Protective effect of Gallic acid against Aβ neuronal toxicity and neuroinflammation for the treatment of Alzheimer's Disease

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***Abstract*- Alzheimer's Disease (AD) is one of the leading causes of death and dementia in elderly people. Previous research indicates that AD may take its roots from Amyloid Beta (AB) aggregation and neuroinflammation. As 3,4,5-trihydroxy benzoic acid, gallic acid, demonstrates its potential to alleviate AD induced cytotoxicity and oxidative stress, this study researched the effect of gallic acid on pathological formation of AD. Gallic acid was tested against AB induced cytotoxicity, neurons apoptosis, and lipopolysaccharide (LPS) induced neuroinflammation, via a variety of biological assays, including cell viability assays, cell mitigation assays, and ELISA assays. According to the data and statistical analysis, gallic acid attennued the neuronal apoptosis by reversing the cytotoxic effects induced by AB. Moreover, gallic acid also reduced the production of AB by significantly down regulating the protein expression of Amyloid precursor protein. Gallic acid indicated its effects on neuroinflammation since it significantly inhibited LPS induced pro-inflammatory cytokines released from immune cells. All the results demonstrated that gallic acid has a positive impact on AD by inhibition of AB aggregation and AD induced cytotoxic effects, as well as anti-inflammatory effects. Overall, gallic acid gives new insight into the treatment of AD.**

I. Introduction

Alzheimer's Disease (AD) is the most predominant cause of neurodegenerative dementia and one of the leading sources of morbidity and mortality in the aging population [1].

Two hallmarks for the pathological formation of AD are known: (1) the extracellular plaques formed by amyloid-beta (Aβ) aggregates and (2) the intracellular tangles formed by hyperphosphorylated tau proteins[2]. Aβ, an amino acid commonly found in the amyloid plaques with AD, forms tangles in between the synapses of neurons in the hippocampus, blocking the communications between neurons and resulting in memory and learning impairments[3]. Since Expression of Amyloid precursor protein (APP) determines the production of Aβ, overexpression of APP results in excess Aβ production and thus aggregation [3]. In addition toAβ aggregation, bacterias induce microglia to secret pro-inflammatory cytokines, resulting in neuron inflammations, which eventually leads to neuron apoptosis [4]. Apoptosis has a significant negative impact on the developments of AD symptoms [2].

Gallic acid (GA) has been researched for its anti-inflammatory effects and effects on alleviating cell apoptosis in allergic research, obesity, and cancer. *In vivo* and *in vitro* studies on attenuating effects of GA on cytotoxicity and inflammation suggest a possible therapeutic application of this agent in other cytotoxicity-induced inflammatory diseases. Since AD is associated with neuron loss, as a result, researching GA 's effects on cytotoxicity, neuron survival rates, inflammatory responses will greatly reflex GA's effects on healing AD [5].

Thus, the goal of this study is to identify possible mechanisms by which GA may alleviate and target AD pathogenesis and symptoms and to study the possibility of this chemical to both decrease plaque aggregation and neuroinflammation, as well as reduce cell death and inflammatory cytokines induced by Aβ and bacteria.

II. Methods

Gallic acid, 3,4,5-trihydroxy benzoic acid, the powder was obtained from Sigma-Aldrich. It was then diluted with distilled water to create 10uM and 1uM concentrations.

HTB-11 human neuroblastoma cells (ATCC, USA), 3-day transfer, inoculum 3×10^5 cells (ATCC), and Raw 264.7 murine macrophage-like cells (ATCC) were used as neuron cell models to study the effects of 3,4,5-trihydroxy benzoic acid on the effects on neurons apoptosis and amyloid-beta induced cytotoxicity.

To assess the alleviative effects of GA on neuron apoptosis, cell proliferation assays were used to determine neuronal survival rates. Active cells reduced the yellow tetrazolium MTT into purple formazan. Spectrometer measured the absorbance and cells survival rates under the wavelength of 595nm after 24 hours incubation.

To analyze the effects of GA on Aβ production and neuroinflammation, Enzyme Linked Immunosorbent Assay (ELISA)assay was performed on cytokines and protein, including APP, IL-1β, TNF-α, IL-4, and IL-10 following the manufacturer's protocol (BosterBio, Pleasanton, California). A microplate absorbance reader is used for results analysis.

P-values, calculated from two-tailed T-tests, were employed to determine the statistical significance of the results. $P<0.05$ indicates statistically significant results.

III. Results

GA was found to have a positive effect in treating Alzheimer's diseases as it alleviates neuro apoptosis, attenuates inflammation, and reduces the overproduction of Aβ.

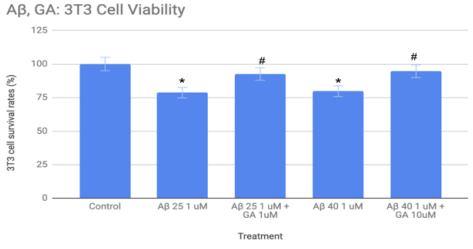


Figure 1. Fragment Aβ25 and full-length Aβ40 toxicity treated with GA; 10uM GA is the most effective in treating Aβ40 with 94.61 ± 0.096% for 24 hours. Error bars denote standard deviation. * indicates statistical significance, $p<0.05$, when data is compared to control, and # indicates statistical significance, $p<0.05$, when the data is compared to the group only treated with Aβ25 1uM or Aβ40 1uM.

Cells have the lowest survivability when treated with Aβ25 1uM, with a survival rate of 78.57%. Both treatments of Aβ25 1uM and GA 10uM and Aβ40 1uM and GA 10uM increase cell survivability. Moreover, treatment of Aβ40 1uMand GA 10uM has the highest survival rate of 94.96%, while the treatment of Aβ25 1uM and GA 10uM is slightly lower in survival rate with 92.50%. This suggests that GA may be more effective at targeting full-length Aβ cytotoxicity (figure 1).

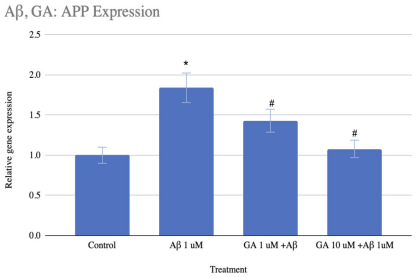
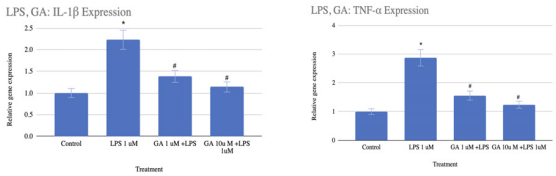


Figure 2. ELISA for APP gene expression under concentration treatment of GA. The most effective treatment is GA 10 uM + Aβ 1uM. The error bar represents standard error. * indicates statistical significance, p<0.05, when data is compared to control, and # indicates statistical significance, p<0.05, when the data is compared to the group only treated with Aβ 1uM.

Aβ 1 uM increased APP expression to 1.84 times the normal APP expression level. Both concentrations of GA significantly reduced APP expression level, while GA 10uM is the most effective by reducing APP expression to only 1.08 times the normal APP expression level. This suggests that GA is prominent in reducing Aβ stimulative effect on APP expression, and the higher concentration may be more effective (figure 2).



Figures 3a and 3b. ELISA for IL-1β and TNF-α gene expression under GA concentrations treatment; most effective treatment for most IL-1β expression is GA 10uM +LPS 1uM, with relative gene expression of 1.14±0.15, and the most effective treatment for TNF-α expression is GA 10uM + LPS 1uM, with relative expression of 1.24±0.13. Error bars represent standard errors. * indicates statistical significance, p<0.05, when data is compared to control, and # indicates statistical significance, p<0.05, when the data is compared to the group only treated with LPS 1uM.

IL-1β and TNF-α are two pro-inflammatory cytokines. Bacterial LPS increases both of their expressions to 2.23 times and 2.87 times their normal expression levels. Treatment with both concentrations of GA has a significant impact on decreasing the cytokines expression levels. GA 10um + LPS 1uM is the most effective, bringing both IL-1β and TNF expression to 1.14 times and 1.24 times their normal expression levels. This suggests GA can potentially reduce the inflammation triggered by toxins, such as LPS, and higher concentration may be a more effective option (Fig 3a & 3b).

IV. Discussion

Data from the experiment indicates that GA can be a potential treatment due to its effects to target the molecular mechanisms of AD. Aβ aggregation and resultant neuron apoptosis has been understood as the main cause of AD, and in the experiment, GA significantly alleviates cell death that was originally induced by Aβ. This can be translated into that GA has an alleviative and protective effect against Aβ-induced cytotoxicity.

APP is known to be the protein that makes excess Aβ and responsible for the Aβ aggregation in AD patients. The overexpression of APP will cause the excess production of Aβ, and a large amount of Aβ will induce APP expression again. Data from the experiment shows that when Aβ is used alone, the APP expression becomes significantly greater than its normal expression. However, GA shows its potential effect to decrease and reverse the overexpression of APP and thus reduce the production of Aβ since the expression level of APP is greatly reduced after GA is used with Aβ. Moreover, a greater concentration of GA reduces more APP expression in the experiment, implying that a high concentration of GA may be a more effective option for APP overexpression.

Pro-inflammatory cytokines, such as IL-1β and TNF-α, are overexpressing and causing the neuron's death in neuroinflammation. The data from the experiment indicates that LPS, when added alone, will greatly increase the expression of IL-1β and TNF-α, indicating that LPS very likely causes serious inflammation in the cells. However, treatment with GA reduced the overexpression of IL-1β and TNF-α to close-to-normal levels, suggesting that GA may reverse and alleviate the inflammatory responses that LPS induced. As a result, GA will be a possibility to target bacterial LPS induced inflammatory effects and neuron apoptosis.

V. Conclusion

Overall, this research investigated the effects of GA on AD. The result indicates that GA can be a prominent option to alleviate neuron death, prevent overproduction of Aβ, attenuate inflammation. It ultimately indicated that GA has the potential to target and prevent Aβ tangles. As a result, GA can be considered as a potential treatment based on the ways to attack the symptoms and pathologies of AD.

Future studies need to test GA effects in vivo AD models, such as C.elegans or experimental rats. It will be crucial to test the GA effect in vivo too since the impacts and performances of the chemicals may be different when used in the human body.

VII. Acknowledgments

I would like to thank Dr. Wei Zhu for all his help and the amazing opportunity to conduct research.

VI. References

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