UNIVERSITY OF CALIFORNIA SAN DIEGO

Development of Cobinamide as a Potential Therapeutic Agent for Radiation-Induced Oxidative Stress in Human Cells

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

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Professor Stephanie Mel

2019

The Thesis of Stephen Sue-Young Chang is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California San Diego

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DEDICATION

I would like to dedicate this to my family for always supporting me and allowing me to pursue my interests.

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ACKNOLWEDGEMENTS

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Results are currently being prepared for submission for publication of the material. Boss, Gerry; Chang, Stephen; and John Tat. The thesis author will be co-author of this material.

ABSTRACT OF THE THESIS

Development of Cobinamide as a Potential Therapeutic for Radiation-Induced Oxidative Stress in Human Cells

by

Stephen Sue-Young Chang

Master of Science in Biology

University of California San Diego, 2019

Professor Gerard R. Boss, Chair
Professor Immo E. Scheffler, Co-Chair

Radiation poisoning has become an increasingly larger public health concern due to the proliferation of nuclear technology. No effective treatment for radiation poisoning exists. Radiation is believed to cause damage to cells through a process known as radiolysis, which generates reactive oxygen species (ROS). Cobinamide, a vitamin B_{12} precursor, has been

shown to be an effective scavenger of ROS. We therefore hypothesized that cobinamide could be utilized as a potential therapeutic for radiation sickness. We first found that cobinamide acts as a ROS scavenger, and then found that it may also act as a superoxide dismutase mimetic. *In vivo* experiments were then performed to determine if cobinamide could recue cells from radiation toxicity. Experiments with HEK293A cells showed that cobinamide partially rescued cells from radiation toxicity in both, radioprotective and radiomitigative manners. Results suggest that cobinamide can be further developed as an effective radiation poisoning therapeutic.

Introduction

1.1 Gamma (γ) Radiation

Radiation has played an increasingly larger role in human society since its discovery in the 19th century. It is utilized as a source of energy, light, heat, and communication among others. But, in addition to beneficial uses, radiation has also been utilized as a weapon due to the biological and chemical effects it can have when absorbed into the body. Radioactive contamination from nuclear disasters has also been a major public health concern.

Ionizing radiation can be split into two categories based on their biological effects: directly ionizing and indirectly ionizing [1]. Directly ionizing radiation occurs when a particulate has enough kinetic energy that it can directly affect the atomic structure of the medium the particulate is passing through. Indirectly ionizing radiation is not produced from a particulate with high kinetic energy, but from the generation of secondary electrons after photons are absorbed. Indirect ionizing radiation has been shown to mainly damage cells by reacting with the aqueous intracellular environment, thereby generating reactive oxygen species (ROS) [2].

Gamma (γ) rays, a type of electromagnetic radiation, are a prime example of indirect ionizing energy. γ radiation can be emitted when a radioactive atom decays [1]. Major sources of γ rays are cosmic rays, fallout from either nuclear bombs or power plants, “dirty” bombs containing radioactive material, radiation therapy treatments, as well as naturally occurring elements that undergo radioactive decay [1].

1.2 Gamma Radiation Mechanism

γ rays have high photon energies that can be harmful. When a γ ray is absorbed into a cell, the energy of the photon can excite an electron and eject it from its orbital path [1]. Occasionally, the energy of the photon is not enough to eject an electron but instead gives the electron a higher energy state. This process is called “excitation”. In brief, when γ radiation is absorbed it can produce charged (a.k.a. ionized) molecules, free electrons, and excited molecules. The ionized and excited molecules can then fragment into free radical species that

will react with other molecules [1]. This generation of charged particles and free radicals is extremely detrimental to cells.

When γ rays are absorbed into cells, there is a high likelihood of them hitting water molecules because water comprises ≥70% of a cell's mass, instigating water radiolysis [3]. The mechanism of water radiolysis is reviewed by Le Caër (Figure 1 [4]). When ionizing radiation strikes H_2O, a three-stage reaction occurs. First, the physical stage occurs roughly 1×10^{-15} of a second after the γ ray interacts with the cell, causing H_2O molecules to either be excited or ionized into $H_2O^+ + e^-$. Then, in the physicochemical stage which occurs on a time scale of femto- to picoseconds, these excited H_2O molecules and the ionized H_2O^+ molecules react with the surrounding H_2O molecules to generate radical hydroxyl species, H_3O^+, as well as $H\cdot$. Finally, the chemical stage is where all these molecules diffuse into solution and then react with each other and the surrounding water molecules, generating more free electrons and more radical species such as $HO\cdot$ and $HO_2\cdot$. This process also generates other reactive oxygen species (ROS) such as O_2^- and H_2O_2.

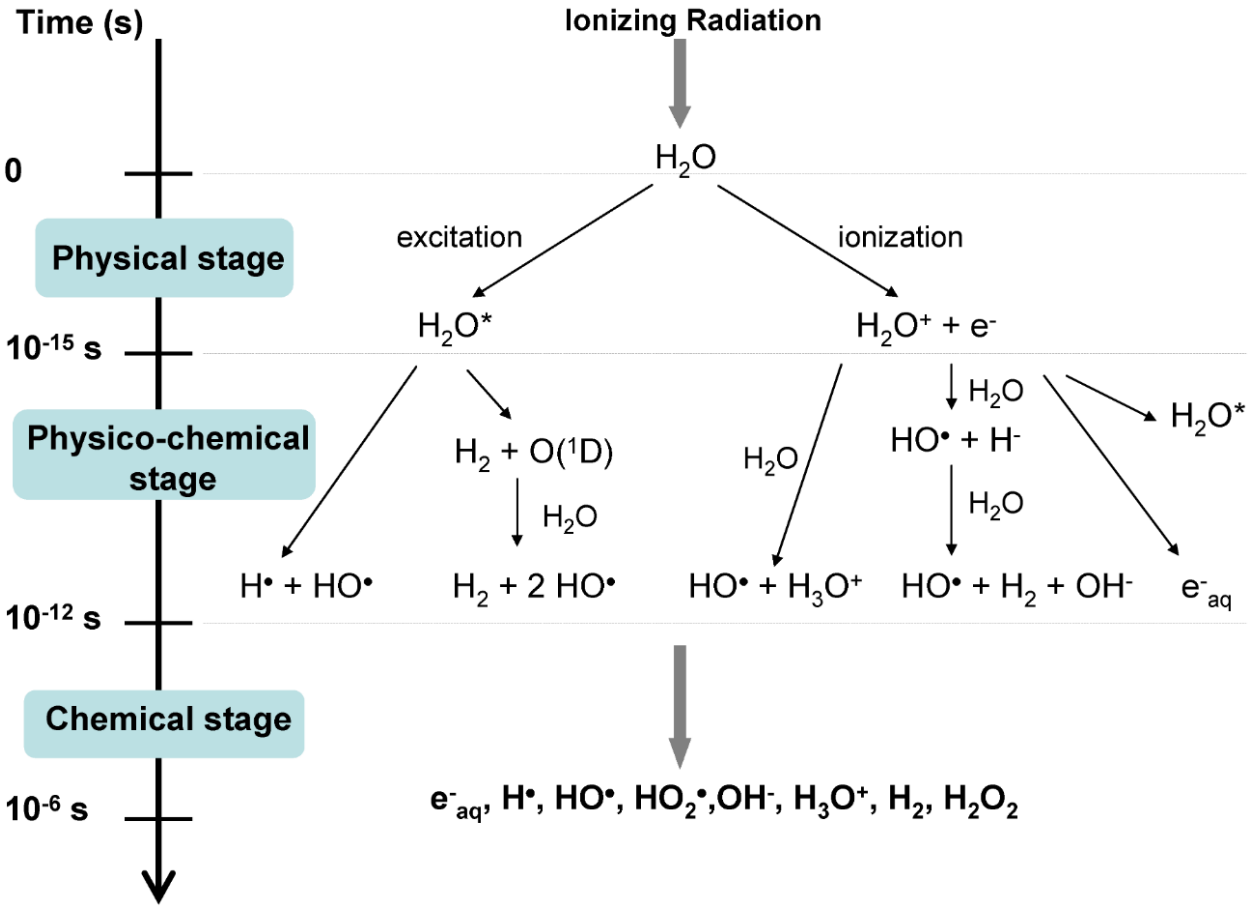

Figure 1. The mechanism of the radiolysis of water [4]. The generation of reactive oxygen species is hypothesized to be the primary driver of radiation-induced oxidative damage after gamma irradiation.

e^-_{aq}, HO·, OH^-, HO_2·, and H_3O^+, are highly reactive and can generate more of the same species. These species will also immediately react with any biomolecules in their vicinity, such as nucleic acids, proteins, and lipids, producing oxidative damage [5] This mechanism is reviewed by Azzam et al. [2]. Radiation-induced damage initiates signaling events that will repair the damage, permanently alter cellular physiology, or kill the cell [5]. Although cells are able to utilize endogenous enzymes such as superoxide dismutase (SOD) to detoxify and remove ROS, irradiation often produces such an excess of ROS that they overwhelm endogenous detoxification systems, rendering cells permanently damaged. While the initial generation of ROS from γ radiation happens on the order of femto- and picoseconds, oxidative damage may still occur days or months after the initial radiation event due to continuous

generation of ROS [6]. Roughly 60 ROS per nanogram of tissue are created in less than a microsecond when hit by γ rays [2].

1.3 Radiation Poisoning Symptoms

When a person is exposed to high doses of penetrating radiation in a short period of time, the ensuing illness is characterized as acute radiation syndrome (ARS). Symptoms of ARS mainly affect the hematopoietic, gastrointestinal, and neurovascular systems [7].

Radiation absorbed by the subject is measured in rad or gray (Gy). One rad is equivalent to 0.01 joules of energy absorbed per kilogram of tissue; 1 Gy is equivalent to 100 rad, which is the same as 1 joule of energy deposited per kilogram.

Hematopoietic deficiency manifests after the subject is exposed to ≥2 Gy [7]. Lymphocytes are depleted first because they are the most sensitive to radiation; granulocyte and platelet levels will decline over a few days [7]. Mature red blood cells decline slower and over a longer period of time than the aforementioned cell types [7]. Nausea, vomiting, headache, red skin, and fever constitute early symptoms [5]. Symptoms that occur later are usually due to loss of hematopoietic cells; such as an impaired immune system, bleeding, and slow clotting. Death will occur within weeks or months following exposure. About half of all people exposed to a dose higher than 3.5 Gy will die within 60 days [8].

Gastrointestinal (GI) syndrome is seen at doses around 6 to 10 Gy, with the early onset-symptoms being much more severe. Within one to two hours after exposure, nausea, anorexia, vomiting, and abdominal cramp pain will likely occur [8, 9]. Death from GI distress is usually due to multisystem organ failure, sepsis, and complications due to bleeding [8, 9]. People exposed to doses of 6 to 10 Gy will usually expire in a matter of weeks [5].

The nervous system is the least sensitive to the effects of radiation exposure. Due to this, neurovascular syndrome does not usually occur unless the absorbed dose is over 10 Gy. At this point vomiting is suppressed while fever and headache are present. At higher dosages, altered reflexes, dizziness, contusions, disorientation, ataxia, and loss of consciousness are

common [8, 9]. ~35 Gy damages large blood vessels, leading to circulatory collapse and other comorbidities [8]. ≥50 Gy will lead to death within 48 hours but sans the hematopoietic and GI syndromes since insufficient time will have passed for these syndromes to manifest [8].

1.4 Treatment of Acute Radiation Sickness

Patients will be put into one of three treatment categories depending on the exposure dosage. The first category of treatment is minimal intervention; the second is aggressive supportive care; and the third is aggressive supportive care as well as palliative care [8, 9]. There is no cure for radiation exposure. Therefore, treatments are meant support the body as it attempts to recover from the damage caused by radiation exposure.

ROS generated from radiation exposure are a major source of cellular damage, leading to the aforementioned symptoms. For this reason, an ROS scavenger could, in theory, mitigate the damage done by radiation.

1.5 Radio-protectant and Radio-mitigative Agents

Currently, amifostine is the only FDA approved radioprotective agent available to patients who are going to be exposed to radiation, such as cancer patients undergoing radiation therapy. Amifostine acts as an ROS scavenger in cells. Due to amifostine's side effects, such as hypocalcemia, nausea, hypotension, and vomiting, amifostine treatment is highly monitored. However, these adverse effects can be so severe that treatment may cease entirely [10]. Thus, there is still a need to develop novel, nontoxic compounds that can protect from and mitigate the damage caused by radiation.

1.6 Cobinamide

Cobalamin, also known as vitamin B_{12}, is utilized as a cyanide antidote, because it has been shown to bind to cyanide with high affinity [12,13]. Cobalamin is made up of a cobalt ion that has six coordination sites, and four of those sites are coordinated to nitrogen groups that make up a planar corrin ring. A 5,6 dimethylbenzimidazole nucleotide tail occupies the fifth coordination site, and the sixth is available to bind with potential ligands. The available

coordination site can contain additional R groups, such as aminotetrazole, to prevent an unintended ligand from binding with cobalamin.

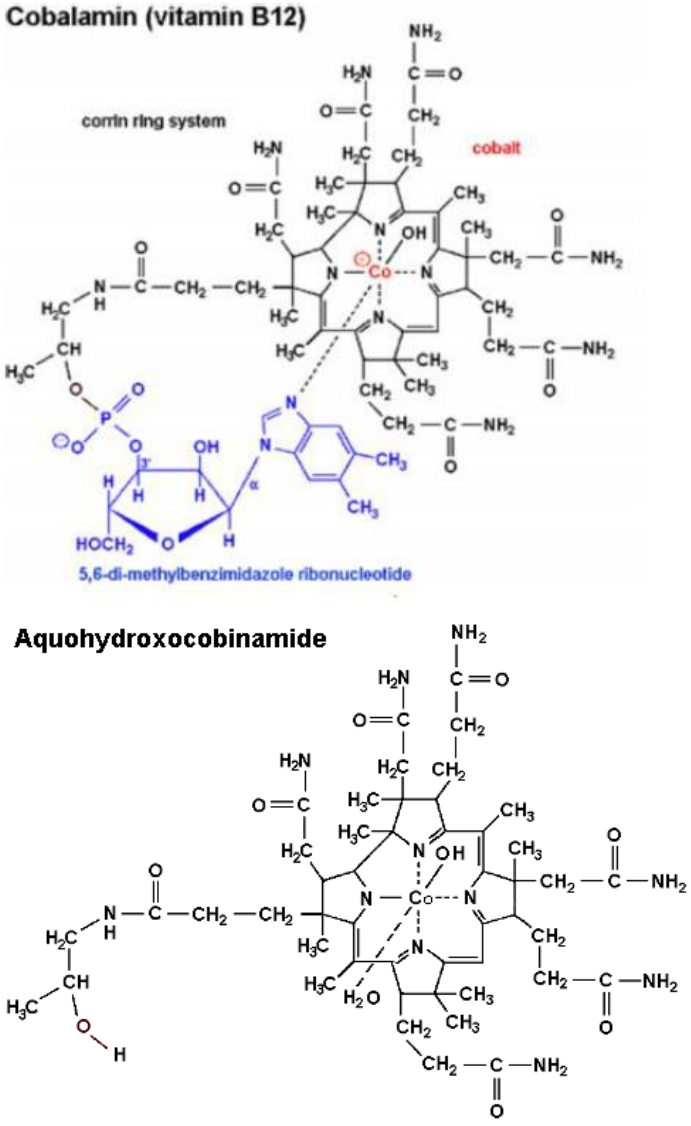

Figure 2. The structure of cobalamin and cobinamide [11]. The cobalt ion highlighted in red indicates the center of the corrin ring. The group highlighted in blue represents the 5,6 dimethylbenzimidazole nucleotide tail, occupying the fifth coordination site. The removal of this nucleotide tail results in the structure of cobinamide. The hydroxyl group attached to the cobalt ion is replaced when a ligand with higher affinity is encountered. For the following experiments, multiple forms of cobinamide were utilized. Cobinamide with two hydroxyl groups coordinated to the cobalt atom was utilized, as well as cobinamide with two aminotetrazole groups coordinated to the cobalt atom.

Cobinamide, the immediate biological precursor of cobalamin, lacks the 5,6 dimethylbenzimidazole nucleotide tail. This allows cobinamide to have two potential ligand binding sites (as opposed to one for cobalamin). The removal of the tail also removes the negative trans-effect that is seen in cobalamin and allows for cobinamide to have a higher binding affinity for ligands [14]. Cobinamide has also been shown to bind to nitric oxide with a binding affinity more than 100 times greater than cobalamin. [15].

In addition to nitric oxide, workers in the Boss Lab showed that cobinamide could bind to superoxide. Mechanistically, superoxide actively reduces cytochrome C. But this effect would be neutralized in the presence of cobinamide if cobinamide could bind to superoxide. To test this hypothesis, Dr. Sameh Ali et al carried out spectrophotometric analysis of samples containing cytochrome C, superoxide, and cobinamide. Increased cytochrome C absorbance correlates with increased reduction by superoxide. Ali et al. saw that addition of cobinamide reduced cytochrome C absorbance in a dose-dependent manner (Figure 3). It was therefore concluded that cobinamide could react with superoxide.

1.7 Central hypothesis

If γ radiation induces ROS and cobinamide is an ROS scavenger, then cobinamide could decrease the effects of radiation-induced oxidative damage through directly scavenging and sequestering ROS.

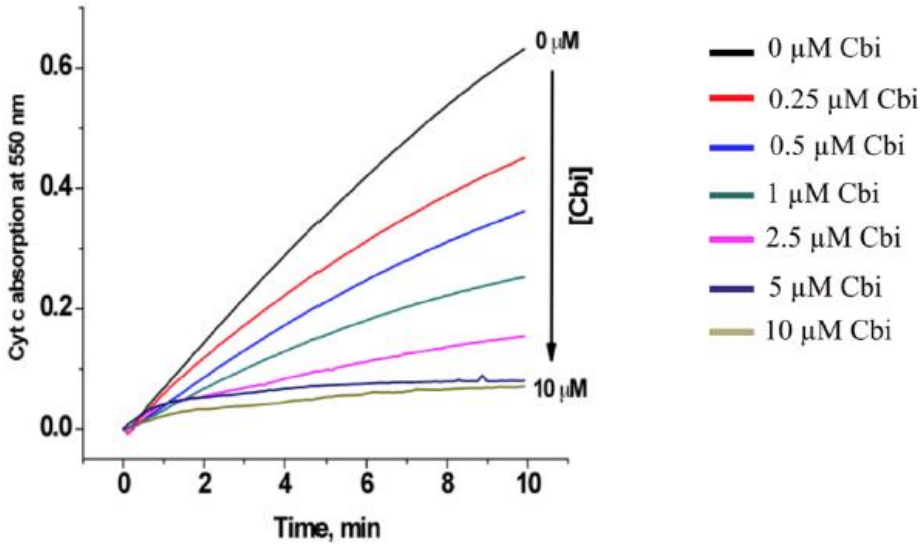

Figure 3. Cytochrome c absorbance. Absorbance reduced with cobinamide addition, indicating cobinamide potentially neutralizes O_2^-. Cytochrome c absorbance was measured for 10 minutes at 550 nm. Different colors correlate to the concentration of Cbi used in the sample.

MATERIALS AND METHODS

2.1 Materials

Aminotetrazole cobinamide, referred to as cobinamide throughout the text, was synthesized from aquohydroxl cobinamide by adding the aminotetrazole ligand. A caesium-137 irradiator in the Medical Teaching Facility (MTF) at University of California, San Diego was utilized for all irradiations. Hypoxanthine was obtained from Sigma-Aldrich (hypoxanthine, H9636, Sigma-Aldrich, St. Louis, MO, USA). Xanthine oxidase was obtained from Sigma-Aldrich (xanthine oxidase from bovine milk, X476-5UN, Sigma-Aldrich, St. Louis, MO).

2.2 Cell Culture

HEK293A cells were grown in Gibco DMEM 1X (Dulbecco's Modified Eagle Medium), which contained 4.5 g/L glucose, L-glutamine, and sodium pyruvate. Medium was additionally supplemented with 10% fetal bovine serum (FBS) obtained from Sigma-Aldrich. Cells were kept at 37 °C at 5% CO_2 atmosphere. HEK293A cells were derived from human embryonic kidney cells and obtained from ATCC.

2.3 Cell counting studies

HEK293A cells were plated into 24-well plates, only in the 16 wells that make up the perimeter of the plate, at 14,000 cells/well 24 hours prior to treatment. Four conditions were tested: untreated, irradiated alone, cobinamide alone, or irradiation combined with cobinamide. Experiments were performed in duplicates at least three times. Cells were incubated with respective conditions for indicated times, before cobinamide was removed, and the cells were released for 24-hours in fresh medium. After 24 hours, cell number was assessed using a hemocytometer.

2.4 pH tracking studies

25 μM cobinamide was plated into 24-well plates lacking cells. pH measurements were taken immediately prior to irradiation, and then immediately after irradiation. pH was measured using an Orion Star A111 pH Benchtop Meter.

2.5 UV-Vis Spectrophotometry

The UV-Vis spectrum of cobinamide was measured using a Kontron 860 Spectrophotometer. Superoxide binding curves were generated at a constant concentration of 25 μM cobinamide.

2.6 Statistical Analyses

One-way ANOVA with matched pairs and Bonferroni correction was used to analyze significance in selected conditions for cell culture experiments. Geisser-Greenhouse corrections were not applied. Conditions considered significant had a p value < 0.05. analyses were performed on Prism 7.0.

RESULTS

3.1 Cobinamide is Reduced in the Presence of Superoxide

We first sought to confirm that cobinamide could react with superoxide. While prior experiments presented in Figure 3 showed that cobinamide reacts with superoxide, the results were characterized by cytochrome C absorbance. Due to this, we did not know if cobinamide was being converted into a reduced state or if another chemical interaction was occurring. To rectify this, we wanted to directly analyze cobinamide's reaction with superoxide by looking at reduced species of cobinamide. To this end, spectrophotometric analysis was conducted on samples containing hypoxanthine, xanthine oxidase, and cobinamide. Figure 4a describes the expected chemical species, where Cbi^{+3} will interact with a superoxide anion generated from catalyzing the reaction of hypoxanthine to xanthine utilizing xanthine oxidase to Cbi^{+2}. Figure 4b describes the physical set-up: 2 mL of 25 µM aquohydroxyl cobinamide and 10 µL of 10 mM hypoxanthine were added to a glass cuvette and deoxygenated with argon gas for 30 mins to remove $O_{2(g)}$. Next, 10 µL of a solution containing 5mg of xanthine oxidase per 50 µL of dipotassium phosphate buffer (pH 7.0) was added to the cuvette. Spectrophotometric analysis was done at time points of 0 time (right before xanthine oxidase addition), 1 minute, 5 minutes, 10 minutes, 15 minutes, 20 minutes, and 30 minutes after xanthine oxidase addition. A slight shift in the cobinamide spectrum is detectable 1 minute after xanthine oxidase addition, and the shift becomes more pronounced as time passed (Figure 4c). Upon comparison to spectrophotometric analysis graphs of Cbi^{+2} generated from reacting Cbi^{+3} with ascorbic acid, it was concluded that the two graphs were almost identical, indicating that cobinamide had been reduced. It was also revealed that cobinamide was reduced at a linear rate after looking at the change in absorbance over the 30 minutes at the wavelength 311 nm. It was concluded that cobinamide is reduced in the presence superoxide.

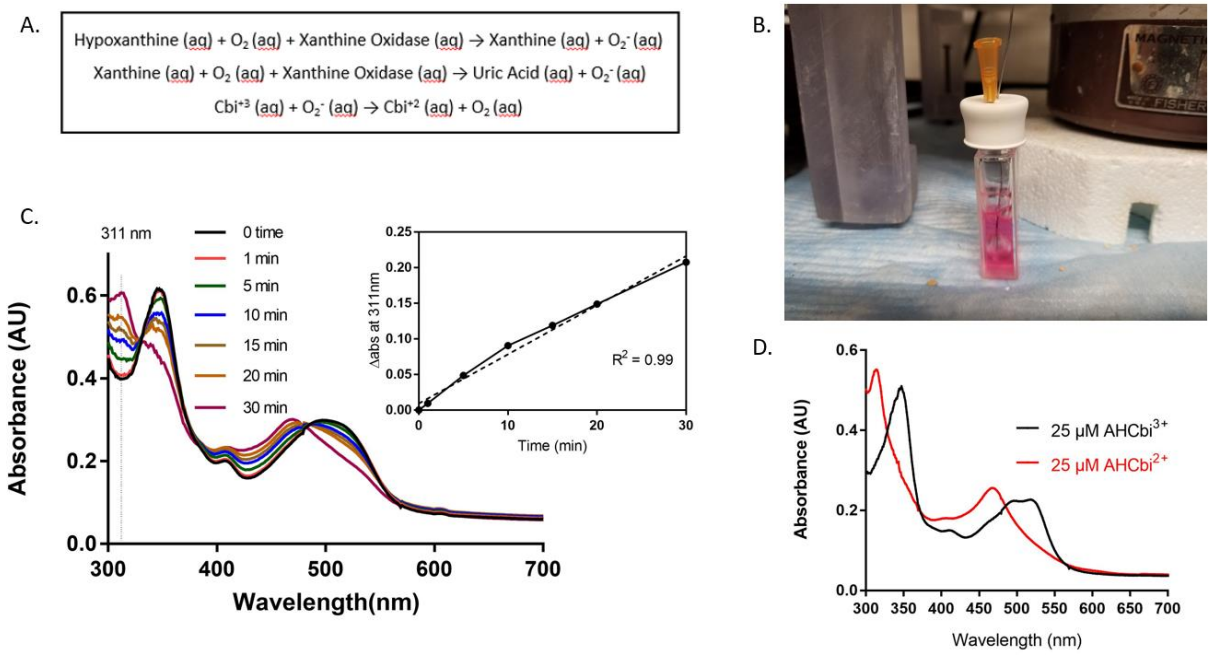

Figure 4. Cobinamide is reduced in the presence of superoxide. (A) Proposed mechanism of cobinamide reduction in the presence of superoxide. (B) The experimental design for the UV-Vis spectrophotometry analysis. The glass cuvette contains aquohydroxyl cobinamide and is covered by a rubber stopper. A needle is inserted into the rubber stopper to relieve internal pressure as argon gas is bubbled through the cobinamide solution to dislodge O_2 gas. (C) Spectrophotometry analysis of cobinamide in the presence of superoxide over a 30 minute time span. (D) Spectrophotometric analysis of cobinamide reduced by ascorbate acid provided by John Tat

3.2 Cobinamide as a Superoxide Dismutase Mimetic

Superoxide dismutase (SOD) is an endogenous defense system against O_2^-, the latter of which are produced upon radiation-induced radiolysis of water. If cobinamide could additionally act as a SOD mimetic, it would then expand the therapeutic value of this chemical. To assess this possiblity, we irradiated 25 µM cobinamide (III) under the hypothesis that after Cbi^{+3} has reacted with superoxide, it would be reduced to Cbi^{+2}. However, Cbi^{+2} would donate an electron to another superoxide anion and two hydrogen ions. In this process, H_2O_2 and Cbi^{+3} will be formed. The generation of H_2O_2 from O_2^- would make cobinamide a SOD mimetic (Figure 5a).

Without an ability to directly measure H_2O_2 levels, we sought to measure changes in pH as a surrogate readout for H_2O_2 production based on our predicted chemical reactions. We found a significant increase in pH in the cobinamide solution after irradiation, whereas the water control remained unchanged (Figure 5b). The evidence suggests cobinamide could potentially act as a superoxide dismutase mimetic.

A.

$$Cbi^{+3}\,(aq) + O_2^{-}\,(aq) \rightarrow Cbi^{+2}\,(aq) + O_2\,(aq)$$

$$Cbi^{+2}\,(aq) + O_2^{-}\,(aq) + 2H^{+}\,(aq) \rightarrow H_2O_2\,(aq) + Cbi^{+3}\,(aq)$$

B.

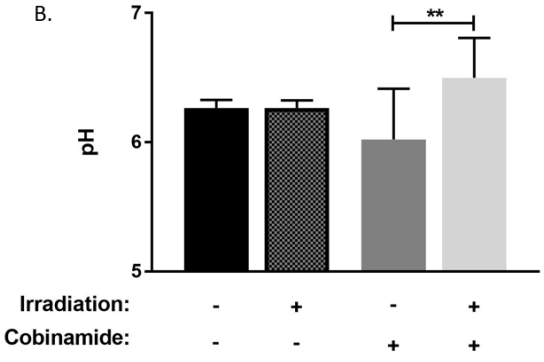

Figure 5: Cobinamide as a superoxide dismutase mimetic. (A) The proposed chemical reaction. (B) Irradiated samples of aquohydroxl cobinamide displayed a rise in pH immediately post-irradiation. The graph is composed of 6 independent experiments. Data are presented as normalized means ± standard deviation. One-way ANOVA with matched pair and Bonferroni correction was used to determine statistical significance; two asterisks (**) represents $p<0.01$.

3.3 Cobinamide is a radioprotective agent

Chemical studies support the notion that cobinamide could be radioprotective via acting as an ROS scavenger and superoxide dismutase mimetic. To test cobinamide's radioprotective efficacy *in vivo*, we used HEK293A cells for these experiments. HEK293A cells are human in lineage, easily manipulative, and grow rapidly. To perform these experiments, cells were first seeded for 24 hours before irradiation. 15 minutes before irradiation, 50 μM cobinamide was administered. Cells were then irradiated. And then cobinamide was removed either 1-hour post-irradiation or 24 hours later. We found that incubation at both 1 hour and 24 hours increased cell counts by 10% but neither time point showed significant difference from each other. Thus, releasing cells 1 hour or 24 hours post-irradiation made no difference, as long as cobinamide was administered prior to irradiation. Most importantly, the findings showed that Cbi could act as a radioprotective agent.

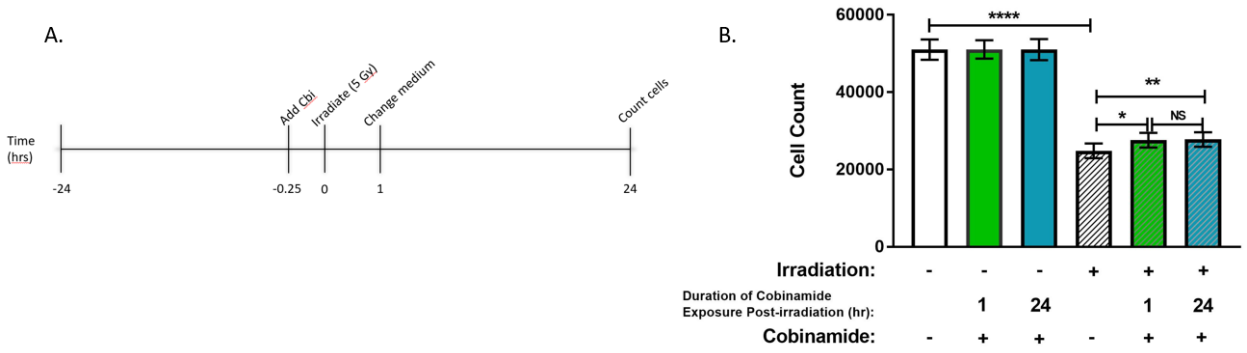

Figure 6: 50 μM cobinamide pre-incubation protected cells from radiation toxicity. (A) Experimental timeline. (B) 50 μM cobinamide pre-incubation increased cell counts by approximately 10% regardless of whether cobinamide was removed 1 hour or 24 hours post-irradiation. The graph is composed of four independent experiments. Data are presented as normalized means ± standard deviation. One-way ANOVA with matched pair and Bonferroni correction was used to determine statistical significance; one asterisk (*) represents $p<0.05$, two asterisks (**) represents $p<0.01$, and four asterisks (****) represents $p<0.0001$

3.4 Cobinamide Pre-Incubation at 100 μM has Time-Dependent Effects on Cell Survival and Cobinamide Toxicity

Therapeutic effects are a function of treatment time and drug concentration. Having been shown that 50 μM cobinamide increased cell count by 10%, we next asked whether pre-incubation with 100 μM of cobinamide would have a larger effect on cell survival. Cells were seeded 24 hours before irradiation. 100 μM cobinamide was administered 15 minutes before irradiation. Cells were then released from cobinamide at various timepoints ranging from immediately after irradiation (t = 0) to 1 hour after irradiation. Cells were counted 24 hours after irradiation. We saw that cells released at the 0^{th} timepoint improved their count by ~50% and suffered no cobinamide-induced toxicity. At time points of 15 min. and 30 min., while cell counts also improved by 50%, unirradiated control cells that had been pre-incubated with 100 μM cobinamide exhibited 5% and 7% decrease in cell number, respectively. More worrisome, while cells released 1 hour after irradiation also improved cell count by ~50%, the unirradiated control cells with cobinamide pre-incubation decreased cell count by approximately 11%. Collectively, while 100 μM cobinamide better protected cells than 50 μM cobinamide, total exposure to 100 μM cobinamide cannot be more than 30 minutes total, highlighting that this potential drug has adverse side effects.

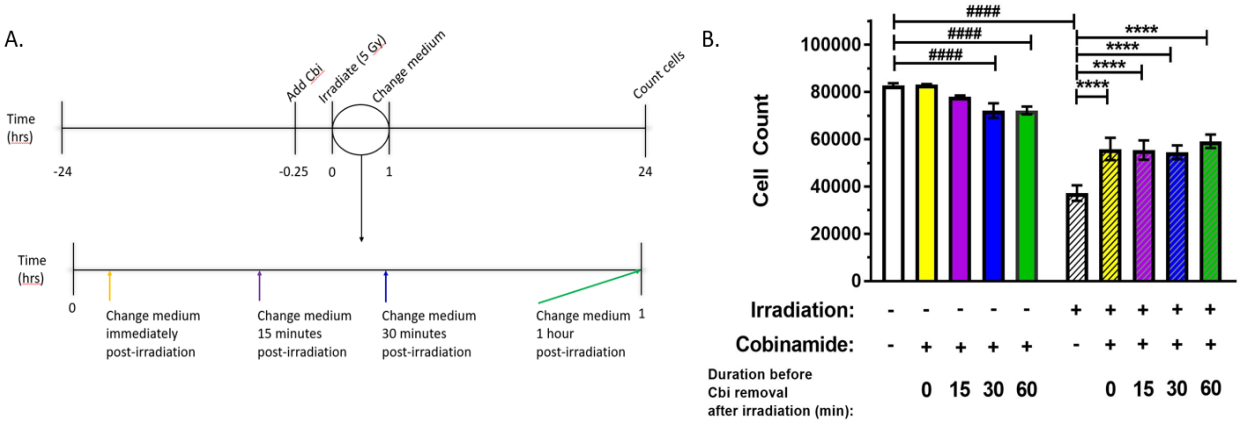

Figure 7: 100 µM cobinamide pre-incubation for 15 minutes improves cell counts by 50%. (A) Experimental timeline. (B) 100 µM cobinamide preincubation significantly improved cell counts but has time-dependent toxic side effects. The graph is composed of three independent experiments. Data are presented as normalized means ± standard deviation. One-way ANOVA with matched pairs and Bonferroni correction was used to determine statistical significance; four asterisks/hash marks (****/####) represents $p<0.0001$.

3.5 Cobinamide Partially Rescues Irradiated Mammalian Cells Up to an Hour After Irradiation

Having been shown that cobinamide has radioprotective properties, we wanted to test whether cobinamide could rescue cells after radiation exposure. After seeding for 24 hours, cells were irradiated with a dosage of 5 Gy (Figure 8a). Cells then received cobinamide immediately after irradiation (t=0), or up to 1 hour after irradiation. Cobinamide remained in the media until cells were counted 24 hours after irradiation. We found that cobinamide administration resulted in a 10% increase in cell counts, even when treatment was delayed by 1 hour (Figure 8b). These results show that cobinamide could serve as a radio-mitigative agent.

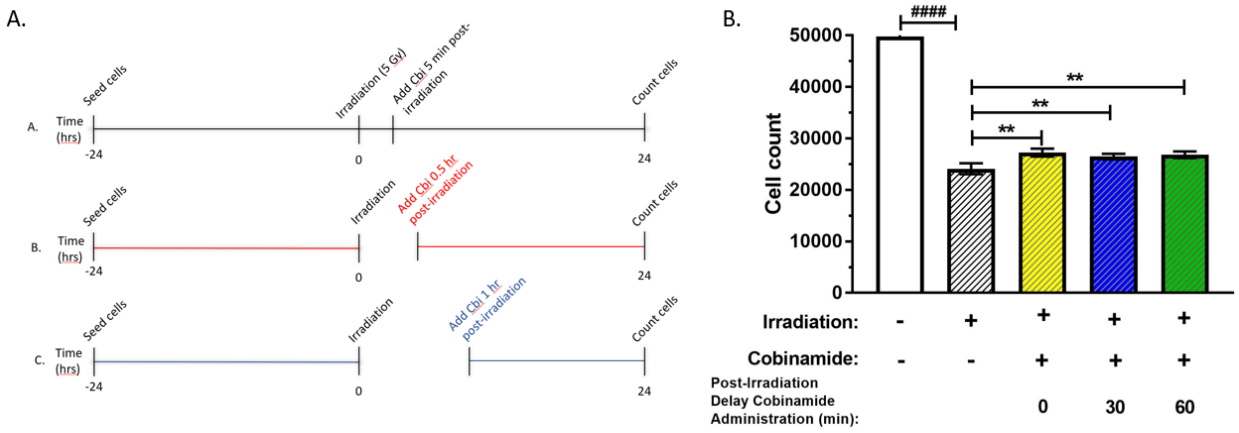

Figure 8: Delayed cobinamide addition partially rescues irradiated cells. (A) Experimental timeline (B) 50 µM cobinamide addition increased cell counts by 10%, even when delayed up to an hour after irradiation. The graph is composed of 3 independent experiments performed in duplicates. Data are presented as normalized means ± standard deviation. One-way ANOVA with matched pair and Bonferonni correction was used to determine statistical significance; two asterisks (**) represents $p<0.01$, and four hash marks (####) represents $p<0.0001$.

Results are currently being prepared for submission for publication of the material. Boss, Gerry; Chang, Stephen; and John Tat. The thesis author will be co-author of this material.

Discussion

Between 1980 and 2013, 2390 cases of radiation exposure of 1 Gy or more were reported, with 190 casualties [16]. Presently, there is no therapy for radiation poisoning, and therefore current treatments rest on decontamination and symptom alleviation. Thus there is a need for an effective therapeutic that can alleviate radiation-induced oxidative damage on a molecular level.

Previous work done by Bryan Nguyen, a Master's candidate in the Boss Lab, showed that cells treated with cobinamide were resistant towards the harmful effects of radiation [17]. While insightful, Nguyen's work exhibited three deficiencies: (1), there was no mechanistic work, (2) he used HeLa and MDA-MB-231 cancer cells, which may be more resistant to radiation damage than non-cancerous cells, and (3) the formulation of cobinamide at that time was less pure. Given that the formulation of cobinamide in the Boss Lab has progressed since the last study on cobinamide and radiation, the purpose of my thesis work, therefore, was to address the weaknesses mentioned above.

We found that cobinamide (III) was reduced in the presence of superoxide, signifying that cobinamide is a ROS scavenger (Figures 3-4). Next, we found that cobinamide could potentially act as a SOD mimetic by producing hydrogen peroxide from superoxide anions. This mechanism expands the therapeutic value of cobinamide. Moreover, given that this cobinamide is constantly being reduced and oxidized, this suggests that cobinamide's therapeutic properties are regenerative and therefore long-lasting (Figure 5). Additional studies are needed to show whether cobinamide truly is a SOD mimetic.

When tested *in vivo*, we found evidence supporting the notion that cobinamide could act as a radioprotective agent (Figures 6-7). Most importantly, we found that preincubation of 100 μM cobinamide for 15 minutes, could improve cell counts by ~50% if the cobinamide was then

removed within 15 minutes post irradiation (Figure 7). Finally, we also wanted to investigate the efficacy of cobinamide as a radiomitigative agent. It was found that delayed treatment of cobinamide even 1 hour after irradiation improved cell counts by 10%. While not large, the improvement is significant and shows a positive trend towards rescue.

In conclusion, radiation poisoning poses serious health threats to humans. Currently, there is no effective radiation poisoning therapy. This study explored the potential development of cobinamide as a radiation poisoning treatment with promising results. Future experiments should focus on determining the mechanism of cobinamide rescue from radiation poisoning. Potential *in vitro* experiments include Western blot analysis of stress-activated kinases, such as JNK, in radiated cells; lipid peroxidation assays; OxyBlotting; and 8-hydroxydeoxyguanosine assays to test for oxidative stress in lipids, proteins, and DNA respectively. Finally mouse survival studies will be useful in determining the effects cobinamide has on radiation poisoning in a complex animal. Success in future experiments could demonstrate cobinamide's potential to be a radiomitigative and radioprotective agent for radiation exposure and may eventually be utilized as a treatment for radiation exposure in clinical settings.

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