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Research Article

Flaxseed Secoisolariciresinol Diglucoside Provides Protection to PC12 Cells Exposed to Beta-Amyloid, a Common Risk Factor for Alzheimer's Disease

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Abstract

Objective: This research determined the effects of antioxidant, secoisolariciresinol diglucoside (SDG), found in flaxseed, on beta-amyloid (BA)-induced oxidative stress in PC12 cells.

Methods: Cells were treated with 50 or 100 µM SDG, 100 U/ml superoxide dismutase (SOD), or 100 U/ml catalase (CAT). Then, cells were treated with 10 µM BA. Reactive oxygen species (ROS) accumulation, cell damage, and cell viability were determined.

Results: SDG decreased ROS levels compared to control cells. BA-exposed cells treated with antioxidants had no differences in cell damage and viability compared to controls, indicating protective effects.

Conclusion: SDG shows promise for reducing oxidative stress caused by beta-amyloid in cells.

Keywords: *Oxidative Stress; Beta-Amyloid; Secoisolariciresinol Diglucoside; Flaxseed; Linum usitatissimum; PC-12*

Chemical compounds: Beta-amyloid (PubChem CID: 145705875); Catalase (PubChem CID: 318693332); Secoisolariciresinol diglucoside (PubChem CID: 9917980); Superoxide dismutase (PubChem CID: 405230653).

Introduction

Secoisolariciresinol diglucoside (SDG), an antioxidant found in flaxseed (*Linum usitatissimum*), has yet to be studied as a protective antioxidant in cells. Flaxseed has gained interest over time because of its nutritional content and health benefits [1-7]. SDG has been shown to decrease cardiovascular disease risk and protect against other diseases including diabetes in mice [2,8]. Additionally, SDG is a phytoestrogen, and as such may be protective against oxidative stress (OS) in Alzheimer's disease (AD) development [9-11]. While SDG does not cross the blood-brain barrier, it has been shown to protect against inflammatory substances crossing it [12]. What is not known is whether SDG can protect against beta-amyloid (BA).

Purpose of the Study

The purpose of this study was to determine if SDG protected PC12 cells exposed to BA from oxidative damage and cell death. PC12 cells, derived from the adrenal medulla of rats, are used in brain research due to their ability to differentiate into neuron-like cells [13,14]. Thus, PC12 cells were used to determine if SDG would reduce cell damages and cell death due to OS from BA.

Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative disease that leads to memory loss, delusions, and loss of cognitive functions. Over 55 million people worldwide (about 1% of the population) are living with a dementia diagnosis according to the World Health Organization, with more than 10 million new cases annually. Alzheimer's disease is the most common form of dementia [15]. However, nearly 60% of persons living with probable dementia are either undiagnosed or unaware of their condition [16]. Alzheimer's disease and dementia are rarely the cause of death for individuals who have these conditions. Instead, the most frequent causes of death are secondary infections such as pneumonia [17], or other chronic conditions that are poorly managed due to poor cognition [18]. Therefore, prevention of Alzheimer's disease is needed to improve quality of life for those at risk.

Alzheimer's disease risk factors are typically divided into modifiable and non-modifiable risk factors. One commonality in all of the modifiable risk factors is oxidative stress. By itself, OS is a risk factor for the development of AD. Oxidation of brain lipids is associated with neurotransmission dysfunction. The oxidation of proteins, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA) lead to cell damage and death related to AD progression [19]. Much of this OS comes from the presence of reactive oxygen species (ROS). ROS are byproducts of oxygen metabolism that result in the production of free radicals. While ROS are required for proper cellular function, in excess they are detrimental to cell health, leading to cell damage and death [20-22]. OS occurs when ROS are produced faster than they can be removed, resulting in overaccumulation of ROS in cells [23].

Another source of OS is the presence of beta-amyloid (BA) [24]. Clumps of BA plaques and tau neurofibrillary tangles in brain tissue are characteristic of AD dementia. BA is formed by the cleavage of mutated amyloid precursor protein [25]. The accumulation of BA is toxic to cells and causes high levels of OS [23,25]. The damage caused by the dual challenge of BA and ROS accumulation leads to neurodegeneration and cell death [23].

Antioxidants in Alzheimer's prevention

Antioxidants inhibit oxidative damage, and protect cells from overaccumulation of ROS. Enzymatic antioxidants such as superoxide dismutase (SOD) and catalase (CAT) naturally occur in cells and protect cells from regular ROS levels. Dietary antioxidants also help protect cells against oxidative damages by replenishing cellular antioxidants and through the action of phytochemicals [26-28]. Various dietary antioxidants have been explored for their potential to protect against BA-induced cell damage and death related to AD [29-34]. While many dietary antioxidants show promise as OS preventatives, the evidence is conflicting at best, and bleak at worst for treating Alzheimer's disease and dementia once it has developed. Even more concerning is that many of these antioxidants are sold as dietary supplements, which may have side effects or drug-interactions [27,35]. SDG, found in flaxseed, is one of these compounds. However, it shows evidence for protecting against other forms of oxidative stress influenced by lifestyle and may be protective against BA [8,36].

Materials and Methods

Materials

Reagents were purchased from MilliporeSigma (Saint Louis, MO) unless specified. PC12 cells were obtained from American Type Culture Collection (Manassas, VA). Assay kits were purchased from BioVision [37,38]. The ROS Detection Assay Kit measured intracellular ROS levels, which indicate OS. A Lactate Dehydrogenase (LDH) Assay Kit evaluated LDH levels in cells, measuring damage to the cell membrane. An MTT Assay Kit measured cell viability and death. Purified SDG was used as the treatment of interest at two concentrations.

Methods

The PC12 cells were grown in a growth medium (RPMI-1640, 10% heat-inactivated horse serum, 5% fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM glutamine) at 37°C in a 5% CO_2 incubator (Sheldon Manufacturing, Cornelius, OR). When cells reached 80 - 90% confluence, the clusters were broken up with fine needle aspiration using a syringe with a 22g needle. The

dispersed cells were counted, and $2x10^5$ cells/mL were seeded in Collagen IV-treated 96-well plates and incubated for 48 hours at 37°C in a 5% CO_2 incubator. Following the 48-hour incubation period, cells were treated with 100 µL of either plain RPMI or one of the antioxidants: SDG (50 or 100 µM), SOD (100 U/mL), or CAT (100 U/mL) for 2 hours. Plain RPMI was the background control, while SOD and CAT were antioxidant controls. SDG treatment occurred at two levels, one that has previously been shown to be effective as an antioxidant and suggested to be achievable *in vivo* (100 µM), and half that concentration [39]. All treatments were completed in triplicate. After the antioxidant treatment, cells were treated with 100 µL of either 10 µM BA or Plain RPMI for 24 hours. After BA treatment, ROS levels, LDH levels, and cell viability were determined using assay kits.

Statistical analysis

The average of two independent experiments was used for statistical analysis. Each treatment was completed in triplicate for each experiment. Independent t-tests were performed using SPSS (Version 27.0, IBM Corp., Armonk, NY) to identify if there were any differences between the control and treated cells. An alpha level of 0.05 was used for all analyses.

Results

Our findings revealed that SDG protects BA-exposed PC12 cells against the accumulation of ROS and related cell damage and death. Intracellular levels of ROS, such as hydroxyl, peroxyl, and other ROS, were significantly reduced in BA-exposed cells treated with both 50 µM and 100 µM SDG compared to control cells (Figure 1).

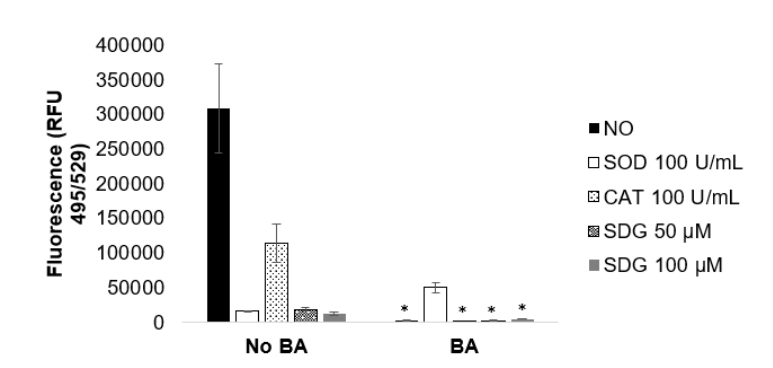

Figure 1: *Reactive oxygen species (ROS) comparison of secoisolariciresinol diglucoside (SDG) to cellular antioxidants in PC-12 cells.*

[1]BA: Beta-Amyloid; SOD: Superoxide Dismutase; CAT: Catalase; SDG: Secoisolariciresinol Diglucoside.

[2]The data represent the mean ± standard error from triplicate wells for each treatment for two independent experiments.

**Significantly different compared to the control, $p \leq 0.05$.*

Cells exposed to BA and treated with SDG at either concentration had no difference in LDH levels compared to the control, suggesting membranes were undamaged (Figure 2). Additionally, SOD-treated cells exposed to BA had a significant reduction in LDH compared to control cells (Figure 2). This finding was opposed to our hypothesis that antioxidants provide a protective effect against BA-induced cell damage.

In addition, cells that were exposed to BA and treated with SDG had the same levels of cell viability as control cells that faced no BA challenge (Figure 3). This finding supports our hypothesis that SDG would protect against BA-induced cell death.

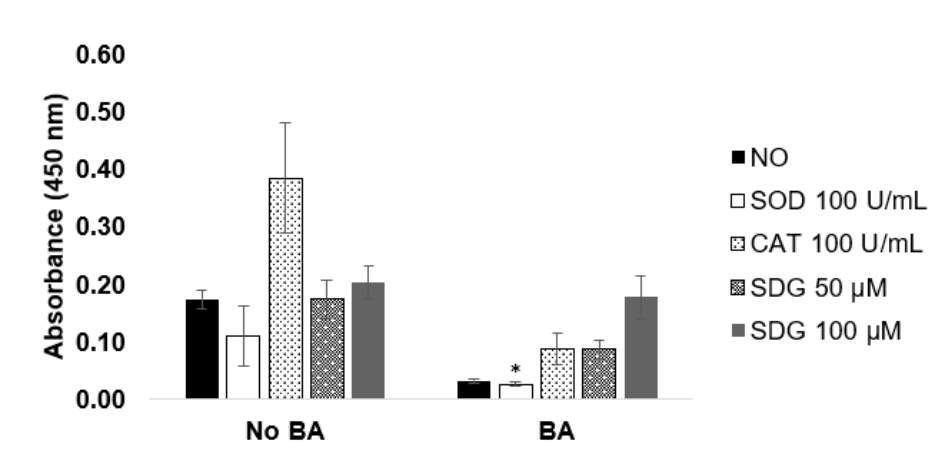

Figure 2: *Differences in lactate dehydrogenase (LDH) levels in cells treated with secoisolariciresinol diglucoside compared to cellular antioxidants.*

[1]BA: Beta-Amyloid; SOD: Superoxide Dismutase; CAT: Catalase; SDG: Secoisolariciresinol Diglucoside.

[2]The data represent the mean ± standard error from triplicate wells for each treatment for two independent experiments.

**Significantly different compared to the control, p ≤ 0.05.*

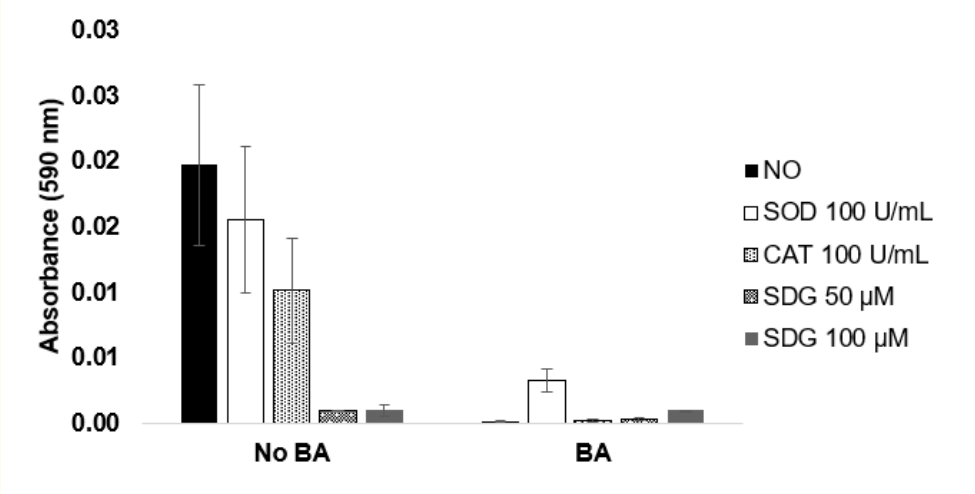

Figure 3: *MTT assay for cell viability between cells treated with secoisolariciresinol diglucoside or cellular antioxidants.*

[1]BA: Beta-Amyloid; SOD: Superoxide Dismutase; CAT: Catalase; SDG: Secoisolariciresinol Diglucoside.

[2]The data represent the mean ± standard error from triplicate wells for each treatment for two independent experiments.

Discussion

ROS were lower in cells treated with SDG compared to control cells in our study, suggesting that SDG protected them against oxidative stress, damage and death. LDH is released when the plasma membrane is damaged. Thus, increased LDH in supernatant fluid or the cytosol indicates cytotoxicity. LDH levels in our cells were not significantly different than control cells, suggesting no differences in damage to BA-exposed cells. This was most likely induced by the protective effects of the SDG treatment. There was also significant LDH reduction in cells exposed to BA with no antioxidant treatment (Figure 2). No difference in cell damage between SDG-treated and control cells indicates that SDG was protective against BA-induced cell damage. We also observed a decrease in cell damage in SOD-treated cells exposed to BA, indicating that the SDG and SOD both protected against BA-induced cell damage. However, there was also significantly less damage in cells with no antioxidant treatment that were exposed to BA when compared against control cells with no BA exposure. This result could be from the small scale of our study, or potentially from aggregation of cells, which were prone to clumping and may have produced inconsistent cell numbers in each well.

Limiting cell damage ultimately helps protect cells against cell death, which suggests that while there was apparently less damage to BA-exposed cells that had no antioxidant protection, these cells may have died outright from BA exposure before damage could accumulate. Our results showed a protective effect of SDG against cell death, as cells that were exposed to BA and treated with SDG had the same levels of cell viability as control cells that faced no BA challenge. Previous research found protective effects of various antioxidants against BA-induced cell death [29-34]. We observed similar results in our LDH and cell viability assays, where SDG protected cells against damage and death caused by BA.

Conclusion

Our findings indicate the potential of SDG, an antioxidant found in flaxseed, as a protective compound against BA-induced OS by limiting the accumulation of ROS, thus reducing cell damage and retaining cell viability in BA-exposed PC12 cells. This has implications for AD research. BA accumulation is one characteristic of AD and leads to much of the ROS-associated cell damage and death associated with AD progression. Adding flaxseed to the diet could help prevent the development of AD and slow down its progression. While more research is warranted, this study provides an important stepping-stone in AD research. Future studies would benefit from a larger sample size. More research is needed to determine the mechanisms of AD development and how antioxidants work to prevent disease development.

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Disclosure Statement

There are no declarations of interest to report.

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Data Availability Statement

All data generated by this study are included in this manuscript and its figures.

Authorial Contributions

Annika Rotvold Stone: writing- original draft preparation; investigation; Kelly Burdett Parker: investigation; writing- revision, reviewing and editing; Yeong Rhee: Conceptualization, methodology, supervision, project administration, funding acquisition, data curation, formal analysis; writing- review and editing.

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