Evaluation of Tea Extract as an Inhibitor of Oxidative Stress in Prostate Cells

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SUMMARY

Oxidative DNA damage is caused by free radicals eroding the cell membrane and directly attacking DNA molecules in the cell. This DNA damage plays an important role in the development of cancer. Antioxidants remove free radical intermediates, thereby preventing cell membrane erosion and subsequent DNA damage. Tea leaves are rich in antioxidants, and studies conducted on green tea have reported numerous health benefits; however, those studies left out other types of tea. We evaluated the ability of various tea extracts to protect prostate cells from hydrogen peroxide induced oxidative damage. Cells were treated with black, oolong, green, and white tea extracts and then exposed to hydrogen peroxide for two days, followed by a quantification of viable cells using an MTS assay. Because treatment with white tea extract yielded the greatest cell viability, we hypothesized that an abundant antioxidant in white tea, epigallocatechin-3-gallate (EGCG), might also protect cells from oxidative stress. We found that, indeed, pre-treatment of prostate cells with 50 mg/mL EGCG led to a significant increase in cell viability when compared to the control group (fold change of 2.90), based on MTS assay absorbance. These data suggest that antioxidants in white tea extract, including EGCG, protect prostate cells from oxidative stress in culture, which is suggestive of the health benefits of white tea.

INTRODUCTION

In 2017, it is estimated that 1.7 million new cancer cases were diagnosed and 600,000 people died of cancer in the United States alone [1] . Cancer begins when cells in a part of the body start to divide uncontrollably. Unlike normal cells, cancer cells escape from cell death and can continue to divide and expand. If the cancer cells metastasize, or spread, to vital organs or tissues, they can disrupt the normal function of those organs and tissues, eventually leading to the patient's death.

Oxidative DNA damage plays an important role in the development of human cancers, including prostate cancer, and is caused by an oxidation reaction involving free radicals [2]. A free radical is an atom with an unpaired electron that attacks normal atoms to steal an extra electron. The victim atom, left with an unpaired electron, attacks another atom

to become stable which, in turn, starts a chain reaction. The chain reaction can erode the cell membrane and free radicals can directly attack DNA molecules, causing DNA damage, including DNA sequence mutations. In normal cells, DNA damage either triggers cell arrest to allow DNA repair response or activates cell death processes if too much damage has been sustained. If unrepaired, these mutations are propagated to daughter cells when the damaged cell divides. As the cell accumulates mutations, the chances of it acquiring a mutation in a gene important for growth or programmed cell death (or apoptosis) increase, which may lead to unchecked growth and cancer [3].

An antioxidant is a molecule that inhibits the oxidation reactions by removing free radical intermediates, thereby preventing DNA damage. They do so by being oxidized themselves and donating their extra electron to free radicals, which stabilizes them [4]. Antioxidants can be found in foods such as tea, tomatoes, berries, and beans, and one study indicated a possible beneficial role for tomato sauce-based entrees in the treatment of patients with prostate cancer [5] . In addition to cancer, antioxidants are also believed to prevent other diseases, such as heart disease, cardiovascular disease, Alzheimer's disease, and arthritis [6].

Tea leaves are rich in antioxidants. Many studies have been conducted on green tea and reported numerous health benefits [7]. However, those studies left out other types of tea, which may be more promising than green tea. For example, white tea has a higher level of epigallocatechin-3-gallate (EGCG) (8.00 g/100 g of white tea, 6.75 g/100g of green tea), a form of antioxidant which could provide better protection again oxidative stress than other types of tea [8]. In this study, various tea extracts were evaluated to determine which type of tea will best protect cells from oxidative stress. Human prostate tissues are vulnerable to oxidative stress. In this study, we choose RWPE-1 cells, the immortalized human normal prostate epithelial cell line, as the model system. The different types of tea (green, white, black, and oolong) were the independent variable, and the fold change in viable cells, relative to the control group, was the dependent variable. Since white tea is thought to have the most antioxidants, we hypothesized that if hydrogen peroxide were used to induce oxidative stress, the group treated with the white tea extract would receive better protection, resulting in a higher detection of viable cells, than other types of teas. The control group consisted of cells which received an equal amount of the
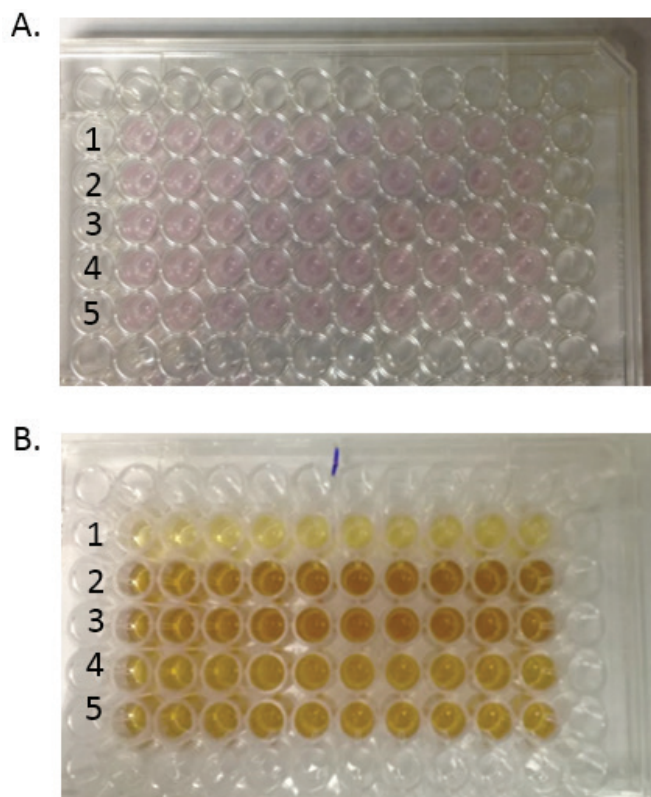
Figure 1: Images of the 96-well plate before (A) and after (B) MTS assay. 1: Control, 2: white tea, 3: green tea, 4: black tea, 5: oolong tea.

solvent that we used to make the tea extracts.

This study also explored the mechanism by which white tea extract provided the cells with the best protection under oxidative stress. Since EGCG is a major antioxidant in white tea [9], we hypothesized that if hydrogen peroxide was used to induce oxidative stress, the group treated with the highest concentration of EGCG would receive the best protection and more cells would survive. The concentration of EGCG (0, 1, 10, 25, and 50 µg/mL) was the independent variable, and the fold change in viable cells, relative to the control group, was the dependent variable. The control group was the group of cells which received 0 µg/mL of EGCG.

RESULTS

Since 0.2 µM hydrogen peroxide has been reported to induce oxidative stress and subsequent cell death in multiple cell lines (Burkhardt et al.), we performed a preliminary study to determine whether this dose would trigger oxidative stress in RWPE-1 cells [10]. We treated the cells with 0.2 µM of hydrogen peroxide for 24 hours and observed the cells under a light microscope. We observed massive cell death and the number of viable cells was much less than we initially seeded. We further stained the cells with trypan blue solution, a stain indicating dead cells with a damaged cell membrane, but not healthy viable cells, and confirmed that such a dose can kill the majority of RWPE-1 cells.

The effect of different tea extract types on protecting the prostate cells upon oxidative stress was determined by the MTS assay. The MTS assay is a colorimetric assay for sensitive quantification of viable cells. The underlying principle is that the NAD(P)H-dependent dehydrogenase enzymes in metabolically active cells reduce MTS tetrazolium compound into a colored formazan product, which can be quantified by measuring the absorbance at 490-500 nm. The assay was frequently used for the analysis of cytotoxic compounds like anticancer drugs and many other toxic agents. Before treatment, all wells were a light pink color (**Figure 1A**). After the MTS assay was completed, each well became a yellow or brown color (**Figure 1B**). The darker color indicated higher cell viability. Prostate epithelial cells were subjected to 5 different treatments: solvent (as a control), green, white, black, or oolong tea extract. After 24 hours of incubation, cells were challenged with hydrogen peroxide, and then incubated for an additional two days. Viable cells in each well were quantified by MTS assays. The absorbance at 490 nm of the control group was set to 1.00, and the other groups were normalized to the control group based on their average MTS assay absorbance (**Figure 2**). All the groups treated with tea extracts showed positive effects on cell protection against oxidative stress. Based on MTS assay absorbance relative to the control group, the white tea-treated group had a 2.26-fold change in viable cells and the green tea-treated group showed a 2.08-fold change in viable cells. To a lesser extent, the black tea and oolong tea-treated groups displayed a 1.63-fold and 1.58-fold change in viable cells, respectively. There was a statistically significant difference in viable cells between the control and each tea extract-treated group (**Figure 2**). Among the four tea extract treatment groups, the white tea treated group had the most viable cells, supporting the hypothesis.

Since white tea exhibited the highest protective effect against oxidative stress (**Figure 2**) and EGCG is a major antioxidant and prominent catechin in white tea [5], we hypothesized that if hydrogen peroxide was used to induce oxidative DNA damage, EGCG would protect cells in a dose-dependent manner. To test this hypothesis, the effect of different levels of EGCG on protecting the prostate cells from oxidative stress was tested. Prostate epithelial cells were treated with different concentrations of EGCG. After 24 hours of incubation, cells were treated with hydrogen peroxide, and incubated for an additional two days. Live cells in each well were quantified by MTS assays. The average MTS assay absorbance at 490 nm for each treatment group was normalized to the control group. All the treatment groups showed a protective effect against oxidative stress (**Figure 3**). The group treated with 50 µg/mL of EGCG had a 2.90-fold change in average viable cells with a standard deviation of 0.089, while the 25 µg/mL EGCG group showed a 1.95-fold change in average viable cells with a standard deviation of 0.074, relative to that of the control group. To a lesser extent, the 10 µg/mL and 1 µg/mL EGCG-treated groups displayed
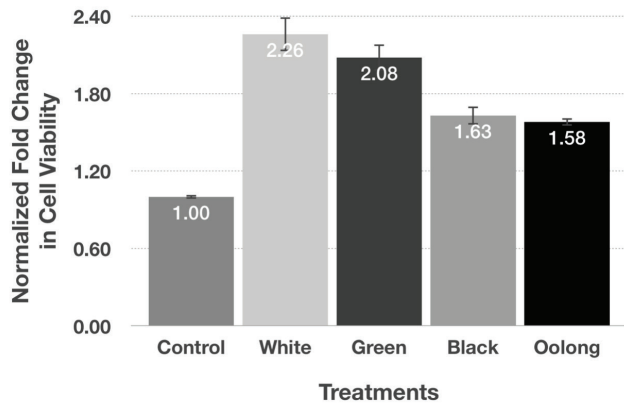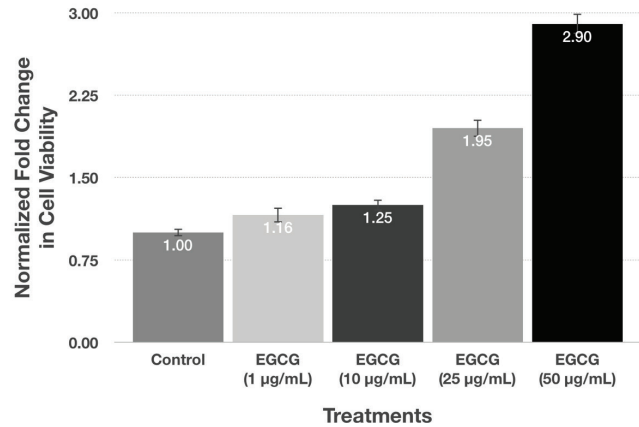
Group	Control	White	Green	Black	Oolong
Average Fold Change (relative to control)	1.00	2.26	2.08	1.63	1.58
Standard Deviation	0.015	0.132	0.102	0.070	0.034
p-Value		1.8 x 10^-10	4.9 x 10^-11	1.2 x 10^-10	1.9 x 10^-15

Figure 2: The effect of various tea extracts (white, green, black, oolong, or solvent only) on protecting prostate cells from hydrogen peroxide-induced oxidative stress. The data are represented as fold changes in viable cells in relation to the control group. Bars represent the mean values; error bars represent the standard deviation.

Group	Control	EGCG (1 ug/mL)	EGCG (10 ug/mL)	EGCG (25 ug/mL)	EGCG (50 ug/mL)
Average Fold Change (relative to control)	1.00	1.16	1.25	1.95	2.90
Standard Deviation	0.034	0.068	0.051	0.078	0.093
p-Value		2.7 x 10^-5	1.1 x 10^-9	8.6 x 10^-6	1.7 x 10^-15

Figure 3: The effect of different concentrations of the antioxidant EGCG on protecting prostate cells from hydrogen peroxide-induced oxidative stress. The data are expressed as the fold changes in viable cells in relation to the control group. Bars represent the mean values; error bars represent the standard deviation.

a 1.25-fold and 1.16-fold change in average viable cells, respectively. The standard deviation for those two groups were 0.049 and 0.064, respectively. Therefore, the higher the EGCG level, the better the protection. A linear regression t-test performed on the logarithmic relative viable cells produced a high r-squared value of 0.988, indicating strong positive correlation between EGCG concentration and cell viability as assessed by the MTS assay. Among the four treatment groups, the 50 μg/mL treatment resulted in the most viable cells, supporting the original hypothesis.

DISCUSSION

The two main purposes of this study were to determine the effect of different types of tea on protecting prostate cells against oxidative stress and to investigate the mechanisms underlying this effect. In this study, the effect of four different types of tea extract on the protection of prostate cells from oxidative stress was assessed. According to the results, when prostate cells were treated with white tea extract, it resulted in the highest cell viability, followed by green tea, black tea, and oolong tea. While all four types of tea helped to protect the cells from oxidative damage, white tea extract exhibited the highest protective effect. Since average survival rate of the white tea group was the highest, the hypothesis was supported. To further explore the working mechanism by which white tea has the highest protection effect against oxidative stress, the effect of different concentrations of EGCG, a major antioxidant in white tea, on protection of

prostate cells from oxidative stress was assessed. While all concentrations of EGCG tested helped to protect prostate cells from oxidative damage, the 50 μg/mL treatment exhibited the highest protective effect, suggesting that the EGCG in the white tea plays an important role in protecting the prostate cells from oxidative assault experimentally.

Related studies regarding antioxidants and how they could prevent oxidative DNA damage and their health benefits have been reported. One study experimented on how the antioxidant melatonin could help protect cells against oxidative DNA damage induced by Chromium (III) and hydrogen peroxide [11]. Another study found that consumption of teas rich in catechins could reduce body fat [12]. In another study, Dr. Assuncao found that the catechins in green tea slows down the brain function decline during aging [13]. However, this was the first study to evaluate the protective effect of different types of tea on protecting prostate cells from oxidative stress.

Since white tea contains multiple antioxidants, this study could be expanded on by exhaustively testing different antioxidants to determine which has the greatest protective effect on prostate cells. Furthermore, future studies should evaluate DNA damage directly in oxidative stress-insulted cells in the presence and absence of tea extracts and in alternate cell lines. The goals of this study were to explore the health benefit of teas, especially white teas, and to elucidate their underlying mechanisms. This study could benefit everyone in the community and could inspire future investigations.

Since oxidative DNA damage is closely associated with many human diseases, this study should be of great importance towards improving human health.

METHODS AND MATERIALS
Preparation of a variety of tea extracts

To make tea extracts, 5 grams (g) of each tea (black, green, white, and oolong tea) were added to 10 milliliters (mL) 70% ethanol. These tubes were then wrapped with aluminum foil and shaken once a day for one week while being stored in a dark place.

Preparation of different concentrations of EGCG

Four different concentrations of EGCG (1 microgram (µg)/mL, 10 µg/mL, 25 µg/mL and 50 µg/mL) were tested. To make a 5 milligram (mg)/mL stock solution of EGCG, 5 mg of EGCG (Catalog#:E3768, Sigma) was dissolved in 1 mL of distilled water and filtered through a 0.2 micron filter. To make a 2.5 mg/mL stock solution of EGCG, 250 µL of 5 mg/mL of EGCG was mixed with 250 µL of sterilized distilled water. To make a 1 mg/mL stock solution of EGCG for group B, 100 µL of 10 mg/mL of EGCG was added to 400 µL of sterilized distilled water. To make a 0.1 mg/mL stock solution of EGCG, 490 µL of sterilized distilled water was added to 10 µL of 5 mL/g of EGCG.

Cell treatments

RWPE-1 cells, immortalized human normal prostate epithelial cells, were maintained in Keratinocyte-SFM medium supplemented with recombinant human Epidermal Growth Factor (rhEGF) and Bovine Pituitary Extract (BPE) at 37 oC in an incubator under 5% CO2 conditions. To determine the effect of various tea extracts on protecting cells from oxidative stress, five groups were set up: a control group and 4 treatment groups with each treated with 1 of the 4 tea extracts. Prostate epithelial cells (RWPE-1 cells) were counted by hemocytometer and then diluted to 2.5 x 104/mL. 200 µL of diluted cells were seeded in 50 wells of a 96-well plate (10 trials per group), and incubated at 37 oC. On the following day, 10 µL solvent (70% ethanol) was added to each of the control wells and 10 µL of each tea extract was added to each of the treatment wells. After 24 hours of incubation, cells were treated with 0.2 µM of hydrogen peroxide, and then incubated for an additional two days. Viable cells in each well were quantified by MTS assay according to protocol below.

To determine the effect of different concentrations of EGCG on protecting cells from oxidative stress, 5 groups were set up: 0, 1, 10, 25, and 50 µg/mL of EGCG. Prostate epithelial cells were diluted to 2.5 x 104 /mL. A volume of 200 µL of diluted cells were seeded in 50 wells of a 96-well plate (10 trials per group) and incubated at 37 °C in an incubator. A volume of 2 µL of 0.1 mg/mL of EGCG stock solution was added to each well with ten trials (Figure 1A). The same procedure was followed for the remaining solutions. The ten control trials were treated with 2 µL of sterilized distilled water. After 24 hours of incubation, each well of cells was treated with 0.2 micromoles (µM) of hydrogen peroxide. The cells were incubated for an additional two days. Live cells in each well was quantified using MTS assay. The relative number of viable cells was calculated by dividing the average absorbance of each test group by the average absorbance of the control group.

MTS assay

20 µL of MTS solution was added to each well. Cells were incubated for four hours. Absorbance of each well was read at 490 nm and absorbance of culture medium containing 10 µL of 70% ethanol was subtracted from that of the cells. The average absorbance of each group was calculated. The relative viable cells were calculated by dividing the average absorbance of each test group by the average absorbance of the control group.

Statistical analysis

A t-test was performed between the control group and each experimental group. A linear regression t-test was performed to determine a correlation between EGCG level and relative viable cells.

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