fermentation. (Holden & Cunningham, 2018) Molecular Hydrogen can be consumed in a variety of ways including drinking water or injecting MH water, inhaling H₂, taking a bath in Hydrogen water, putting MH saline in the eyes etc. Because of its specific antioxidant qualities, MH exerts anti-inflammatory and anti-apoptotic effects and is now a special cytoprotective agent (Strocchi & Levitt, 1992) Primary cause of many illnesses and aging is an excessive buildup of peroxides generated during metabolic processes. Body needs a specific mechanism to combat these peroxides. Redox balance is achieved by organisms through natural elimination of peroxides by MH, a reducing agent with no negative side effects. (LeBaron et al., 2024) Discusses antioxidant activity of MH links psychological consequences of MH with the free radical aging theory and validates that MH may

have an incalculable impact on a variety of disorders. (Cader et al., 2021) By selectively lowering harmful oxygen radicals (*OH and ONOO-) it is suggested that a mechanism by which MH may function as a therapeutic antioxidant and prevent cerebral ischemia reperfusion injury. (Meredith et al., 2014) Initially, researchers concentrated mostly on oxidative stress related acute and chronic organ damage such as that shown in animal studies of drug toxic injury or ischemia reperfusion injury in critical organs like liver and heart. In order to confirm therapeutic benefits of MH inhalation, researchers primarily employed a variety of damage models at this time. Additional studies on drinking MH enriched water started to surface. (Ohta, 2015) as well as research on using gut microbes to increase MH replenishment. (Xie et al., 2014) Because of this classification the majority of researchers have concentrated on MH antioxidant mechanisms (Jahng et al., 2012) However, researchers have suggested that MH has a signaling function that could be engaged in metabolic processes and even give bacteria energy which expands ideas on how MH research might progress. (H. Liu et al., 2023) The most significant area of study in diving hyperbaric medicine is kinds of gases that humans can breathe with MH being one of the most essential gases in this subject. For the first time, suns group focuses on biological effects of MH and its usage in medicine, demonstrating importance of MH in Chinese medicine, Qins recent review summarizes 51 clinical trials involving 1213 subjects in four areas of MH biomedicine, basic research, exercise, dermatology and healthcare. They established the standardized laboratory for MH in molecular biology which has published multiple placebo-controlled population trials providing crucial clinical evidence for translation of MH into medicine. (Schroeter et al., 2006) In vitro and in vivo studies, MH can prevent growth of gliomas by promoting the differentiation of glial progenitor cells. (Zheng et al., 2021) There is now an academic association for MH medicine with around 400 members. Current clinical research on MH is still being developed today and size of these trials is progressively growing. MH inhalation device has been added to the Chinese National medical products Administration's new medical device development process due to its advantageous biosafety and convenience. Furthermore, MH has been authorized as a dietary supplement in Japan.

5. Discussion of Different routes and Applied Molecular Hydrogen Passing into Targeted Organ

MH is not created endogenously as is well known. Human body does not have any enzymes involved in hydrogenase action. However, under healthy conditions the colonic bacteria in human body may produce roughly 12L of MH each day. Therefore, for example the concentration of MH in a normal person's final breath is approximately 5-10 ppm. (Rochette et al., 2023) Many studies have shown that increasing the amount of MH gas produced by intestinal bacteria may be a useful therapeutic approach for a number of illnesses. Additionally, there are a number of ways to provide external MH including direct integration, injection, drinking and breathing as shown in Figure 8.

5.1 Inhalation of hydrogen gas

One of the simplest methods of administering hydrogen is direct inhalation such as through a ventilator circuit, nasal cannula or facemask. This is a simple and fast way. Studies have shown that 1-4% MH gas administered by inhalation is a successful treatment for radiation injury. (Ge et al., 2022) 50% helium, 49% hydrogen and 1% oxygen make up hydrex a breathing gas mixture. It is frequently used for decompression during deep diving in order to avoid decompression sickness and nitrogen narcosis (Ji et al., 2010).

5.2 Oral administration of hydrogen water

Drinking enhanced MH is a more practical and secure approach that can be readily delivered anywhere even though inhaling hydrogen gas may have an immediate effect. (Wu et al., 2023) However, aluminum walls have a comparatively longer retention period for H₂ gas. (Y. Zhang et al.,

2023) When animals with inflammatory bowel illness were given drinking water enriched with hydrogen, there was a noticeable reduction in symptoms of intestinal inflammation. Additionally, research on the use of MH water in dentistry showed that it was useful in treating periodontitis (Zeng et al., 2023).

5.3 Injection of hydrogen saline

A certain hydrogen concentration can be delivered precisely using hydrogen saline injection. In experimental animals, injecting MH dissolved saline intravenously or intraperitoneally has shown remarkable effectiveness. (Zanini et al., 2021) As a result, this approach has also been used in real clinical settings. A prospective, nonrandomized open label study is being conducted.

5.4 Hydrogen bath and eye drops

MH may readily pass through skin and go throughout body through blood flow because of its miniscule molecular size. One way to incorporate hydrogen into the body in daily is to bathe in water that has dissolved hydrogen gas. (Qiu et al., 2021) Researchers create MH enriched eye drops by dissolving MH in saline and then applying drops straight to the surface of eye. (Hu et al., 2024) Researchers stated they constantly applied MH enriched eye drops during radiation periods, vitreous body MH concentration rose right away while radiation induced -OH level dropped instead.

6. Radioprotective performance and others biological recovery of molecular Hydrogen:

Previously Discussed that radiation has both direct and indirect effects. Damage to biomolecules like DNA is a direct effect. (Hutchinson, 1985b) Oxidative damage from -OH oxidizes a variety of biological components and this oxidation results in the production of more secondary free radicals. (S.-Y. Li et al., 2023) However, by selectively scavenging the vast amounts of -OH produced in the living body where MH an inert molecule can shield living from oxidative damage caused by radiation. Few studies describe the specific mechanisms of MH despite the fact that its radioprotective effects have been validated in previous finding. (Oike et al., 2016) The potential mechanisms of MHs radioprotective effects from these chapter will be covered in this part.

6.1. Antioxidant Effects

-OH which is generated in significant amounts during irradiation is selectively scavenged by MH and this scavenging can be seen as a direct consequence of MH's radioprotective actions. In cell free systems the fenton reaction and water radiolysis yield OH which can be decreased by MH. (Guo et al., 2016) Demonstrated that in both in vitro and in vivo tests MH dramatically lowers the -OH generated by radiation. However, when considering total ROS. (J. Li et al., 2017) Demonstrated that MH dramatically lowers the generation of ROS caused by radiation in both in vitro and in vivo tests indicating that MH's radioprotective effects include the selective removal of -OH. We must take into account how MH provides radioprotection -OH has a broad range of non-specific reactions. Compared to DNA, amino acids, sugars and GSH the rate at which -OH reacts with MH in aqueous solution is substantially slower. (M. Liu et al., 2019) Theoretically more MH must be present in the nucleus than other solutes in order to MH to react with -OH. High intracellular diffusion rates of MH may need to be taken into account in aqueous solutions with a lot of solutes including culture medium and buffer solutions even if more thorough research is required to reconcile these discrepancies. Another possibility is that the nucleus has a distinct rate of reactivity between MH and -OH. The anticancer effects of radiation may be diminished if we believe that the selective removal of -OH is the sole mechanism behind the radioprotective properties of MH. However, MH did not lessen the antitumor effects of radiation in both reports of clinical trials looking at radioprotective effects in cancer patients instead MH improved the oxidative stress related index, indicating that while MH's antioxidant effect may be responsible for its radioprotective effects other biological defense systems

such as hormones and radiation protective enzymes may also be at play. (J. Li et al., 2017) In addition to this we noted the direct scavenging of $\cdot\text{OH}$ may not be only way that MH has radioprotective effects, host mediated antioxidant and anti-inflammatory systems may also be activated. (Sharp, 1970) The fact that patients inhaled MH gas after IMRT but not before suggests that the radioprotective effects of MH may involve an indirect influence on $\cdot\text{OH}$ rather than a direct one.

6.2. Anti-Inflammatory Effects

Cytokines such as IL-1, TNF- α and IFN- γ are crucial markers of oxidative and chronic inflammatory damage following radiation exposure. (McKelvey et al., 2018) In the injured skin MH dramatically lowered the levels of MDA and IL-6. According to a recent review mitochondrial DNA experiences oxidative stress due to $\cdot\text{OH}$ generated in the mitochondria. Oxidized mtDNA starts a chain reaction that releases inflammatory cytokines from nucleotide binding and oligomerization domain like receptor family pyrin domain containing 3 NLRP3 to IL-1 and IL-18. The scavenging of $\cdot\text{OH}$ produced in mitochondria may be a method by which MH induced amelioration of chronic inflammatory disorders occurs (Mandal, 2010).

6.3. Anti-Apoptotic Effects

Oxidative damage and inflammation are also intimately linked to apoptosis or radiation induced cell death. According to published research MH's anti apoptotic properties provide a radioprotective effect on radiation induced cell or animal models. (Mei et al., 2014) Antiapoptotic effects of MH on radiation on injury models have been assessed using the TUNEL assay and quantification of caspases-3,8,9 which are crucial proteases for apoptosis. It can also be evaluated by looking at the expression of apoptosis inducing protein Bax and apoptosis inhibiting proteins Bcl-xL and Bcl-2. MH suppresses cytochrome c release, mitochondrial depolarization and actions of PARP, caspase3,9. They also stated that MH prevents apoptosis by preventing the upregulation of Bax and recovering from downregulation of Bcl-xl and Bcl-2 (Suman et al., 2018).

6.4. Regulation of Gene Expression

Enhancement of SOD and CAT is intimately associated with Nrf2 an endogenous antioxidant regulator. Furthermore, Nrf2 possesses biological protection properties including increasing the activity of HO-1 which has cytoprotective properties like anti-oxidation and anti-inflammation. (S. Pei et al., 2024) Using a model developed by irradiating mice the moderating effects of MH on gastrointestinal diseases have been investigated. It is found that a small intestine microarray analysis MH inhibited the expression of MyD88.(Zang et al., 2024) Next to, A rat model of radiation induced myocardial injury MH controls the expression of miRNAs implicated in myocardial oxidation, hypertrophy or fibrosis. This finding imply that MH has indirect radioprotective effects via controlling gene expression and displaying anti-inflammatory, anti-apoptotic and antioxidant properties in addition to its direct action of scavenging $\cdot\text{OH}$ (MacVittie et al., 2000).

7. Prospects of Molecular Hydrogen as a Radioprotective Agent in Medical Science.

Even if the amount of radiation exposure is minimal radiation damage happens during medical diagnostic procedures including CT scans and radiotherapy for cancer patients. As a result, efforts are underway to create safe and efficient radioprotective medicines, nonetheless amifostine is the only one that is currently approved for clinical usage. (N. Lee et al., 2007)During radiotherapy this medication shields the healthy tissues surrounding malignancies from radiation harm. Normal tissues absorb amifostine quickly but tumors absorb it more slowly. Thus, it is believed that irradiation within a few minutes of administering amifostine protects normal tissues in a selective manner. Amifosines radioprotective actions are mediated through the scavenging of free radicals generated during irradiation such as $\cdot\text{OH}$. (González et al., 2009) However, due to a number of safety concerns and

dose dependent adverse effects including hypotension, nausea and vomiting, amifostine is not regarded as a helpful radioprotective drug. (Gudkov et al., 2015) Acute cerebral infarction has been treated clinically with a -OH scavenger. Basic research on this medications radioprotective effects has been carried out using experimental animals and cultured cells based on its processes.(Reiter et al., 1997) However, as a radioprotective agent it has not yet been employed in clinical settings. Furthermore, the potential of antioxidants such melatonin, vitamin C, vitamin D and vitamin E as radioprotective agents has been investigated. (Jamialahmadi et al., 2024) They are not as safe as MH though as systematic reviews and meta-analyses have shown that taking too much of these antioxidants raises mortality. On the other hand, MH is now a new antioxidant with anti-inflammatory, anti-allergic and anti-apoptotic properties. Numerous illnesses such as cancer, sepsis, cardiovascular disease, brain and nerve disorders diabetes and metabolic syndrome can be prevented or treated using MH. (He et al., 2024) MH can be administered intravenously as MH dissolved saline consumed as MH rich water or inhaled as MH gas among other forms. Although each of these administration techniques has unique properties inhaling MH gas produce most MH in a time dependent manner. This can be explained by fact that in comparison to other delivery methods, maximum blood and tissue concentrations in MH gas inhalation are lower and their area under curve is incredibly high. MH inhalation is deemed appropriate for usage as a radioprotective agent. Furthermore, there are no safety concerns with MH because it doesn't healthy tissues and cells and no adverse effects have been seen in clinical testing. (B. J. Lee et al., 2010) Our diet and lifestyle determine how much MH is created by the MH producing bacteria in our large intestine. However, majority of MH is expelled through fast or expiration since it is not utilized by our bodies. MH diffuses from stomach and intestinal walls to the surrounding organs and tissues when it is ingested as MH water. It also circulates throughout body through the bloodstream.(Holden & Cunningham, 2018) Similarly when MH is inhaled as gas it diffuses from the lung tissues to the tissues and organs around it before being carried by the blood throughout the body. Since MH is a very small molecule its physical characteristics enable it to diffuse into the cytoplasm and easily cross cell membrane. MH is able to quickly reach nucleus and mitochondria to provide protection. The blood brain barrier is also easily crossed by MH. Regarding MH as a radioprotective agent it has been demonstrated outstanding effectiveness in irradiated cells and animal model enhanced quality of life for patients with radiation therapy for liver cancer and decreases bone marrow damage in patients with end stage cancer receiving IMRT. As a free radical scavenger, amifostine has been utilized in therapeutic settings. Another scavenger, edaravone has also been studies as a possible radioprotective drug. (Weber et al., 2008) Despite, MH is safe and effective. MH might be a perfect radioprotective drug with therapeutic applicability based on the aforementioned characteristics. Direct removal of -OH is one potential mechanism for MH's radioprotective properties. Nevertheless, indirect mechanisms of -OH must also be taken into account. MH demonstrated anti-inflammatory, anti-apoptotic and gene expression regulating properties in addition to antioxidant ones in cellular and animal models used to study radioprotective effects. Through regulation of gene expression through intracellular reactions, MH might potentially also indirectly carry out its antioxidant effects. MH's direct impact on radioprotection that is removal of -OH is well understood but its indirect effects are still unknown and need more research.

Conclusion

In search of perfect radioprotectant researchers have never stopped. The sole radioprotectant approved for use in humans where amifostine is a sulfhydryl molecule that has demonstrated good radioprotective properties. However, a number of adverse effects including nausea, vomiting and hypertension. Certain natural antioxidant such as vitamins and flavonoids has poorer radioprotection but fewer harmful effects while radiation could acquire or intrinsic continues to be a major clinical issue that restricts its effectiveness and MH is a common option for treating radio resistance. Radiation simultaneously triggers several prosurvival signaling pathways that prevent induction of

apoptosis, encourage DNA repair and adaptive significantly lessen the amount of radiation induced lethality in cancer cells. Among them key roles of ATM, ATR and DNA-PK signaling are to support DNA repair and cell cycle checkpoint activation in cancer cells whereas main roles of AKT, ERK1/2 and NF- κ B signaling are to prevent apoptosis induction in the irradiation cancer cells. Furthermore, cell cycle checkpoint response is positively regulated by EKR1/2 and AKT signaling which also aid in DNA repair. Furthermore, HIF-1 α reduces radiation damage in cancer cells via promoting angiogenesis, autophagy, CSCs and reprogramming energy metabolism. Moreover, by encouraging the transcription of numerous genes necessary for cell survival, DNA repair and CSCs, YAP signaling adds to inherent radio resistance. These signaling pathways work together to both increase radio resistance and shield cancer cells from radiation damage. However, by shielding skin cells from radiation induced DNA and mitochondrial damage and MH gas prevents radiation. By increasing antioxidant enzymes activity, it lessens the damage that reactive ROS and ensuing release of pro-inflammatory cytokines cause which in turn lowers the incidence of apoptosis. MH potential in radiation management has been investigated in a number of research because it's incredibly low molecular weight and MH may enter mitochondria and cell nuclei quickly. It shields cells from radiation damage by specifically scavenging dangerous free radicals such as hydroxyl radicals and peroxy nitrite anions. So, non-irradiated cells the administration of MH decreased membrane potential depolarization. Impact might result from MH's ability to stop electron leakage in the electron transport chain which stops it from reacting with oxygen to produce ROS. MH may prevent radiation through exposure to high concentrations which immediately lowers partial pressure of oxygen in tissue hence reducing ROS formation and radiation damage as well as antioxidant, anti-apoptotic and anti-inflammatory already discussed.

Conflicts of Interest

The authors have no relevant financial or non-financial interests to disclose.

Author contribution

The first draft of the manuscript was originally written by Md. Sadikuj Jaman and all authors commented on the manuscript. All authors contributed to the study's conception and design. Md Maniruzzaman made table arrangements, data collection and analysis, figure design and material preparation. All authors read and approved the final manuscript.

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