World Scientific Research Journal Volume 6 Issue 10, 2020
ISSN: 2472-3703 DOI: 10.6911/WSRJ.202010_6(10).0010

Decreasing of the Level of the Alzheimer's Amyloid β Peptide with the Ginseng Saponin Metabolite, Compound K

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Abstract

Alzheimer's disease is a common and serious progressive neurological disease that influences human beings. Previous study has shown that three kinds of specific ginsenosides, Rg1, Rg3, Re, are found to decrease the level of the toxic Alzheimer's amyloid β peptides, Aβ40 and Aβ42. This study investigates the effect of compound K, the ginseng saponin metabolite, on the level of Aβ40 and Aβ42 by using Culture Chinese hamster ovary 2B7 cells and three-to-four month old female mice. The possible results are treatment with increasing levels of compound K leads to increasing, decreasing, or the same levels of Aβ40 and Aβ42. The result of this study could lead to further investigation and discussion of the new, beneficial ginseng saponin metabolite. Future studies could focus on finding more functions of compound K and distinguishing the different effects between ginseng saponin metabolite and ginsenosides.

Keywords

Amyloid β peptide, Ginseng, Compound K, Rg1, Rg3, Re, Ginsenosides.

1. INTRODUCTION

Alzheimer's disease is one of the progressive neurological diseases that leads to the dementia, which is defined by the loss of neurons of the brain and the degeneration of language, memory, and other cognitive abilities of a person [1, 2]. It is reported that Alzheimer's disease becomes the sixth cause of death in America, which will even be the fifth one, and over 16 million people are unable to take care of patients in their families for totally approximately 18.6 billion hours [3]. Until now, there is still no effective drug or treatment that could help prevent or cure this kind of disease [4]. Researchers only find out some ingredients in Western medicine and Chinese medicine which are likely to treat the symptoms or restrain the expressions of some activations in a certain degree. In order to explore and help solve the serious problem people are facing now, I specifically focus on and do the research into one of traditional Chinese medicines -- Ginseng, and distinguish whether some parts or metabolites of it could effectively help solve the problem of Alzheimer's disease.

Ginseng is a kind of traditional herbal medicine that has comprehensive pharmacological actions [5]. The main active ingredients inside ginseng are ginsenosides, which have certain effects on some of neurological diseases, cancers, etc [6]. Based on the differences of position and number of sugar moiety between different kinds of ginsenosides, they can be divided into three groups, which are A-Panaxadiol group, B-Panaxatriol group, and C-Oleanolic acid group [7]. Rg1, Rg3, and Re, three common types of ginsenosides in B-Panaxatriol group, were recently found to effectively decrease the level of the Alzheimer's amyloid β peptide, especially the level of toxic Aβ40 and Aβ42, which is good for patients [8]. At the same time, a type of ginseng saponin metabolite, compound K, is also realized to be beneficial to patients in certain areas [9]. As a result, whether the compound K could decrease the level of Alzheimer's amyloid β peptide as strong as Rg1 could become a question.

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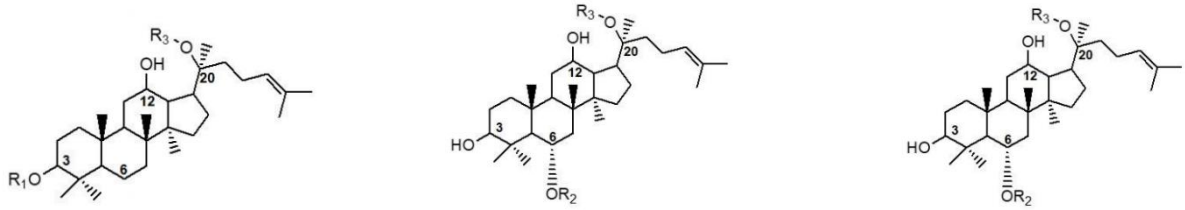
In the work, I investigate the effect of compound K in the level of the Alzheimer's amyloid β peptide by using both in vitro and in vivo conditions. We predict that treatment with increasing levels of compound K leads to decreasing levels of Aβ40 and Aβ42 by using mice and cell lines, and sandwich ELISA assay.



Rg3 Re Rg1

R1: Glc-Glc R3: H R2: Glc-Rha R3: Glc R2: Glc-Xyl R3: Glc

Figure 1. The structure of specific ginsenosides [11]

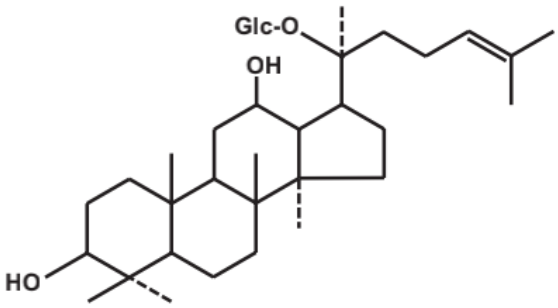


Figure 2. The structure of compound K [8]

2. EXPERIMENT DESIGN OF THE RESEARCH

The experiment will need extracts of American ginseng and Chinese ginseng [8]. It is going to use Rg1, Rg3, Re, and Compound K in both experiments. We will first evaluate the purity and structure of each ginsenoside and metabolite before having the in vitro cell culture [8].

2.1. Cell Culture

Culture Chinese hamster ovary (CHO) 2B7 cells in Ham's F-12 medium by using 10% calf serum, penicillin, and 100 μg/mg Zeocin [8]. Put CHO 2B7 cells in 96-well plates, which wells have same concentration of DMSO, and mix with ginsenosides and metabolite in different concentration for 3 hours [8]. Then, remove the medium, analyze the level of Aβ peptide, do the assessment of LDH release and evaluate the changes in cellular metabolism of rest of cells [8]. Use the pair of antibody, 2.1.3.35.86/4G8 and 33.1.1/13.1.1, to detect Aβ40 and Aβ42, and use the method of sandwich ELISA to measure the level of them [10]. Compare the results of samples and the standards of synthetic Aβ40 and Aβ42. The final results will be means ± standard error of triplicate culture wells [8].

2.2. Animal Model

Use several female mice that are three to four months old and contain the human APP gene, which could lead to Alzheimer's disease, to test the effects of Rg1, Rg3, Re, and compound K [8]. First, dissolve these compounds in DMSO, and put them into the 0.9% sodium chloride [8]. Then, dose the mice with oral administration, which volume is 8 ml/kg of weight [8]. The final concentration of DMSO should be less than 2.5% after using 25 mg/kg of weight of Rg1, Rg3, Re, and compound K [8]. After 18 hours, the mice will be killed, and we will analyze their brains [8].

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In order to analyze the level of Aβ, we will use 70% (150 mg tissue/ml) formic acid solution to dounce the hemibrains [8]. Convey the homogenates to the chilled ultracentrifuge, and spun the ultracentrifuge at 100,000 g for one hour at 4°C [8]. Collect the supernatants, neutralize it with 1.0 M Tris base, 0.5 M NaH2PO4, and 0.05% NaN3 (1:20), and analyze them by using ELISA which is stated before [8]. The experiment is done for four times, and the value in each time will be normalized by the different number of control animals[8]. Thus, the final results will be means ± standard error.

3. RESULTS

The experiment could lead to three different results, which are listed in the following paragraphs. Different results shows different directions of the future works.

3.1. By the Increasing Levels of Compound K, the Levels of Aβ40 and Aβ42 in Medium of CHO 2B7 Cells and the Brains Decrease

The positive controls of the experiments are Rg1, Rg3, and Re while the negative control is no drug at all. Comparing with the initial levels of Aβ40 and Aβ42 that do not use any drug or compound, both experiments show that the levels of Aβ40 and Aβ42 decrease greatly. As the concentration of compound K increases, the decreasing of levels of Aβ40 and Aβ42 becomes larger and larger, which supports the hypothesis of the experiment. It could show that compound K has great effects on controlling the peptide of Alzheimer's disease, which helps patients in a certain degree. Compound K can be evaluated more deeply and even be successfully used.

3.2. By the Increasing Levels of Compound K, the Levels of Aβ40 and Aβ42 in Medium of CHO 2B7 Cells and the Brains Do Not Change

The positive controls of the experiments are Rg1, Rg3, and Re while the negative control is no drug at all. After the experiments, there is no or no marked change in the levels of Aβ40 and Aβ42 in the sample. It is as similar as the result that we do not use any drug. It means that compound K may not have effects on controlling the expression of Aβ40 and Aβ42 in Alzheimer's disease, which does not support the hypothesis. Compound K is different from some ginsenosides, and it may not have this kind of function. However, the reasons that lead to this result could be external and environmental. We should repeat the experiments and change some of the parameters to make sure the functions of compound K.

3.3. By the Increasing Levels of Compound K, the Levels of Aβ40 and Aβ42 in Medium of CHO 2B7 Cells and the Brains Increase

Table 1. The possible results of the percentage of Aβ42 that accumulates in CHO 2B6 cells after 3-hour treatment

The ingredient Aβ42 (% of control)	25 μM	50 μM	100 μM	200 μM
No drug	\	\	\	\
Compound K	?	?	?	?
Rg1	-	-	-	-
Rg3	-	-	-	-
Re	-	+	-	\

The positive controls of the experiments are Rg1, Rg3, and Re while the negative control is no drug at all. The Aβ40 and Aβ42 levels in brains of mice and the Aβ40 and Aβ42 levels in medium

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of CHO 2B7 cells increase in the experiments. Compound K not only does not have great effects on decreasing the levels of Aβ40 and Aβ42, but also have negative effects on the expression of Aβ40 and Aβ42. It contradicts to the hypothesis and shows that it may even cause the aggravation of Alzheimer's disease. Compound K could not be used to control the levels of Aβ40 and Aβ42. It has negative results comparing to those of no drugs or other ginsenosides.

(The results are based on the previous study [8]. \:The level of Aβ42 is as same as the initial level. ?: The level of Aβ42 is unknown. -: The level of Aβ42 decreases compared to the initial level. +: The level of Aβ42 increases compared to the initial level.)

Table 2. The possible results of the percentage of Aβ40 that accumulates in CHO 2B6 cells after 3-hour treatment.

The ingredient Aβ40 (% of control)	25 μM	50 μM	100 μM	200 μM
No drug	\	\	\	\
Compound K	?	?	?	?
Rg1	+	\	\	\
Rg3	-	-	-	-
Re	+	\	-	\

(The results are based on the previous study [8]. \:The level of Aβ40 is as same as the initial level. ?: The level of Aβ40 is unknown. -: The level of Aβ40 decreases compared to the initial level. +: The level of Aβ40 increases compared to the initial level.)

Table 3. Influence of the oral administration of ginsenosides and metabolite on Aβ42 in brains of mice

Aβ42 (% of vehicle control) The ingredient	No drug	Compound K	Rg1	Rg3	Re
	\	?	-	-	-

(The results are based on the previous study [8]. \:The level of Aβ42 is as same as the initial level. ?: The level of Aβ42 is unknown. -: The level of Aβ42 decreases compared to the initial level. +: The level of Aβ42 increases compared to the initial level.)

Table 4. Influence of the oral administration of ginsenosides and metabolite on Aβ40 in brains of mice

Aβ40 (% of vehicle control) The ingredient	No drug	Compound K	Rg1	Rg3	Re
	\	?	-	-	-

(The results are based on the previous study [8]. \:The level of Aβ40 is as same as the initial level. ?: The level of Aβ40 is unknown. -: The level of Aβ40 decreases compared to the initial level. +: The level of Aβ40 increases compared to the initial level.)

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4. DISCUSSION OF THE RESEARCH

Previous study has tested the effect of several specific ginsenosides on the concentration of Aβ, which shows that the level of Aβ40 and Aβ42 decreases in the medium of CHO 2B7 cells and in the mice's brain.8 This study is based on the previous study, and the experiments are designed similarly. It is designed to explore the effect of ginseng metabolite, compound K, and compare the results of it with those of the effective ginsenosides in order to know whether it is effective enough to be used in treatment. One possible result confirms that compound K has effects on decreasing the levels of Alzheimer's amyloid β peptides. Another possible result shows that compound K is not effective to control or treat Alzheimer's disease. The final possible result means that compound K has negative effects on Alzheimer's amyloid β peptides. Possible results are discussed in the following parts.

The first possible result shows that compound K has similar effects on decreasing the levels of Aβ40 and Aβ42 as other kinds of ginsenosides. It is the expected result, which supports the hypothesis and helps explore the effective ingredients or drugs for patients who have Alzheimer's disease and the research in this area. However, before the use of this kind of treatment, we might do some related experiments to know more about compound K. We should also learn more about the side effect of compound K and other types of ginsenosides in order to make sure the safety of treatments.

The second result means that while increasing the level of compound K, the levels of Aβ40 and Aβ42 do not decrease. It does not support the hypothesis of the experiment. It demonstrates that compound K might not have positive effects on this kind of expression. The failure of experiments is likely to be happened because of the wrong method of using compound K. It might be effective if we do not use the oral administration to the mice. We might change the way of use of the ingredients or compounds in order to find out whether this compound is no use in the level of Aβ in all aspects.

The final result could be shocked and contradict to the hypothesis because compound K has negative effects on controlling the levels of Aβ40 and Aβ42 although compound K has already been found that it has positive effects on inhibiting the expression of microglial activation[9]. The different results in different kind of experiments and expressions could lead to further problems of discovering this kind of ginseng metabolite. We should find out the structure of compound K and determine which part of it could be beneficial to inhibiting the negative expressions or inciting the positive expressions.

5. CONCLUSION

Generally, this study explores the effect of compound K on the levels of Aβ40 and Aβ42 by using the experiments of CHO 2B7 cells and the brains of mice. The results could illustrate whether compound K could decrease the Aβ40 and Aβ42 levels, which shows whether compound K could be as useful as Rg1, Rg3, and Re in treatment of Alzheimer's disease. However, people focus on different kinds of ginsenosides for many years, and they know too less about compound K. People should quickly know and define the functions of compound K for more explorations. Then, we could easily and clearly explain the effects of compound K on Alzheimer's disease and even different kinds of diseases. In the future, if people could learn more about which specific kind of cell that has great effects on controlling the levels of Aβ40 and Aβ42, we might extract that kind of cell and make good use of it. Lastly, the experiment process might be improved or simplified in the future in order to be done more easily. These might make a big difference in future development of Alzheimer's disease.

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202010_6(10).0010

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