

Short Communication

Neuroprotection of soyabean isoflavone co-administration with folic acid against β -amyloid 1-40-induced neurotoxicity in rats

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Soya isoflavones (SIF) and folic acid (FA) both confer the biological properties of antioxidation; however, the mechanism of their antioxidant effect on nervous system development is unclear. Our purpose is to investigate the neuroprotective effects of SIF, FA or co-administration of SIF with FA against β -amyloid 1-40 ($A\beta$ 1-40)-induced learning and memory impairment in rats. In the present study, the learning and memory ability of rats and the amount of amyloid-positive neurons in the cerebral cortex and hippocampal CA1 area were measured. The levels of total antioxidant capacity (T-AOC), glutathione (GSH) and glutathione peroxidase (GSH-Px) in serum and brain tissue were also measured. The results showed that intracerebroventricular administration of $A\beta$ 1-40 resulted in a dramatic prolongation of the escape latency; however, in the SIF, FA and SIF + FA treatment groups, the functional deficits of learning and memory were significantly improved. Moreover, after $A\beta$ 1-40 injection, the levels of T-AOC and GSH were profoundly decreased, suggesting a decline of antioxidant activity in the rats. However, intragastric pre-treatment with SIF, or FA, or SIF + FA resulted in a significant increase of antioxidative activity. SIF, or FA, or SIF + FA treatments also reversed the $A\beta$ 1-40-induced increase in the amount of amyloid-positive neurons. These results suggest that: (1) learning or memory impairment in experimental rats was caused by $A\beta$ 1-40, which is probably attributed to $A\beta$ -induced oxidative damage and deposition of β -amyloid peptides in the brain; (2) pre-administration of SIF and/or FA may prevent the pathological alterations caused by $A\beta$ 1-40 treatment and the neuroprotective effects of SIF and/or FA are indicated.

Soyabean isoflavones: Folic acid: Neuroprotection: β -Amyloid peptide

β -Amyloid-peptide ($A\beta$) has long been considered as the chief constituent of senile plaques observed in the brain of patients with Alzheimer's disease⁽¹⁾. It has been reported that $A\beta$ 25-35 injection can cause mild learning and memory deficits in animals⁽²⁾. This assertion has also been supported by other experiments *in vivo*⁽³⁾.

The intake of soyabean isoflavones (SIF) has been proved to be associated with decreased risk of some kinds of diseases. However, there is a paucity of basic research exploring the mechanism of their actions in the mammal central nervous system. Although there is solid evidence from models of familial amyotrophic lateral sclerosis and ischaemia, supporting the idea that SIF may produce some extent of neuroprotection^(4,5), there remains less understanding whether they have similar neuroprotective effects against $A\beta$ -induced neurotoxicity. Additionally, it has been well established that folic acid (FA) deficiency may lead to many neurological and psychological disorders including impaired memory function⁽⁶⁾ and Alzheimer's disease⁽⁷⁾, whereas FA supplementation can improve cognitive function⁽⁸⁾. The exact principles of neuroprotective actions exhibited by FA are intangible.

Recently, a growing number of reports have indicated the involvement of oxidative stress in the occurrence of $A\beta$ -induced neurotoxicity^(9,10). Given the previously reported notion that dietary sources of sufficient antioxidants are critical for preventing oxidative damage⁽¹¹⁾, it seems that the antioxidant ability of FA and SIF is, at least partially, responsible for its neuroprotective roles. Nevertheless, whether SIF or FA can diminish oxidative damage during the development of learning and memory impairment and the neuroprotection of SIF co-administered with FA *in vivo* were not mentioned.

In the present study, we first investigated the negative influences exerted by intracerebroventricular administration of $A\beta$ 1-40 upon the learning ability and memory function of adult rats using the water maze test. Thereafter, neuroprotective effects of intragastric administration of SIF, FA or co-administration of SIF with FA were systemically evaluated using immunohistochemistry. In addition, the antioxidant activity changes in the serum and brain tissues of rats were further investigated to elucidate the possible neuroprotective mechanisms of SIF, FA or co-administration of SIF and FA in rats.

Abbreviations: $A\beta$, β -amyloid peptide; FA, folic acid; GSH, glutathione; GSH-Px, glutathione peroxidase; SIF, soya isoflavones; T-AOC, total antioxidant capacity.

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Materials and methods

Animals

The experiments were performed on seventy-five adult male Wistar rats (250 ± 30 g at the beginning of the experiment). The animals were provided by the Laboratory Animal Centre of Capital Medical University and handled in accordance with the guidelines established by the Chinese Committee on Experimental Animal Supervision.

All the rats were randomly divided into five groups: (1) control group (intracerebroventricular administration of physiological saline); (2) A β 1-40 group (intracerebroventricular administration of A β 1-40 (10 μ g) alone); (3) SIF group (intra-gastric pre-administration of SIF (160 mg/kg body weight per d) for 2 weeks before A β 1-40 treatment); (4) FA group (intra-gastric pre-administration of FA (0.7 mg/kg body weight per d) for 2 weeks before A β 1-40 treatment); (5) SIF and FA group (co-administration of SIF (160 mg/kg body weight per d) and FA (0.7 mg/kg body weight per d) for 2 weeks before A β 1-40 treatment).

Surgery

All experimental rats were first anaesthetised by intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). Then physiological saline (10 μ l) or A β 1-40 (10 μ g/10 μ l) was injected, respectively, into the targeted cerebral ventricle (anteroposterior – 1.2 mm from Bregma, mediolateral 2.0 mm, dorsoventral – 4.0 mm) according to the rats' solid localisation spectrum of a graph.

Morris water maze-learning and memory ability

On the eighth day after the injection of A β 1-40, the learning and memory ability of the rats was tested by an assessment of the rats' behaviour in a Morris water maze. These animals were released from four randomly assigned start positions respectively. Each rat was given training each day for four consecutive days to find the hidden platform and the full acquisition time course was recorded. The data on day 5 were recorded. The final readout included the escape latency, the distance to arrive at the hidden platform, and the frequency of the rat spanning the quadrant where the platform laid.

Immunohistochemistry

The SABC (streptavidin-biotin complex) immunocytochemical method was used to measure the amount of amyloid-positive cells in the cerebral cortex and hippocampal CA1 area. Leica Qwin Standard V2.8 image analysis software (Leica, Cambridge, UK) was applied to calculate the amount of A β 1-40-positive expressed neurons.

Measurements of antioxidative status

Brain tissue homogenates prepared from each group of rats were centrifuged at 4000 g for 15 min and the supernatant fraction of the homogenates was then used for the neurochemical assay. The serum was also collected from all experimental rats for the neurochemical assay. The levels and/or activities of total antioxidant capacity (T-AOC), glutathione (GSH) and

GSH peroxidase (GSH-Px) in the serum and brain tissue were analysed using the measurement kit. T-AOC activity, GSH and GSH-Px levels in the serum and brain tissue were as determined according to the guidelines of the kit.

Statistical analysis

Data were expressed as mean values and standard deviations. Statistical comparisons were performed by one-way ANOVA. The acceptable level of significance was set at $P < 0.05$.

Results

A β 1-40-treated rats exhibited significantly prolonged escape latency compared with that in the control group. However, the escape latency of rats in the FA, SIF, or SIF co-administered with FA groups was much shorter than that of the A β 1-40-treated group with statistical significance. No significant differences were found in the distance and frequency of rats spanning the platform between the five groups. Furthermore, A β 1-40-treated rats showed a substantial increase in the number of A β -positive cells in both the cerebral cortex and CA1 area of hippocampus formation. However, in the SIF-, FA- and SIF + FA-treated rats, a significant decrease was observed in the number of A β -positive cells in each tested region in comparison with that in the A β 1-40-injected group. In the A β 1-40-treated group, the level of T-AOC and the activity of GSH in the serum decreased dramatically compared with that in the control group. Pre-treatment of these animals with intragastric administration of SIF, FA or SIF co-administered with FA for 14 d before A β 1-40 injection led to an apparent increase in T-AOC level and GSH activity in the serum. However, GSH-Px activity showed no significant difference. A β 1-40 injection profoundly reduced T-AOC level and GSH activity in the brain tissues. Pre-treatment with FA, SIF or FA co-administered with SIF for 14 d before A β 1-40 injection clearly increased T-AOC activity and GSH level. However, GSH-Px activity showed no significant difference (Table 1).

Discussion

The present study suggests that intragastric administration of SIF and/or FA can prevent the neurotoxicity induced by A β 1-40, and this neuroprotection may be achieved by increasing the antioxidant effect and decreasing the numbers of A β -positive neurons, and eventually improving the learning and memory ability of rats.

The Morris water maze is a classical method to evaluate learning and memory accurately with different treatments⁽¹²⁾. The results of the Morris water maze showed that A β 1-40-treated rats exhibited significantly prolonged escape latency compared with that of the control group. SIF and/or FA could alleviate the change induced by A β 1-40, especially in the group injected with SIF alone. However, no significant differences in the total distance and the frequency of animals spanning the platform were found between the five groups. These results indicate that SIF and/or FA could alleviate A β 1-40-induced impairment of spatial learning.

The cerebral cortex and hippocampus are important regions that process learning and memory. In the present study,

Table 1. Effects of soya isoflavone (SIF) co-administration with folic acid (FA) on the learning and memory impairments of rats damaged by β -amyloid peptide (A β)

(Mean values and standard deviations for fifteen rats)

Group†...	Control		A β 1-40		FA		SIF		SIF + FA	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Latency (s)	10.15**	3.95	14.99††	5.15	11.67*	2.90	10.41**	2.72	11.41*	3.69
Distance (cm)	244.66	129.83	304.42	127.51	286.72	118.26	265.09	125.14	279.02	93.87
Frequency of crossing the platform	5.13	0.99	5.83	2.64	3.50	1.23	4.25	2.82	4.71	2.81
Serum T-AOC (U/ml)	11.20*	1.58	9.35†	0.84	11.10*	0.99	12.14**	0.96	12.37**	2.01
Serum GSH (mg/l)	143.03*	7.20	117.84†	6.53	143.64*	23.81	149.38**	16.35	155.05**	16.50
Serum GSH-Px (U)	317.78	17.38	310.66	13.43	301.11	4.51	320.47	12.31	312.87	16.93
Brain tissue T-AOC (U/ml)	3.09**	0.28	2.40††	0.23	2.87*	0.29	2.82*	0.37	2.99*	0.60
Brain tissue GSH (mg/l)	123.79*	8.44	103.66†	10.66	131.30**	16.32	126.78**	15.36	131.51**	21.65
Brain tissue GSH-Px (U)	5.43*	0.48	3.51	1.73	3.14	1.31	3.20	1.21	3.64	1.30
Number of amyloid-positive neurons in cerebral cortex	34.25*	26.64	83.75†	16.11	44.00*	15.13	42.80**	16.13	41.60**	17.64
Number of amyloid-positive neurons in hippocampus CA1	45.00*	26.6	134.20†	32.82	48.30*	20.01	40.00**	14.29	47.10*	14.70
Brain weight (g)	4.87	0.52	4.87	0.37	4.44	0.15	4.79	0.39	5.32	0.63

T-AOC, total antioxidant capacity; GSH, glutathione; GSH-Px, GSH peroxidase.

Mean value was significantly different from that of the A β 1-40 group: * P <0.05, ** P <0.01.Mean value was significantly different from that of the control group: † P <0.05, †† P <0.01.‡ Control group, intracerebroventricular administration of physiological saline treatment; A β 1-40 group, intracerebroventricular administration of A β 1-40 (10 μ g) alone; SIF group, intragastric pre-administration of SIF (160 mg/kg body weight per d) for 2 weeks before A β 1-40 treatment; FA group, intragastric pre-administration of FA (0.7 mg/kg body weight per d) for 2 weeks before A β 1-40 treatment; SIF + FA group, co-administration of SIF (160 mg/kg body weight per d) and FA (0.7 mg/kg body weight per d) for 2 weeks before A β 1-40 treatment.

A β -positive neurons in the cerebral cortex and hippocampus CA1 in rats were numbered to investigate the toxicity of A β 1-40 and the neuroprotection of SIF and/or FA. The amount of A β -positive neurons reflects the toxicity of A β 1-40. The present results showed that SIF and/or FA can reduce the number of A β -positive neurons both in the cerebral cortex and hippocampus CA1, which indicates that SIF and/or FA could alleviate the toxicity of A β 1-40.

The strong relationship between oxidative stress and A β prompted us to investigate the oxidative damage of neurons induced by A β 1-40. We found that A β 1-40 could lower T-AOC and GSH levels, indicating the oxidative damage induced by A β 1-40 due to changes in redox homeostasis and reduction of antioxidant ability both in serum and brain tissue.

SIF and FA are both diet-derived antioxidants, which are critical in protection against oxidative damage⁽¹³⁾. SIF have been described to decrease antioxidant enzyme activities in tissues and erythrocytes of experimental rats⁽¹⁴⁾. FA has also been proved to establish strong antioxidant activity⁽¹⁵⁾.

The antioxidant enzyme system of cells plays an important role in oxidative stress. Antioxidant enzymes such as GSH, GSH-Px and superoxide dismutase are all antioxidant molecules that act as redox homeostasis keepers *in vivo*⁽¹⁶⁾. Therefore, the agent that exhibits a regulatory effect on these antioxidant molecules is suggested to be the promising prevention or treatment strategy for oxidative impairment. In the present study, we further investigated the neuroprotection of SIF and/or FA on A β 1-40-damaged neurons, so the level of oxidative damage-related molecules was measured experimentally in rat serum and brain tissues. The present results demonstrated that SIF show neuroprotection by up-regulating the antioxidant level both in serum and brain tissues. Compared with the A β 1-40 treatment group, the T-AOC activity and GSH level in the FA, SIF, and FA co-administered with SIF

groups were all increased, which indicates that SIF and/or FA can alleviate oxidative damage by maintaining redox homeostasis and increasing antioxidant activity both in serum and brain tissues.

However, the protective effects of SIF and FA in combination in the Morris water maze test, the number of A β -positive cells and the antioxidative parameters were not significantly different from isolated effects. The reason may be related to the isolated protective effects of SIF and FA that were so strong that the combination effect was not prominent, while the protective effect was enhanced when the isolated effects were not sufficient.

In addition, previous studies have indicated that SIF exhibit antioxidant activity and modulate the enzymic antioxidant defence system by increasing resting erythrocyte superoxide dismutase activity and restoring the altered redox homeostasis⁽¹⁷⁾. FA supplementation can improve the total plasma antioxidant capacity in haemodialysis patients⁽¹⁸⁾.

All together, these results were consistent with our findings that SIF, FA, or SIF co-administered with FA performed strong antioxidant activity *in vivo*, which may be the possible mechanism of the neuroprotective effect of these food-derived antioxidants.

Conclusions

Learning or memory impairment in experimental rats was caused by A β 1-40, which can probably be attributed to A β -induced oxidative damage and deposition of A β in the brain. Pre-intragastric administration of SIF and/or FA can inhibit the neurotoxicity induced by A β 1-40 *via* increasing the antioxidant effect in both serum and brain tissues and decreasing the number of A β -positive neurons, finally improving the learning and memory ability of rats.

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The authors have no conflicts of interest to declare.

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