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Article

Impact of gadodiamide (Omniscan) on a beef liver catalase *ex vivo* model

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SUMMARY

Gadolinium is used in Magnetic Resonance Imaging scans to enhance image quality and allow doctors to more effectively diagnose patients. It is administered to patients through intravenous injection of gadolinium-based contrast agents (GBCAs). Although most of the injected gadolinium is excreted from the body soon after it is administered, studies have shown that some gadolinium remains in the body for extended periods of time. In patients with renal dysfunction, gadolinium retention has been associated with Nephrogenic Systemic Fibrosis, and even in patients with normal kidney function, long-term gadolinium deposits have been observed in the brain following exposure to GBCAs. The specific biochemical effects of gadolinium retention remain largely unknown, but it is possible that gadolinium may impact enzymatic activity. The present experiment investigated the effects of gadodiamide (brand name Omniscan), a common GBCA, on the function of catalase, a crucial biological enzyme that converts hydrogen peroxide into water and oxygen. We exposed beef liver to various concentrations of hydrogen peroxide both with and without gadodiamide, and we determined the V_{max} and K_m values of the catalase. We found that the presence of gadodiamide did not significantly inhibit catalase, which we hypothesize is due to the chelating agent present in gadodiamide. This work has important implications for understanding the biochemical effects of GBCAs and their potential impact on patient health.

INTRODUCTION

Gadolinium is a transition metal. In its third oxidation state, gadolinium (Gd^{3+}) exhibits strong paramagnetism due to its seven unpaired electrons (1). Gadolinium is therefore useful in magnetic resonance imaging (MRI) scans because it can change the magnetic properties of surrounding water molecules, thereby enhancing the clarity, brightness, and contrast of the images (2). The improved image quality allows doctors to better diagnose inflammation, cancer, blood vessel disease, and many other conditions (3).

Like all transition metals, gadolinium is toxic to the human body in low concentrations (3). Therefore, doctors administer gadolinium to patients undergoing MRI scans through gadolinium-based contrast agents (GBCAs), in which the gadolinium ion is tightly bound to chemicals called chelating agents (3). Chelating agents mitigate the toxicity of gadolinium but preserve its ability to enhance MRI visualization (3). After a patient undergoes an MRI scan, the vast majority of the gadolinium is excreted by their kidneys within 24 hours (3). However, gadolinium retention can occur, in which some gadolinium remains in the body for months to years after an MRI (4). In patients with impaired renal function, the retained gadolinium can cause a potentially fatal disease called Nephrogenic Systemic Fibrosis (NSF), which is characterized by the thickening and hardening of the skin and can lead to restricted motion of the joints (5). Severely afflicted individuals can lose the ability to walk (5). NSF can even affect other organs of the body such as the heart, lungs, and esophagus (5). In addition, even in people with normal renal function, research studies have shown that patients who received GBCA injections had long-term gadolinium deposits in their brains (2).

Studies have shown that some GBCAs are associated with NSF while others are not (6). The key difference appears to be how tightly the chelating agent binds to the gadolinium (6). For GBCAs with a weaker binding chelating agent, more gadolinium becomes free and displaces essential trace metals such as copper, zinc, and iron (6, 7). Free gadolinium is also linked to excessive production of collagen, triggering the fibrosis characteristic of NSF (5). Therefore, certain GBCAs are no longer used in some countries. For example, England no longer allows the use of the GBCA gadodiamide (brand name Omniscan), while other countries, such as the United States, continue to allow its use (8).

The purpose of the present experiment is to investigate the effect of GBCAs on enzymatic activity. For this study, we chose the enzyme catalase because it is an important biological enzyme present in nearly all living organisms exposed to oxygen, and its activity is easy to quantify because it produces gas (9). Catalase is responsible for the decomposition of hydrogen peroxide (H_2O_2) into water and oxygen gas (10). H_2O_2 is a reactive oxygen species that is a natural by-product of oxygen metabolism (10). Elevated H_2O_2 is associated with many types of cell damage, including DNA and protein disruption, and has been implicated in the development of Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and numerous other disorders (10). Moreover, we chose the GBCA gadodiamide for the study because it is one of the GBCAs with a chelating agent that weakly binds gadolinium and has been associated with the vast majority of NSF cases (11).

To quantify the effect of gadodiamide on catalase activity, we evaluated the rate of change of the height of foam during the H_2O_2 decomposition reaction. This foam is composed of bubbles of oxygen gas produced by the catalase reaction. Historically, biologists have characterized enzymatic activity in terms of V_{max}, the maximum rate of reaction attained when the enzyme sites are saturated with substrate, and K_m, the

concentration of substrate that permits an enzyme to achieve half of V_{max} (12). Prior studies have shown that other transition metals inhibit catalase (13). Since transition metals have similar properties, we hypothesized that gadolinium, and thus gadodiamide, would also inhibit catalase, significantly impacting the V_{max} and K_m values of catalase.

RESULTS

To determine the effect of gadodiamide on catalase activity, we combined beef liver (rich in catalase), gadodiamide, and 10 mL of H_2O_2 at concentrations of 3%, 1.5%, 0.75%, and 0.375%. We also repeated this procedure replacing gadodiamide with tap water **(Figure 1)**. Two negative controls – one for the treatments with gadodiamide and one for the treatments with tap water – were conducted without hydrogen peroxide using 10 mL of tap water. Catalase activity was measured as the rate of change of the foam height over the first 20 seconds of the hydrogen peroxide decomposition reaction. Foam height indicated the amount of oxygen gas generated by catalase. To determine the V_{max} and K_m values of catalase in the presence and absence of gadodiamide, we used a Lineweaver–Burk plot.

Both negative controls resulted in a reaction rate of 0 mL/s, confirming that the foam height in the treatments with hydrogen peroxide was attributed to the decomposition reaction and not alternative factors **(Figure 2)**. Both with and without gadodiamide, the reaction rate increased as H_2O_2 concentration increased (with gadodiamide: 0.11 M = 0.89 mL/s, 0.22 M = 1.28 mL/s, 0.44 M = 2.48 mL/s, 0.88 M = 4.87 mL/s; without gadodiamide: 0.11 M = 0.87 mL/s, 0.22 M = 1.25 mL/s, 0.44 M = 2.42 mL/s, 0.88 M = 4.59 mL/s) **(Figure 2)**.

Both with and without gadodiamide, as $1/H_2O_2$ concentration increased, 1/reaction rate increased at a constant rate **(Figure 3)**. The trendlines have large R^2 values of 0.9788 and 0.9794 for with and without gadodiamide, respectively, indicating that linear models closely fit our data **(Figure 3)**. With gadodiamide, the trendline has the slope of 0.1133 s·M/mL, the y-intercept of 0.1457 s/mL, and the x-intercept of -1.2860 1/M (Figure 3). Without gadodiamide, the trendline has the slope of 0.1152 s·M/mL, the y-intercept of 0.1543 s/mL, and the x-intercept of -1.3394 1/M **(Figure 3)**. A one-way analysis of covariance (ANCOVA) performed between the regression lines in the Lineweaver–Burk plot demonstrated that there was no statistically significant difference between either the slopes (p = 0.93) or the y-intercepts (p = 0.82) for the treatments with and without gadodiamide **(Figure 3)**.

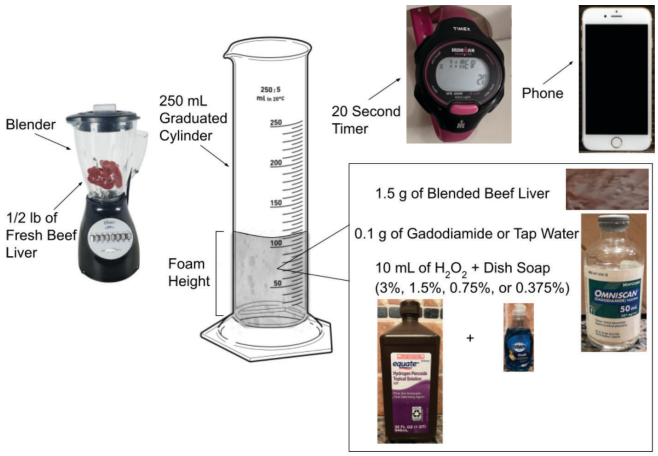

Figure 1: Overview of the experimental design. A half-pound of fresh beef liver was liquefied in a blender. 1.5 g of the blended liver, 0.1 g of gadodiamide or tap water, and 10 mL of H_2O_2 and dish soap solution (concentration 3%, 1.5%, 0.75%, or 0.375%) were combined in a 250 mL graduated cylinder. A watch timer was set for 20 seconds, after which time a photograph of the graduated cylinder was taken using a phone camera. The height of the foam produced by the reaction was measured from the image using the gradations on the graduated cylinder.

DISCUSSION

Our results contradict the original hypothesis that gadodiamide significantly inhibits the enzyme catalase. For typical enzymes, at low substrate concentrations, the reaction rate increases rapidly with increasing substrate concentration because many enzyme sites are available to bind the substrate (12). However, at high substrate concentrations, when most enzyme binding sites are filled, the reaction rate slows and ultimately plateaus when the enzyme is saturated with the substrate (12). The value of this plateau is V_{max} (12). In the current study, the H_2O_2 concentrations we tested were not high enough for the reaction rate of catalase versus the substrate concentration graph to plateau, so we used a Lineweaver–Burk plot to determine the numerical values of V_{max} and K_m. Without gadodiamide, we calculated values of V_{max} = 6.48 mL/s and K_m = 0.75 M. With gadodiamide, the data yielded the values of V_{max} = 6.86 mL/s and K_m = 0.78 M.

There are three main types of enzyme inhibition: competitive, noncompetitive, and uncompetitive (14). The type of inhibition relates to the impact of the inhibitor on the enzyme-substrate binding and whether the inhibitor binds to the active or allosteric enzyme sites (14). Competitive inhibitors increase the K_m and do not affect the V_{max}, noncompetitive inhibitors decrease the V_{max} and do not affect the K_m, and uncompetitive inhibitors decrease both the V_{max} and the K_m (14). Notably, no type of inhibition raises the V_{max}, as the V_{max} represents the maximum reaction rate of the enzyme, and increasing it would not be an inhibitory effect. In the current study, according to the computations based on the Lineweaver–Burk plot, the presence of gadodiamide resulted in a higher Vmax value than when it was absent, indicating that gadodiamide does not inhibit catalase. To determine if the differences in the V_{max} and K_m in the presence and absence of gadodiamide were significant, we used an ANCOVA, which showed that neither the slopes nor the y-intercepts were significantly different ($p > 0.05$) **(Figure 3)**. Since the V_{max} and K_m are computed from these slopes and y-intercepts, these results suggest that gadodiamide does not significantly impact the V_{max} and K_m values of catalase and thus does not inhibit catalase.

Our original hypothesis was that gadodiamide would inhibit catalase activity. It was based on previous studies that showed that many transition metals inhibit catalase. For example, Aydemir *et al.* investigated catalase inhibition by zinc, iron, and copper (15). Zinc sulfate, iron (III) chloride, and copper (II) sulfate were shown to inhibit catalase by 50% at concentrations of 0.016 M, 0.015 M, and 0.012 M, respectively (15). Since transition metals have similar properties, including toxicity to the human body at low concentrations, we predicted that gadodiamide would inhibit catalase similarly to zinc, iron,

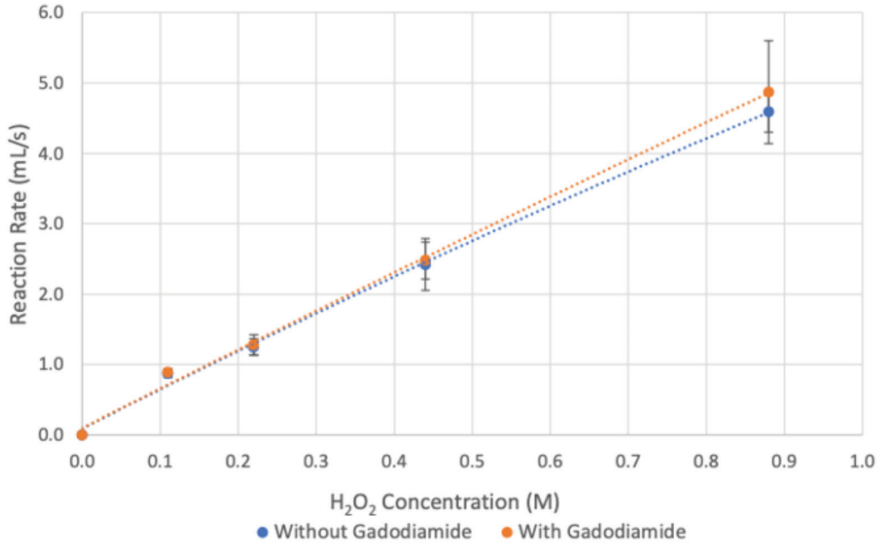

Figure 2: Effect of H_2O_2 concentration on the reaction rate for the H_2O_2 decomposition reaction as catalyzed by the enzyme catalase. H_2O_2 concentrations of 0 M (0%), 0.11 M (0.375%), 0.22 M (0.75%), 0.44 M (1.5%), and 0.88 M (3%) were tested for the treatments both with and without gadodiamide. Reaction rate was calculated by dividing the foam height after 20 seconds had elapsed on a watch timer. The dotted curves indicate polynomial trendlines, where the orange curve represents treatments with gadodiamide and the blue curve represents treatments without gadodiamide. Data points are the average of five replicates. Error bars indicate average absolute deviation (AAD).

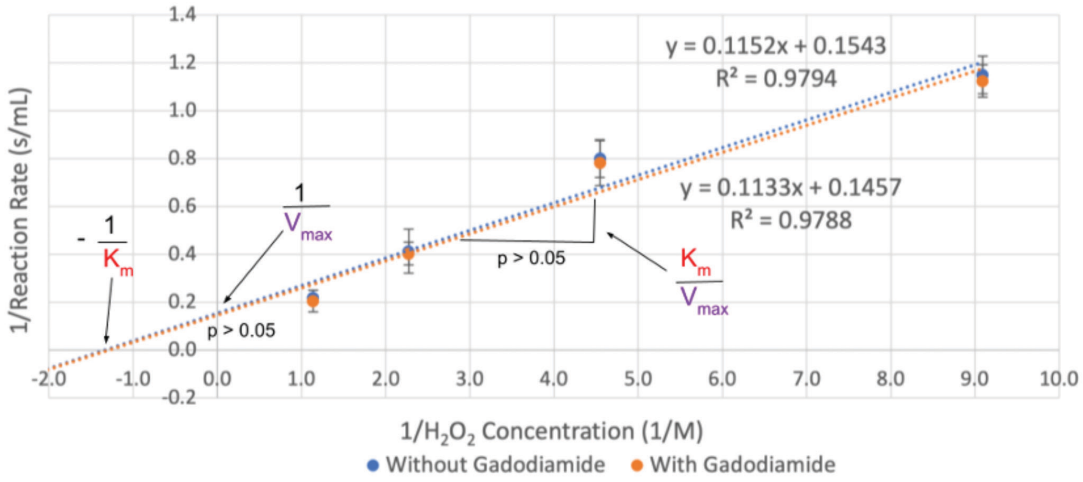

Figure 3: Lineweaver–Burk plot indicating the effect of $1/H_2O_2$ concentration on 1/reaction rate for the H_2O_2 decomposition reaction as catalyzed by the enzyme catalase. $1/H_2O_2$ concentration and 1/reaction rate were determined by computing the reciprocals of the H_2O_2 concentrations and reaction rates, respectively, displayed in **Figure 2**. The dotted fits indicate linear trendlines, where the orange line represents treatments with gadodiamide and the blue line represents treatments without gadodiamide. The x-intercepts represent $-1/K_m$, the y-intercepts represent $1/V_{max}$, and the slopes represent K_m/V_{max}. Data points result from five replicates. Error bars indicate AAD. The *p*-values between the slopes and the y-intercepts were calculated using ANCOVA.

and copper.

One possible reason why our experiment did not show inhibition of catalase may have been insufficient concentration. In the current study, the gadodiamide was diluted 1:100, resulting in a final concentration of 0.005 M. In previous studies, the concentrations of zinc, iron, and copper that caused a 50% reduction in catalase activity were about 2.5-3 times higher than the tested gadodiamide concentration in our experiment (15). However, investigating a higher gadodiamide concentration may not be clinically relevant. In medical practice, the standard dose of gadodiamide is 0.1 mmol/kg injected intravenously (16). Soon after injection, the contrast agent distributes throughout the extracellular space. Given the typical human extracellular volume of 0.25 L/kg, the gadodiamide concentration would be expected to be 0.0004 M after equilibration through the extracellular space (17). Therefore, the concentration of gadolinium in the present *ex vivo* experiments is roughly an order of magnitude higher than the equilibrium concentration of gadolinium, which should have been sufficient to demonstrate inhibition if there was any.

Nonetheless, the fact that our *ex vivo* experiments did not show any significant inhibition of catalase does not entirely exclude the possibility that gadodiamide biochemically inhibits

catalase *in vivo*. While the concentration of gadodiamide used in the *ex vivo* experiments is higher than the estimated steady-state concentration of gadodiamide *in vivo*, it is possible that certain cells are exposed to higher concentrations than other cells. For example, during the initial injection, blood cells near the injection site would be exposed to gadodiamide at higher concentrations. The same would be true of kidney and urinary bladder cells that are involved in gadolinium excretion. Also, it is possible that some cells could actively transport gadodiamide into the intracellular space, thereby increasing its concentration above its steady-state concentration. Therefore, a higher gadodiamide concentration than that tested in the present experiment could potentially be clinically relevant and may inhibit catalase.

A potential limitation of the current study is the fact that the catalase reaction was observed for only 20 seconds, while a longer exposure may have yielded different results. The gadolinium in gadodiamide is bound to a chelating agent designed to mitigate its toxicity (3). The presence of the chelating agent may prevent the gadolinium from inhibiting catalase. Notably, zinc, iron, and copper were not bound to chelating agents when catalase inhibition was demonstrated by Aydemir *et al.* (15). Prior studies suggest that GBCA toxicity may be related to transmetallation, a process by which gadolinium dissociates from its chelating agent and displaces other naturally occurring transition metals in the body (6, 7). The likelihood of transmetallation increases the longer the GBCA remains inside the body (6). In patients with impaired renal function, the excretion of the GBCA is prolonged for days and weeks (5). This extended exposure is thought to exacerbate transmetallation and to be an important factor in the development of NSF (5). Perhaps the present ex vivo study did not detect catalase inhibition because the catalase enzyme was exposed to gadodiamide for only 20 seconds. Inhibition would have potentially been observed if the activity of catalase were assessed after it had been incubated with gadodiamide for several hours or even several days, a time period that may have allowed transmetallation to occur.

There were a number of sources of uncertainty in the experiment. Aydemir *et al.* previously showed that many environmental conditions can substantially affect catalase function, including pH, temperature, and ionic strength (15). To address this source of error, the *ex vivo* studies could have been performed so that these factors were controlled to mimic *in vivo* conditions. Moreover, another source of uncertainty involved the quantification of reaction rate by foam height. Some oxygen gas produced by the reaction may have exited the graduated cylinder and not been counted in the foam height. Additionally, although dish soap was added to mitigate this effect, some bubbles may have broken before the end of the observation period, preventing them from being considered in the foam height. Also, the foam height was sometimes uneven across the graduated cylinder, and determining which height to measure could have resulted in uncertainty. To rectify these errors, oxygen gas production could have been measured using water displacement. Furthermore, although the mass of beef liver per trial was kept constant at 1.5 g, the surface area was not as precisely controlled. Differences in the size of the pieces of liver between trials could have affected the results – smaller pieces would have greater surface area and would result in more catalase being exposed to hydrogen peroxide. To address this uncertainty, a more effective beef liver homogenization technique could have been implemented, perhaps by using a filter to decrease the variability of the size of the pieces of liver.

The present experiment fits into a broader context of investigating the impact of GBCAs on enzymatic activity. GBCAs were introduced in 1988 and have been administered to hundreds of millions of MRI patients across the globe over the past few decades (18). It is important to examine the potential biochemical and long-term effects of these agents. In this experiment, catalase was chosen as a model enzyme due to its biological prevalence and importance, but human cells contain numerous enzymes. It is possible that enzymes other than catalase could be more sensitive to gadolinium and be inhibited by gadodiamide, a prospect for future experimentation. In addition, gadodiamide is only one GBCA, and future studies could examine the effects of other GBCAs, particularly those of different chemical structures and bonding natures. Gadodiamide is characterized as linear and non-ionic, but other GBCAs that could be investigated are linear and ionic, macrocyclic and non-ionic, and macrocyclic and ionic (6). Furthermore, an important future experiment could replicate the current study, but incubate the catalase with gadodiamide for an extended period of time to be more representative of *in vivo* conditions. If this experiment showed inhibition of catalase, perhaps it would be necessary for the medical community to reconsider the administration of GBCAs for MRI scans, particularly gadodiamide and other compounds with weak binding chelating agents. This assessment could be especially relevant for neurological disorders, as gadolinium retention has been confirmed in the brain, and catalase inhibition could result in oxidative stress, a phenomenon linked to many devastating neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (2, 10). These studies may help radiologists assess whether the enhanced MRI images are worth the risks associated with the administration of GBCAs.

In summary, the current *ex vivo* study did not show inhibition of catalase by gadodiamide. However, the toxicity of gadodiamide in patients with renal dysfunction, the known inhibitory effects of other transition metals, and the phenomenon of transmetallation suggest that perhaps incubating gadodiamide with catalase for a prolonged period of time may be more effective in detecting potential catalase inhibition.

MATERIALS AND METHODS

Beef Liver

One half pound of fresh beef liver was prepared in a blender (Oster, 14 speed, all metal drive). The blender was set to "Grind" for 30 seconds, then "Liquefy" for 15 seconds. Both "Grind" and "Liquefy" were executed on the "High" setting.

H_2O_2 Stock Solution and Dilutions

A stock solution was made with 600 mL of 3% H_2O_2 (equate, Hydrogen Peroxide Topical Solution) and 1/4 teaspoon (1.25 mL) of dish soap (Dawn Ultra). Dish soap was added to increase the stability of the bubbles so the volume of oxygen gas produced could be more accurately quantified. The 3% H_2O_2 and dish soap solution was stirred to allow the dish soap and H_2O_2 to mix fully. This solution represented the

0.88 M H_2O_2 solution. To obtain the 0.44 M H_2O_2 solution, 200 mL of 0.88 M H_2O_2 solution was combined with 200 mL of tap water. To obtain the 0.22 M H_2O_2 solution, 200 mL of 0.44 M H_2O_2 solution was combined with 200 mL of tap water. To obtain the 0.11 M H_2O_2 solution, 200 mL of 0.22 M H_2O_2 solution was combined with 200 mL of tap water.

Preparing and Tesing the Reactions

To prepare the reaction, 1.5 g of blended beef liver was added to a 250 mL graduated cylinder. For the treatments with gadodiamide, 0.1 g of Omniscan (gadodiamide) Injection (NYCOMED, 287 mg/mL) was added to the graduated cylinder. For the treatments without gadodiamide, 0.1 g of tap water was added to the graduated cylinder. For all treatments, 10 mL of H_2O_2 (concentration 0.88 M, 0.44 M, 0.22 M, or 0.11 M) was immediately poured into the graduated cylinder, and a photograph (iPhone, camera app) of the cylinder was taken after 20 seconds had elapsed on a watch timer. The foam height in the photograph was estimated using the gradations on the graduated cylinder. The reaction rate was calculated by dividing this foam height by the waiting time of 20 seconds. For the negative control with gadodiamide, this procedure was performed with 1.5 g of beef liver, 0.1 g of gadodiamide, and 10 mL of tap water. For the negative control without gadodiamide, this procedure was performed with 1.5 g of beef liver, 0.1 g of tap water, and 10 mL of tap water. Five trials were conducted for each H_2O_2 concentration both with and without gadodiamide.

Computing V_{max} and K_m

A Lineweaver–Burk plot was used to calculate the V_{max} and K_m values of catalase in the presence and absence of gadodiamide. A Lineweaver–Burk plot is a graph of 1/reaction rate versus 1/substrate concentration for an enzyme (14). In ideal conditions, the graph is linear (14). According to enzyme kinetics, the x-intercept of the line is equivalent to $-1/K_m$, the y-intercept of the line is equivalent to $1/V_{max}$, and the slope of the line is equivalent to K_m/V_{max} (14). In the current study, the trendline without gadodiamide has the y-intercept of 0.1543 s/mL and the slope of 0.1152 s·M/mL. Then the V_{max} = 1/(0.1543 s/mL) = 6.48 mL/s and the K_m = 0.1152 s·M/mL x 6.48 mL/s = 0.75 M. Similarly, the trendline with gadodiamide has the y-intercept of 0.1457 s/mL and the slope of 0.1133 s·M/mL. Then the V_{max} = 1/(0.1457 s/mL) = 6.86 mL/s and the K_m = 0.1133 s·M/mL x 6.86 mL/s = 0.78 M.

Statistical Testing

One-way ANCOVA was performed between the two regression lines ("with gadodiamide" and "without gadodiamide") in the Lineweaver–Burk plot using the VassarStats website (19). k = 2 was selected (two groups of data were being compared), and the four data points for each trendline **(Figure 3)** were input (these data points represented the reciprocal of the average reaction rate that resulted from the five replicates for each nonzero H_2O_2 concentration). For all ANCOVA results, p-values less than 0.05 were considered statistically significant. First, the ANCOVA was used to determine that the slopes of the trendlines were not significantly different. Once the slopes were shown not to be statistically different, ANCOVA was used to compare the y-intercepts (20). This ANCOVA showed that the y-intercepts of the trendlines were not significantly different. Microsoft Excel was employed to produce best-fit polynomial trends **(Figure 2)** and best-fit linear trends **(Figure 3)** (21).

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