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Improvement of Function in the Aging Rat Hippocampus by Administration of Alanine-Glutamine Dipeptide as a Precursor of the Antioxidant Glutathione

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Abstract: Physiological aging and aging due to oxidative stress decrease alanine-glutamine dipeptide concentrations in plasma and hippocampus. These conditions impact the decreased availability of glutamine in intracellular and glutathione levels in the hippocampus. Decrease in hippocampal glutathione level will be followed by decreased function of hippocampal neuron. One way to improve the function of the hippocampus is to increase the availability of glutamic acid in hippocampal neuronal cells. This study was designed to obtain the concentration profile of alanine-glutamine dipeptide in plasma and hippocampus and the repair function in the aging hippocampus is known from structural repairs to the mitochondria of neurons. The experimental rats were assigned into a completely randomized design with 2 x 2 x 2 factorial arrangement and three replications. The first factor was the age of the experimental rats, consisted of two levels i.e., 12 and 24 months. The second factor was oxidative stress consisted of two levels, i.e., without or with oxidative stress. The third factor was the concentration of alanine-glutamine dipeptide administration consisted of 2 concentrations, i.e., 0 and 7%. Administration of alanine-glutamine dipeptide 7% can improve function in the aging hippocampus, both in physiological aging or aging due to oxidative stress in younger or aged rats, in normal or oxidative stress rats. This research concluded that the alanine-glutamine dipeptide 7% gave the best results in increased alanine-glutamine dipeptide plasma and hippocampus, glutathione levels, the repair response of mitochondrial structure that mediates the repair function in the aging hippocampus.

Key words: Alanine-glutamine dipeptide, mitochondria, glutathione, physiological aging, stress oxidative aging, hippocampus

INTRODUCTION

Neurodegenerative disorders associated with increased age or oxidative stress, is clinically characterized by decreased function of the hippocampus (Reddy, 2009). Decline in hippocampal function characterized by decreased concentrations of glutamine or alanine-glutamine dipeptide and glutathione hippocampus to below the normal threshold is correlated with the concentration of glutamine or alanine-glutamine dipeptide plasma. The decrease of both compounds has a correlation with structural damage to neurons, reduced neuronal density, neuronal mitochondrial structure damage, even death of neurons that are indicators of aging (Anonim, 2001; Reddy, 2009). Pathogenesis of aging is characterized by oxidative damage to cell membrane disruption affecting transport mechanisms across the membrane and decrease the availability of metabolic substrates, such as glutamine or alanine-glutamine dipeptide (Speakman *et al.*, 2004). Without any treatment, interference with this mechanism can lead to decreased function of the cells amonnis

horn neurons in the hippocampus. Important indicators of the decline in hippocampal function is decreased viability, increased mortality and damage to neuronal mitochondrial structure (Reddy, 2009).

Aging is inevitable, but may be attempted to be slowed. One way of handling aging is to increase the availability of glutamine or alanine-glutamine dipeptide plasma, hippocampus and glutathione hippocampus above the normal threshold. Availability of glutamine or alanine-glutamine dipeptide plasma, hippocampus and hippocampal glutathione levels is critical to support the integrity and function of neurons that have an impact on improving the function of the hippocampus (Wang *et al.*, 2007; Roth, 2008). A wide selection of anti-aging ingredient has been done to improve the function of the hippocampus with aging, such as the use of glutathione analog, glutathione monoethyl ester, gotu kola leaf, somatotropin and others. Among the antiaging mechanisms of materials is to provide amino acids that can be used to increase glutamine or alanine-glutamine dipeptide plasma and hippocampus. Increase in both of

these compounds can provide increased levels of glutathione, so increase the viability of neurons, decrease the mortality of neurons in hippocampus or reduced and mitochondrial structure damage can be repaired. Compounds that have the potential of this kind is the alanine-glutamine dipeptide (Berg *et al.*, 2006).

Alanine-glutamine dipeptide is another form of glutamine that is known as a provider of intracellular glutamine that is used to support the synthesis of glutathione in the body (Daren *et al.*, 2007; Fernandes *et al.*, 2010; Jun *et al.*, 2006). As a precursor of glutathione, alanine-glutamine dipeptide has a role to provide the amino acid glutamine in the hippocampus. Glutamine can be converted to glutamate and together with cysteine and glycine is used for the synthesis of glutathione (Dringen *et al.*, 2000). Alanine-glutamine dipeptide have stable properties during the run processes in the body, faster experience the process of hydrolysis, were able to cross the blood brain barrier, can be utilized by neurons directly, can increase levels of glutathione, increase the viability of neurons, decrease the mortality of neurons and repair damage to mitochondrial structure hippocampal neurons (Anonim, 2001; Berg *et al.*, 2006; Reddy, 2009).

Administration of alanine-glutamine dipeptide is expected to provide increased concentrations of plasma alanine-glutamine dipeptide and the hippocampus, hippocampal levels of glutathione, can increase the viability of neurons, decrease the mortality of neurons and can improve the structure of mitochondria, both in rats exposed to physiological aging or aging due to oxidative stress. Thus, the alanine-glutamine dipeptide can be used to slow aging and prevent the occurrence of neurodegenerative disease.

This study aimed to obtain concentration profiles alanine-glutamine dipeptide blood plasma and hippocampus, levels of glutathione in the hippocampus and to get a repair response of the structure of hippocampal mitochondria after administration of optimum concentrations of alanine-glutamine dipeptide, in rats exposed to either physiological aging or aging due to oxidative stress.

MATERIALS AND METHODS

The materials used in the research include: commercial pellet feed, physiological saline (NaCl 0.9%), distilled water, alanine-glutamine dipeptide, Krebs solution, perchloric acid, 10% buffered formalin, alcohol, ethanol, paraffin, silol, entellan, propylene oxide, Spur resin, glutaraldehyde, osmium tetra oxide, $K_3Fe(CN)_6$, cacodylate buffer and sucrose. The tools used in research are rats cages and equipment, syringes, a set of surgical instruments, glass cup, measuring cups, glass objects, bottles, test solutions stock, container for organ samples, glass coverings, cabinets ice, freezer, vortex, digital scales, analytical scales, microtomes and

microscopes equipped with digital cameras, water bath, spectrophotometer and TEM.

The experimental rats were assigned into a completely randomized design with 2 x 2 x 2 factorial arrangement and three replications. The first factor was the age of the experimental rats, consisted of two levels i.e., 12 and 24 months. The second factor was oxidative stress consisted of two levels, i.e., without or with oxidative stress. The third factor was the concentration of alanine-glutamine dipeptide administration consisted of 2 concentrations, i.e., 0 and 7%. Test animals used were male rats *Sprague dawley* strain. The study begins with a one-week acclimation rats. During acclimation, rats were fed commercial pellets and water *ad libitum*. Animal models of physiological aging made by means of the untreated rats to oxidative stress. The animal model of aging due to oxidative stress is created in a way not to feed on rats for 6 days, rats were given drinking water *ad libitum* and rats were treated daily activities of swimming in cold water in a bucket, covered, for 15 min. Alanine-glutamine dipeptide be given at a dose result conversion dose of 1.66 g/kg bw/day in human and administered intravenously for 12 days by injecting these compounds in the tail vein after rats were treated without or with oxidative stress. Stock solution prepared daily, stored at 40°C in the refrigerator.

At the end of treatment, the rats were sacrificed and followed by blood sampling and the hippocampus. Determination of alanine-glutamine dipeptide concentration in plasma and hippocampus is done by using a spectrophotometer at a wavelength of 630 nm according to the method that has been done by Wang *et al.* (2010). Determination of glutathione levels by using a spectrophotometer at a wavelength of 520 nm according to the method that has been done by Feoli *et al.* (2010). To complete the concentration of alanine-glutamine dipeptide, glutathione levels and histo-morphological in the hippocampus done making specific histological preparations of mitochondria with the procedure Transmission Electron Microscope (Owen *et al.*, 2007).

The results of the determination of the concentration of alanine-glutamine dipeptide in plasma and hippocampus, the determination of glutathione levels in the hippocampus of physiological aging and aging due to oxidative stress were analyzed by analysis of variance procedure at the level of 5% by using the SAS System software version 9. Concentration of alanine-glutamine dipeptide exogenous, plasma and the hippocampus has a relationship with hippocampal glutathione levels and improvement of mitochondrial structure hippocampal neurons. Repair concentration of plasma alanine-glutamine dipeptide and the hippocampus, hippocampal levels of glutathione and the structure of the mitochondria showed improved function in the aging hippocampus, both in physiological aging or aging due to oxidative stress.

RESULTS AND DISCUSSION

Physiological aging and oxidative stress of aging can cause a decrease in the concentration of plasma alanine-glutamine dipeptide and hippocampus under normal concentration. The decline followed decreased hippocampal levels of glutathione. This study used alanine-glutamine dipeptide optimum concentration in the hope of increasing concentrations of alanine-glutamine dipeptide plasma and hippocampus and increased levels of glutathione in the hippocampus of aging conditions. The results of alanine-glutamine dipeptide observations of plasma and hippocampus and hippocampal levels of glutathione are presented in Table 1 and 2.

Based on the data of Table 1 shows that age or oxidative stress causes a decrease in the concentration of alanine-glutamine dipeptide in plasma or hippocampus and found the interaction between both factors. Increased lifespan of rats, the age of 24 months from 12 months causes a decrease in the concentration of alanine-glutamine dipeptide in plasma or hippocampus, respectively 13.31 and 14.48%. Similarly, the effect of oxidative stress also gave similar results. Oxidative stress in rats causes a decrease in the concentration of alanine-glutamine dipeptide in plasma and hippocampus, respectively 11.39 and 11.48%, lower than normal rats (Table 2). This compound has decreased around 10-15%, both in plasma and in the hippocampus. The oxidative stress that occurs in rats aged 24 months cause a decrease alanine-glutamine dipeptide concentration in plasma and in the hippocampus reached 15.99 and 10.48%, higher than the 12-month old rats. This means the concentration of alanine-glutamine dipeptide in blood circulation and hippocampus has decreased as a result of increased age, oxidative stress, or the interaction between the two. Decrease in the concentration of alanine-glutamine dipeptide in plasma and in the hippocampus followed by reduction of glutathione levels. Increased age or oxidative stress causes a decrease in hippocampal glutathione level reached 26.34 or 7.05% compared to younger age rats or normal rats. Oxidative stress that occurs in old age rats (24 months) resulted in lower levels of glutathione hippocampal 24.77%, lower than normal rats at the same age.

The results of this study provide evidence that increasing age, oxidative stress or the interaction of both an influence on the mobilization of alanine-glutamine dipeptide from storage places in the body into the blood circulation system. Most of the alanine-glutamine dipeptide from the blood circulation system allegedly transported to the hippocampus, because the brain is most in need of substrate glutamine. This condition affects a decrease in alanine-glutamine dipeptide concentration in the blood. In the hippocampus, alanine-glutamine dipeptide is gradually converted into

glutamine and glutamate. These amino acids are then used to support the synthesis of glutathione in the hippocampus. High levels of glutamine requirement causes a decrease in the concentration of alanine-glutamine dipeptide in the hippocampus. Glutathione as an antioxidant system has an important role for cellular defense against free radicals due to increased age, oxidative stress or the interaction between both factors. However, an imbalance between antioxidant capacity with oxidants causing hippocampal function improvement rate was lower than the rate of oxidative damage. Furthermore, these conditions have an impact on the reduction of glutathione levels in the hippocampus. Some studies have collaborated this opinion. Jun *et al.* (2006) reported that the metabolic stress can cause a decrease in the concentration of glutamine, both of which circulate in the blood and present in body tissues. This decline has been known as part of efforts to use the amino acid glutamine to protect tissues from oxidative damage as well as doing repair of morphology and function of tissue. Alanine-glutamine dipeptide as another form of glutamine is also experiencing the same thing. It was further reported that the need for a high glutathione is used to protect cells that have reduced function (cell injury) due to increased age and oxidative stress. This mechanism causes the levels of glutathione in the tissues is lowered, it is also no exception to the hippocampus (Kulkarni *et al.*, 2005; Jun *et al.*, 2006; Lemberg and Fernandez, 2009).

Observed in this study are alanine-glutamine dipeptide concentration in plasma and hippocampus results providing an optimum concentration of alanine-glutamine dipeptide and the interaction between the alanine-glutamine dipeptide with age or oxidative stress. The data in Table 1 shows that the concentration of alanine-glutamine dipeptide in plasma and hippocampus have increased 43.23 and 45.14% compared to control after being given the optimum concentration of alanine-glutamine dipeptide. Increased concentrations of these compounds in plasma and hippocampus were also seen on the results of the interaction between the optimum concentration of alanine-glutamine dipeptide in rats aged 24 months to reach 32.90 and 52.91% compared to controls, whereas in rats older than 12 months each increased 52.66 and 39.10% (Fig. 1). Increased concentrations of alanine-glutamine dipeptide in the plasma and the hippocampus under the influence of the optimum concentration alanine-glutamine dipeptide or the interaction between the optimum concentration of this compound with age induces increased levels of glutathione in the hippocampus.

Glutathione levels of the hippocampus under the influence of the optimum concentration of alanine-glutamine dipeptide seems to have increased 85.76%

Table 1: Mean concentrations of alanine-glutamine dipeptide in plasma and hippocampus in rats exposed to physiological aging and aging due to oxidative stress after administration of the optimum concentration of alanine-glutamine dipeptide

	Oxidative stress	Alanine-glutamine dipeptide (%)	Alanine-glutamine dipeptide plasma (mmol/L)	Alanine-glutamine dipeptide hippocampus (µmol/g wet weight of tissue)	
Age (months)	12	NSO	0	0.510±0.036	5.510±0.050
		S	7	0.780±0.053	7.833±0.076
	24	NSO	0	0.465±0.021	4.817±0.076
		S	7	0.710±0.036	6.533±0.104
	Main factors	NSO	0	0.480±0.020	4.300±0.100
			7	0.670±0.026	6.657±0.140
		S	0	0.437±0.035	4.060±0.053
			7	0.550±0.050	6.100±0.020
		A		*	*
				*	*
G			*	*	
			*	*	
A-S			*	*	
			*	*	
Interactions	G-A		*	*	
			*	*	
	G-S		*	*	
		G-U-S		NS	NS

Data shown is the average value±standard deviation. NS: no oxidative stress, S: Oxidative stress, G: alanine-glutamine dipeptide, A: age. A *(p<0.05): the real effect, NS: No significant effect

Table 2: Mean levels of glutathione in the hippocampus in rats exposed to physiological aging and aging due to oxidative stress after administration of the optimum concentration of alanine-glutamine dipeptide

	Oxidative stress	Alanine-glutamine dipeptide (%)	Glutathione levels (mg/mg wet weight of tissue)	
Age (months)	12	NSO	0	0.0105±0.00016
		S	7	0.0158±0.00056
	24	NSO	0	0.0088±0.00065
		S	7	0.0149±0.00027
	NSO	0	0.0065±0.00039	
		7	0.0142±0.00038	
	S	0	0.0058±0.00045	
		7	0.0138±0.00053	
	Main factors	A		*
				*
G		*		
		*		
A-S		*		
		*		
Interactions	G-U		*	
			*	
	G-U-S		NS	

Data shown is the average value±standard deviation. NSO: no oxidative stress, S: Oxidative stress, G: alanine-glutamine dipeptide, A: age. A *(p<0.05): the real effect, NS: No significant effect

compared to controls. Results with the same pattern also occurs in the interaction between the alanine-glutamine dipeptide with age. Administration of optimum concentrations of alanine-glutamine dipeptide in rats older than 12 months of the hippocampus produce glutathione level reached 58.76%, higher than controls, while rats aged 24 months has increased 125.81% (Table 2). The results of this study provide evidence of a positive correlation between the concentrations of alanine-glutamine dipeptide in plasma and hippocampus with hippocampal glutathione level after being given the optimum concentration of alanine-glutamine dipeptide exogenous. It also found evidence that the rate of decline in alanine-glutamine dipeptide concentration in plasma and hippocampus and hippocampal glutathione levels under normal conditions

can be restored back to normal conditions can be improved even after being given the optimum concentration of alanine-glutamine dipeptide exogenous. This evidence suggests that the optimum treatment concentrations of alanine-glutamine dipeptide is needed in handling exogenous aging caused by the increase in age (physiological aging). The results are consistent with the findings of Mates *et al.* (2002) who reported that administration of exogenous glutamine has an important role in maintaining the balance of plasma and brain concentrations of glutamine and the regulation of brain oxidative metabolism. It was further reported that glutamine is not only important as an energy source but also as precursors of mitochondrial glutamate that is used to increase the synthesis of glutathione in the hippocampus.

The interaction between the optimum concentration of alanine-glutamine dipeptide with oxidative stress results in increased concentrations of alanine-glutamine dipeptide in the plasma and the hippocampus. Rats with oxidative stress results in increased concentrations of alanine-glutamine dipeptide in plasma and hippocampus respectively, 39.69 and 42.31%, higher than controls, but still lower than normal rats that have increased 46.46 and 47.71% (Fig. 1). Increased concentrations of these compounds, either in plasma or the hippocampus is directly proportional to the increase in hippocampal levels of glutathione. Shown in rats with oxidative stress, glutathione levels increased 97.26%, higher than controls, whereas normal rats had increased 76.47%. Difference increased concentrations of these compounds in normal rats or oxidative stress after being given the optimum concentration of alanine-glutamine dipeptide exogenous show an interaction between both factors. Increased concentrations of these compounds in plasma and hippocampus also involves the exogenous and endogenous factors. In a sense,

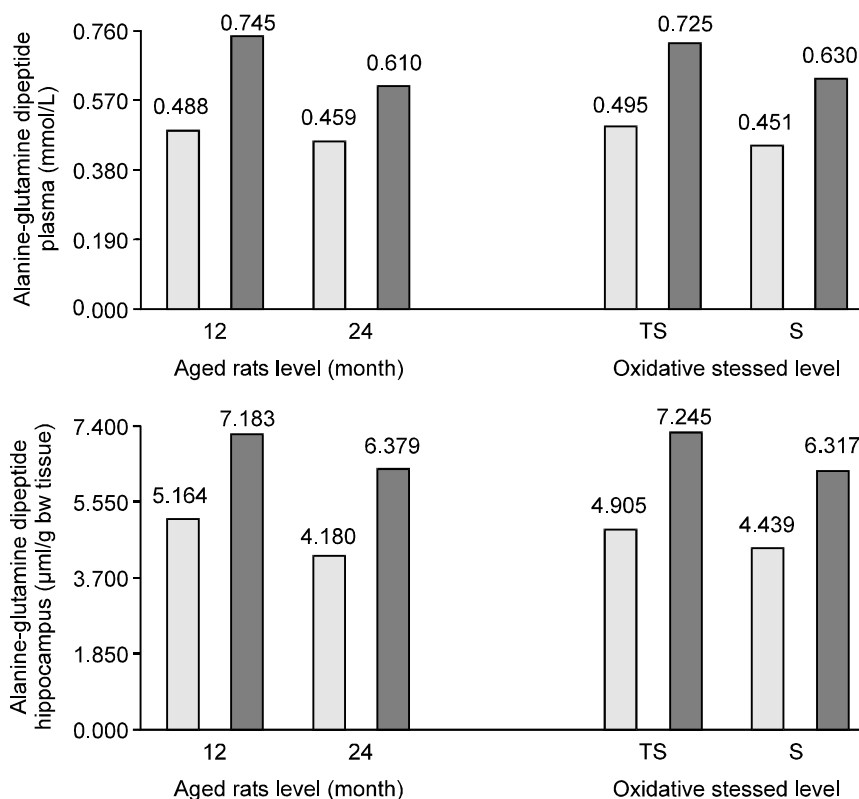


Fig. 1: Concentration of alanine-glutamine dipeptide in plasma and hippocampus result of interaction between the alanine-glutamine dipeptide 0% (black) and 7% (gray) with the level of aged rats or oxidative stress

apart from alanine-glutamine dipeptide exogenous, these compounds can also be derived from the biosynthesis in the body. This evidence is reinforced by the results of a study reported that the alanine-glutamine dipeptide is another form of glutamine that is non-essential amino acids. Non-essential nature means that these compounds can be synthesized in the body (Kulkarni *et al.*, 2005). It was further reported that the digestive tract is the main organ involved in the processing of nitrogen in the body that contributes significantly to the supply of glutamine and alanine-glutamine dipeptide in the blood and various body tissues, including the hippocampus (Lemberg and Fernandez, 2009). Increased concentrations of these compounds in plasma and hippocampus can support the process of merging several amino acids into proteins. Clinically, improvement of the concentration of glutamine, either in circulation or in body tissues may improve nitrogen balance, protein synthesis and tissue morphology, including the synthesis of glutathione in the hippocampus. Similarly, improved levels of glutathione in the body tissues, including the hippocampus is generally used for maintenance of the integrity of the immune system, the integrity of the cellular network and the health of normal tissues (Kulkarni *et al.*, 2005; Lemberg and Fernandez, 2009).

In addition to the response alanine-glutamine dipeptide concentration in blood plasma and the hippocampus, this study also observed the response of the structure of mitochondria in hippocampal neurons. Based on data in Table 3 and Fig. 2 shows that increasing the age to give effect to the changes in mitochondrial structure. Changes in mitochondrial structure at the age of 24 months of normal rats (no oxidative stress) is characterized by the presence of normal mitochondria 33%, mitochondria with partial damage 59% and mitochondria with total damage 8%. The structural changes of mitochondria in rats aged 24 months who received the treatment of oxidative stress characterized by the presence of normal mitochondria, mitochondria with partial damage and total, respectively 0, 26.8 and 73.2%. The results of this study provide evidence that the age old rats (24 months) in normal conditions have changed the structure of mitochondria. Mitochondria with damaged part found to be more dominant than normal mitochondria or mitochondria with total damage. Similarly, in rats aged 24 months who received the treatment of oxidative stress is also changing the structure of mitochondria. Mitochondria are found all abnormal and normal mitochondria was not found. Mitochondria with total damages found to be more dominant than the mitochondria with partial

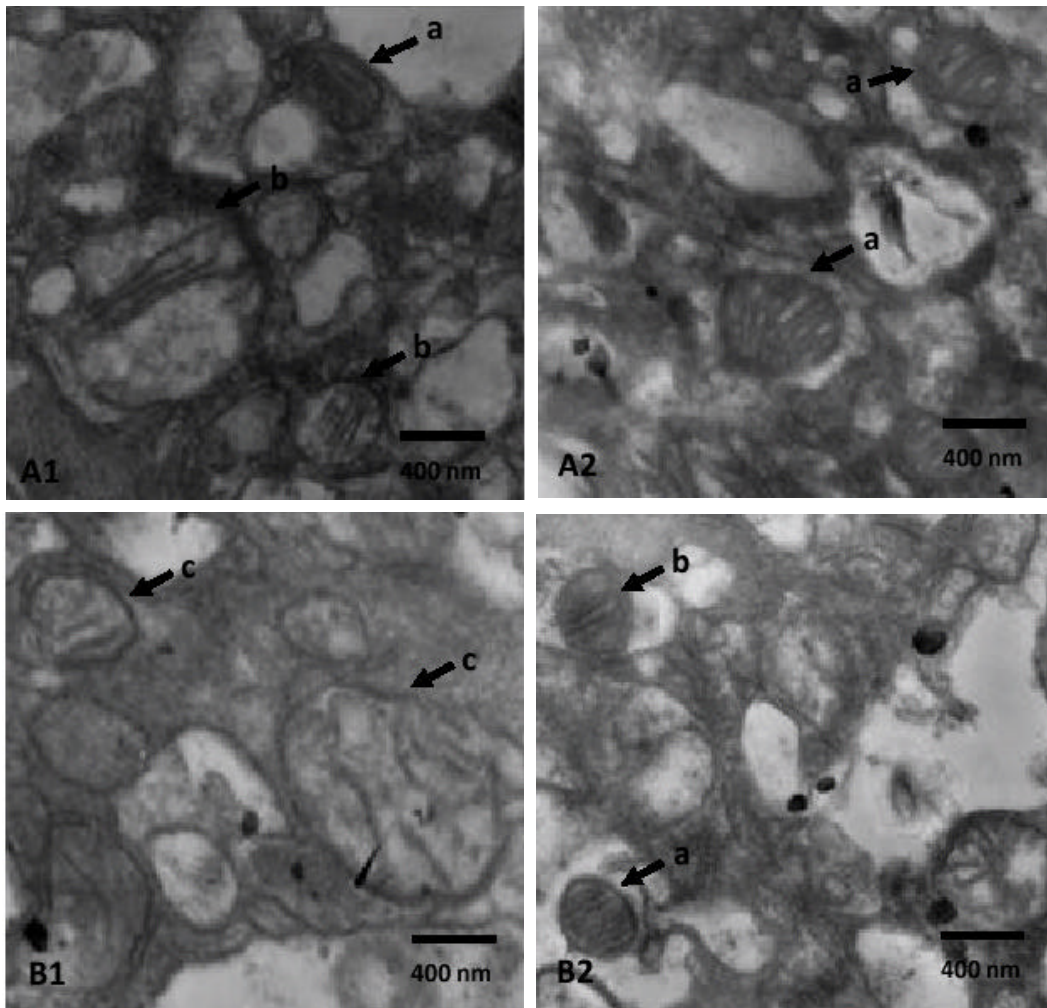
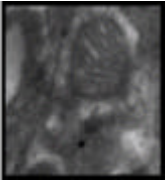
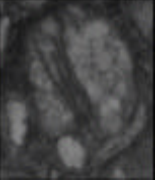
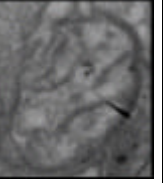


Fig. 2: Mitochondrial profiles hippocampal neurons results from interactions between alanine-glutamine dipeptide 0 or 7% with rats aged 24 months normal or oxidative stress. A1 and A2 respectively the mitochondrial profiles in the hippocampus in normal rats aged 24 months after being given a alanine-glutamine dipeptide 0 and 7%. B1 and B2, respectively the mitochondrial profiles in the hippocampus in oxidative stress rats aged 24 months after being given a alanine-glutamine dipeptide 0 and 7%. The arrows indicate mitochondria. Found a few mitochondria, which includes (a) normal mitochondria, (b) mitochondria with partial damage and (c) mitochondria with total damage

damage. Changes in the structure of mitochondria in old age rats with a high enough damage is the impact of the decline in cellular defense in free radical-mediated decrease in hippocampal levels of the antioxidant glutathione. Decrease in hippocampal glutathione levels due to an increase in age can lead to damage of the organic components of the cell, given the impact of disruption of transport processes across the membrane, decreased mitochondrial efficiency and decrease in cellular energy (Aliev *et al.*, 2009). Decrease in efficiency and cellular energy in the mitochondria gives an overview of mitochondrial damage with partial

damage. McBride *et al.* (2006) reported that structural changes of mitochondria in aging due to increased age is characterized by swelling of mitochondria. Swelling was triggered by a disturbance in the phospholipase, an enzyme which plays a role in mitochondrial phospholipid membrane repair process. Beck *et al.* (2011) reported that mitochondrial swelling is known to contain excessive amounts of cytochrome c that triggers mitochondrial cristae degeneration. Physiological aging is accompanied by oxidative stress can trigger the formation of reactive oxygen species with a higher production rate than normal conditions.

Table 3: Mitochondrial profiles hippocampal neurons results from interactions between alanine-glutamine dipeptide 0 and 7% by age 24 months normal (no oxidative stress) or oxidative stress

Alanine-glutamine dipeptide (%)	Normal		Oxidative stress		Characteristics of mitochondria	Profiles of mitochondrial structure
	0	7	0	7		
Normal mitochondria (%)	33	90	0	41.67	Normal morphology, has a diameter 400-500 nm, having outer and inner membranes and mitochondrial cristae	
Mitochondria with partial damage (%)	59	10	26.8	41.67	Abnormal morphology, has a diameter of more than 500 nm, have swollen (swelling), decreased the proportion of cristae, having outer and inner membranes	
Mitochondria with total damage (%)	8	0	73.2	16.67	Abnormal morphology, has a diameter of more than 500 nm, the mitochondria appeared to be empty or not found mitochondrial cristae, swelling (swelling) followed by membrane damage	

Reactive oxygen species in excessive amounts can cause free radical chain reactions that lead to an imbalance between endogenous antioxidant capacity, such as glutathione and oxidants in the cell, such as superoxide anion, hydrogen peroxide, hydroxyl radicals and others. These conditions can induce mitochondrial damage and impact on the larger disorders in the process of oxidation-phosphorylation, mitochondrial failure in the maintain cellular energy demands and significantly reduced hippocampal neuronal function. Failure of maintenance and energy needs by giving an overview of changes in mitochondrial structure damage to the mitochondria which is dominated by partial or total damage. Changes in mitochondrial structure with partial damage is characterized by degeneration of mitochondrial cristae or swelling size. Mitochondria with total damage is characterized by degeneration of the entire size of the mitochondrial cristae and swelling the size of mitochondria accompanied by the occurrence of damage to membrane phospholipids (Beck *et al.*, 2011). It was further reported that mitochondrial structural damage can occur as a result of an increase in intracellular Ca^{2+} ion-mediated decrease in glutathione levels. This condition affects the mitochondrial membrane leakage triggers mitochondrial structure damage (Green and La Ferla, 2008).

This study also observed at the response of mitochondria in rat hippocampal neurons age of 24 months of normal result the optimum concentration of alanine-glutamine dipeptide. The results showed that the response of hippocampal neurons mitochondria have improved after being given a alanine-glutamine dipeptide 7%. Repair of mitochondrial structure is characterized by the presence of 90% of normal mitochondria, mitochondria with partial damage to 10%, whereas mitochondria with total damage was not found, or 0%. The improvement of mitochondria in rats aged 24 months to the oxidative stress that given the alanine-glutamine dipeptide 7% shows that the concentrations of these compounds are able to provide improved mitochondrial structure in cells of hippocampal neurons. Repair of mitochondrial structure is characterized by the presence of normal mitochondria reached 41.67%, mitochondria with a total damage 16.67%, while mitochondria with a partial damage to be 41.67%. Increase in normal mitochondria and mitochondria with a total damage is evidence that the alanine-glutamine dipeptide 7% affects effective in preventing and repairing the structure of mitochondria is triggered by an increase in age or oxidative stress. Mitochondrial repair can occur through the mechanism of reduction in free radical production mediated by glutathione, maintenance and improvement of the integrity of mitochondrial membrane phospholipids, or the synthesis of mitochondrial membrane phospholipids is catalyzed by the enzyme phospholipase.

There are two metabolic pathways are known to be involved in the repair of mitochondrial structure and function of hippocampal neurons and is linked to the alanine-glutamine dipeptide. The first path associated with the synthesis of glutathione. Glutathione can react directly with free radicals in enzymatic reactions and the electron donor in the reduction or decrease in the production of free radicals is catalyzed by glutathione peroxidase (Dringen *et al.*, 2000). Reduction in free radicals can reduce oxidative damage to biological components of the cell, including the biological component in the mitochondria. Some researchers have reported that increased levels of glutathione can improve cellular integrity and transport mechanisms across cell membranes, improving the process of oxidation-phosphorylation and mitochondrial structure of hippocampal neurons (Sultana *et al.*, 2006; Jun *et al.*, 2006). The second pathway associated with glutamic acid metabolism pathways are directly involved in the synthesis of nucleic acids that contribute to increased expression of phospholipase. The increase of this protein has an important role in the synthesis of mitochondrial membrane phospholipids (Jun *et al.*, 2006; Cruzat and Tirapegui, 2009). Both of these metabolic pathways ultimately affect the decrease in the level of abnormality in the mitochondria. The evidence indicates that administration the optimum concentration of alanine-glutamine dipeptide can increase levels of glutathione, induced improvement of mitochondrial membrane phospholipids and mediates repair of mitochondrial structure and function in rats exposed to physiological aging or aging due to oxidative stress.

Profiles of mitochondria in rats aged 24 months, either normal or oxidative stress results giving alanine-glutamine dipeptide 7% representing of mitochondrial profiles in rats older than 12 months. With the physiological potential of a better, improved mitochondrial structure of hippocampal neurons in rats older than 12 months may occur more rapidly with the degree of improvement is better than rats aged 24 months. An improvement of mitochondrial structure in hippocampal neurons, both in normal rats older age or oxidative stress provide evidence that administration of alanine-glutamine dipeptide 7% can increase levels of glutathione and is able to repair damage to mitochondrial structure, both in rats exposed to physiological aging or aging due to oxidative stress.

Conclusion: Administration of alanine-glutamine dipeptide concentration of 7% can effectively increase the concentration of alanine-glutamine dipeptide in the plasma and the hippocampus, hippocampal levels of glutathione, improved response to mitochondrial structure that mediates the repair function in the aging hippocampus, both physiological aging or aging due to oxidative stress.

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