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Antidepressant-like effect of asiaticoside in mice

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Abstract

In the present study, the potential antidepressant properties of asiaticoside were investigated in male mice in three tests—splash test in the unpredictable chronic mild stress (CMS) model, tail suspension test (TST), forced swimming test (FST)—with clomipramine being a positive control. In the splash test, asiaticoside (10 mg/kg, PO) and clomipramine (50 mg/kg, PO) significantly augmented the frequency of grooming behavior in stressed mice. In the tail suspension test, asiaticoside (10, 20 mg/kg, PO) and clomipramine (50 mg/kg, PO) significantly decreased immobility time. In the forced swimming test, asiaticoside (10, 20 mg/kg, PO) and clomipramine (50 mg/kg, PO) significantly decreased immobility time. These results suggest that asiaticoside may have antidepressant-like action.

Keywords: Asiaticoside; Clomipramine; Antidepressant; Mice; Splash test; Unpredictable chronic mild stress (CMS) model; Tail suspension test; Forced swimming test

1. Introduction

Asiaticoside, a major pentacyclic triterpenoid saponin component of *Centella asiatica* (L.) Urh, has been described to have wound healing, antiulcer, antioxidant and anti-inflammatory activities (Fig. 1). Guo et al. (2004) reported that the mechanisms by which asiaticoside exerted its antiulcer and anti-inflammatory effects may be associated with inhibition of NO synthesis by its inhibitory effect on inducible nitric oxide synthase (iNOS). In our previous studies, asiaticoside was shown to present an anxiolytic-like effect (Si Wei Chen et al., 2006).

Chronic mild stress (CMS) suppresses rewarded-related behaviors in rats and mice. The CMS has a reasonably high degree of validity (Willner, 1984; Willner et al., 1992), and its prolonged duration makes it suitable to examine the effects of chronic treatment with antidepressant drugs. It has been reported that the CMS regimen decreases the consumption of or preference for a sucrose solution (Willner et al., 1987) and induces a degradation of the physical state of the coat (Ducottet et al., 2003; Ducottet and Belzung, 2004). Chronic treatment with antidepressant drugs reverses the hedonic deficits caused by chronic mild stress (Willner et al., 1992). In the context of screening for potential antidepressive agents, mice may be preferable to rats on grounds of cost, size and social organization (Santiago and Paolo, 1995). In the present study we used two parameters: the state of the coat was used to evaluate grooming behavior indirectly whereas the splash test was used to determine this behavior directly (Santarelli et al., 2003).

We also used the tail suspension test (TST) and forced swimming test (FST) to evaluate the antidepressant-like activity of asiaticoside. The TST and FST are well established screening paradigms for antidepressants. A variety of antidepressants are known to reduce immobility time in TST (Steru et al., 1985). The FST, as originally described by Porsolt et al. (1977a,b), is the most widely used pharmacological model for assessing antidepressant activity (Weiss and Kilts, 1998). This is largely due to its ease of use, reliability across laboratories, and ability

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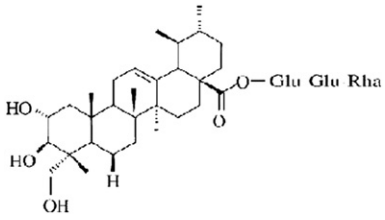

Fig. 1. The structure of asiaticoside.

to detect a broad spectrum of antidepressants (Borsini and Meli, 1988; Weiss and Kilts, 1998).

2. Animals and methods

2.1. Experiment 1

2.1.1. Animals

Male Swiss mice (bred at the Experimental Animal center of Shenyang Pharmaceutical University) Weighing 16–18 g was used in this study. They were kept in the laboratory for two weeks before treatment. In the unpredictable chronic mild stress (CMS) model the stressed mice were individually housed and the non-stressed mice were kept in groups of five in polycarbonate cages (cage size: 25×14×12 cm) and kept in a 12 h light/dark cycle (lights on at 19:00). Food and water were freely available.

The experiments were performed following approval of the committee of Experimental Animal Administration of the University and were in accordance with the National Institutes of Health Guide of the care and use of Laboratory Animals.

2.1.2. Drugs and treatments

Asiaticoside was purchased from Guangxi Changzhou Natural Products Development Co. Ltd (Nanning, China), clomipramine from Jiangsu Nhwa Pharma. Corporation (Jiangsu, China), Tween-80 from Shenyang dongxing Reagent Factory (Shenyang, China).

At the end of two weeks of a drug-free stress exposure mice were assigned to the different groups in a semi-randomized manner, so that the initial coat state and body weight were equivalent in all the groups. The drugs were given daily at 13:30. Asiaticoside (5, 10, 20, 40 mg/kg) and clomipramine (50 mg/kg) were both ultrasonically dispersed in distilled water containing Tween-80 (0.5%), and were prepared freshly on test days and administered PO two weeks after the beginning of the unpredictable chronic mild stress regimen in a volume of 10 ml/kg. Control animals were administered with the corresponding vehicle. The non-stressed mice were also administered PO at the same time with the stressed mice in a volume of 10 ml/kg.

2.1.3. Unpredictable chronic mild stress model (CMS)

The CMS was based on the procedure originally designed by Willner et al. (1992) and adapted to mice (Ducottet and Belzung, 2004). Mice were subjected several times a day for six weeks to one of the following stressors such as damp sawdust, sawdust changing, placement in an empty cage, placement in an empty cage with water on the bottom, switching cages, cage tilting (45 °C), a period of intensity stroboscopic illumination (150 flashes/min), predator sounds for 15 min, inversion of light/dark cycle, lights on for a short time during the dark phase (Ipek and Fazilet, 2005). To prevent habituation and to provide an unpredictable feature to the stressors, all the stressors and/or sequences were administered at different time points every week (see Table 1).

2.1.4. Coat state and body weight

The coat state and body weights of the animals were recorded every Monday before and during the CMS. The evaluation of the coat state was carried out by the assessment of eight different body parts: head, neck, dorsal coat, ventral coat, tail, forepaws, hindpaws and genital region (Ducottet et al., 2003; Ducottet and Belzung, 2004). A score of 0 for a coat in a good state or a score of 1 for a dirty coat were given for each of these areas. Total score was obtained from the sum of the score of each body parts. We presented the total score that we obtained the last week of the stress regimen. The observers who scored the state of the coat were unaware of the treatment of the mice.

2.1.5. Splash test

After 3 weeks of treatment (beginning the sixth week) the splash test was performed. This test was used to evaluate the grooming behavior of both stressed and non-stressed mice. 10% sucrose solution was squirted on the dorsal coat of mice in their homecage (0.35 ml per mouse). Grooming is cleaning the fur of animal by licking or face grooming (strokes along the snout), head washing (semicircular movements over the top of the head and behind the ears), body grooming (body fur licking). The frequency of grooming refers to the number of licking during 5 min. The frequency of grooming was recorded during 5 min after the vaporization of sucrose solution (Ducottet and Belzung, 2004). The observer was unaware of the treatment conditions.

2.1.6. Open-field test

Locomotor activity was studied in mice by open-field test, each animal was placed in the center of a square arena (60×60×35 cm) with a black floor which was divided into 16 equal squares with white line. The apparatus was illuminated with red bulbs (25 w) on the ceiling. The drugs were administered P.O. 60 min prior to testing. The total number of squares entered was recorded for 5 min (Si Wei Chen et al., 2005). We presented the total squares entered that we obtained the last week of the stress regimen. The observers were unaware of the treatment of the mice.

2.2. Experiment 2

2.2.1. Animals

Male Swiss mice (bred at the Experimental Animal center of Shenyang Pharmaceutical University) Weighing 16–18 g were used in this study. Mice were housed in groups of five in polycarbonate cages for at least 10 days prior to testing under a

Table 1
The procedure of the unpredictable chronic mild stress model

	Mon	Tue	Wed	Thu	Fri	Sat	Sun
Week1	Coat state, weighing; (10 h) intensity stroboscopic illumination (11 h–12 h30) Damp Sawdust (15 h–17 h)	Without Sawdust (9 h–11 h), cage Changing (14 h–15 h30)	Light (10 h30–11 h30) 3 Sawdust Changing (13 h30–14 h30)	Damp Sawdust (9 h–10 h30) intensity stroboscopic illumination (15 h)	Cages tilt at 45 °C (10 h30–12 h) Cage Changing (13 h–16 h)	Reversal of the light/dark cycle (after 8 h) reversal of the light/dark cycle	reversal of the light/dark cycle reversal of the light/ dark cycle
Week2	Coat state, weighing; (10 h) Sound Of predator (12 h) cages tilt at 45 °C (15 h–16 h30)	Intensity stroboscopic illumination (9 h30) damp Sawdust (13 h30–15 h30) light (17 h–18 h)	Without Sawdust (10 h–12 h) Sound Of predator (15 h)	Dark (5 h–6 h) 2 Sawdust Changing (10 h–11 h)	2 Cage Changing (9 h–10 h30) 3 Sawdust Changing (14 h30–15 h30)	4 light/dark every 30 min (9 h–11 h30) Light (15 h–17 h) dark (4 h–6 h)	4 light/dark every 30 min (9 h–11 h30) Light (15 h–17 h) dark (4 h–6 h)
Week3	Coat state, weighing; (10 h) Treatment (13 h30) Without Sawdust (15 h–16 h30)	cages tilt at 45 °C (11 h–13 h30) Treatment (13 h30) Light (17 h–18 h)	Damp Sawdust (8 h–11 h30) Treatment (13 h30) Cage Changing (14 h30–17 h30)	Sound Of predator (10 h) Treatment (13 h30) Without Sawdust (15 h30–17 h30)	Light (9 h–9 h30) Treatment (13 h30) cages tilt at 45 °C (16 h–18 h30)	reversal of the light/dark cycle reversal of the light/dark cycle treatment (13 h30)	reversal of the light/dark cycle reversal of the light/ dark cycle treatment (13 h30)
Week4	Coat state, weighing (10 h) Treatment (13 h30) Cages tilt at 45 °C (14 h30–15 h30) (17 h–18 h)	Cage Changing (9 h–11 h) Treatment (13 h30) Light (15 h30–16 h)	Cage Changing (9 h30–11 h30) Treatment (13 h30) Damp Sawdust (14 h30–17 h30)	Sawdust Changing (10 h) Treatment (13 h30) Sound of predator (15 h)	Damp Sawdust (9 h–9 h30) Treatment (13 h30) Bath 15 min (14 h30)	Light (9 h–11 h) Treatment (13 h30) 2 Cage changing (15 h–16 h30)	Treatment (13 h30) 4 light 30 min (15 h15–16 h15–17 h15–18 h15–19 h15)
Week5	Coat state; weighing; (10 h) Treatment (13 h30) without Sawdust (16 h–17 h30)	Cages tilt at 45 °C (10 h–12 h30) Treatment (13 h30) light 30 min (17 h–19 h)	Sound Of predator (9 h) Treatment (13 h30) damp Sawdust (15 h–17 h30)	Without Sawdust (10 h–11 h30) Treatment (13 h30) cages tilt at 45 °C (14 h30–16 h)	Dark (5 h–6 h) Treatment (13 h30) 2 Sawdust Changing (15 h30–18 h)	4 light/dark 30 min (9 h–11 h) Treatment (13 h30) Light (14 h30–16 h30)	Dark (4 h–6 h) 4 light/dark 30 min (9 h–11 h) Treatment (13 h30)
Week6	Coat state; weighing; (10 h) Cages tilt at 45 °C (11 h–13 h30) Treatment (13 h30)	Behavioral Tests Treatment (13 h30)	Behavioral Tests Treatment (13 h30)	Behavioral Tests Treatment (13 h30)	Behavioral Tests Treatment (13 h30)	Behavioral Tests Treatment (13 h30)	Behavioral Tests Treatment (13 h30)

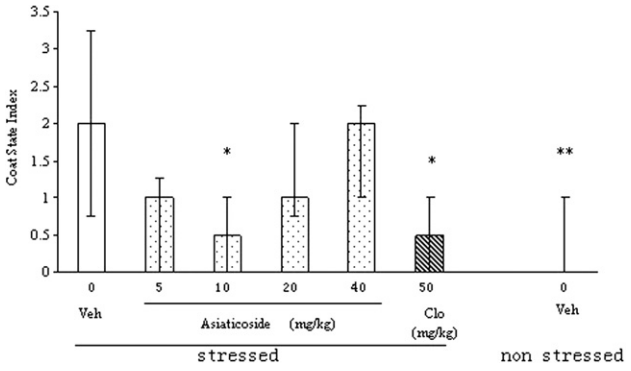

Fig. 2. Effects of asiaticoside (5, 10, 20, 40 mg/kg, PO) and clomipramine (50 mg/kg, PO) on the coat state after the end of the CMS regimen. All of the treatments began after two weeks of stress regimen during the later 4 weeks. Results are expressed as median (interquartile range) of (n=9–10) number of animals. Data are compared by Kruskal–Wallis H followed by Dunn's test when significant differences were detected. *α<0.1. **α<0.05, significantly different when compared to the stressed vehicle [distilled water containing Tween-80 (0.5%), PO].

12 h light/dark cycle (lights on at 19:00). Food and water were freely available. All mice were experimentally naïve.

The experiments were performed following approval of the committee of Experimental Animal Administration of the University and were in accordance with the National Institutes of Health Guide of the care and use of Laboratory Animals.

2.2.2. Drugs and treatments

Asiaticoside (5, 10, 20, 40 mg/kg) and clomipramine (50 mg/kg) were both ultrasonically dispersed in distilled water containing Tween-80 (0.5%). The mice were treated chronically for at least 7 days before test. All drugs were prepared freshly on test days and administered PO in a volume of 10 ml/kg. Control animals were administered with the corresponding vehicle.

2.2.3. Tail suspension test in mice

The total duration of immobility induced by tail suspension was measured according to the methods described by Steru et al. (1985). Briefly, mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6-min period (Rodrigues et al., 2002; Mantovani et al., 2003). Mice were considered immobile only when they hung passively and were completely motionless.

2.2.4. Forced swimming test

Mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25±1 °C. The total duration of immobility was recorded during the last 4 min of the 6-min period. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant-like effect (Porsolt et al., 1977a,b).

2.3. Statistics

The results of the state of the coat and the frequency of grooming were compared by Kruskal–Wallis H followed by Dunn's test when significant difference was detected. Results are expressed as medians (interquartile range) of (n) number of animals. The locomotor activity, the body weight, the immobility time in tail suspension test and forced swimming test with one-way ANOVA, whenever ANOVA was significant, further comparisons between vehicle and drug-treatment groups were performed using the Dunnett's t-test. Results are expressed as the means ±SEM. Analyses were performed using the software SPSS 11.5 for windows. The level of statistical significance adopted was P<0.05.

3. Results

3.1. Coat evaluation and body weight

Effects of asiaticoside (5, 10, 20, 40 mg/kg, PO) and clomipramine (50 mg/kg, PO) on the coat state in stressed group after the end of the CMS regimen are shown in Fig. 2. By the Kruskal–Wallis H test, we observed a significant difference between the groups (H=22.754, P<0.05). By the Dunn's test, We observed a significant difference between non-stressed vehicle and stressed vehicle groups at the end of the CMS regimen (α<0.05). Asiaticoside (10 mg/kg, α<0.1) and clomipramine (50 mg/kg, α<0.1) significantly reversed the degradation on the coat state induced by the CMS in stressed mice when compared to vehicle group.

By the one-way ANOVA, we did not observe a significant difference of the body weight between non-stressed mice and stressed mice. The results are presented in Table 2.

3.2. Splash test

Fig. 3 illustrates the total frequency of the grooming behavior during the splash test in mice after the end of the CMS regimen. The test of Kruskal–Wallis H revealed a

Table 2
The effects of drugs on the body weight and locomotor activity after the Unpredictable chronic mild stress

Environment	Treatment (mg/kg)	Body weight (g)	Locomotor activity (squares crossed/5 min)
Non-Stressed	Vehicle	32.7±0.8	63.7±7.1
	Clomipramine (50)	31.3±1.1	62.7±5.6
	Asiaticoside (5)	35.4±1.7	57.5±7.9
	Asiaticoside (10)	33.1±1.7	48.9±7.3
	Asiaticoside (20)	36.0±1.8	55.2±8.3
	Asiaticoside (40)	36.5±1.6	58.8±36.3
Stressed	Vehicle	32.0±0.9	63.2±10.0
	Clomipramine (50)	35.9±2.1	58.7±8.4
	Asiaticoside (5)	34.9±1.0	68.0±14.2
	Asiaticoside (10)	35.4±1.4	62.1±9.8
	Asiaticoside (20)	34.8±1.3	62.7±11.4
	Asiaticoside (40)	36.3±4.9	45.2±5.6

Results are shown as the means±SEM. (n=9–10).

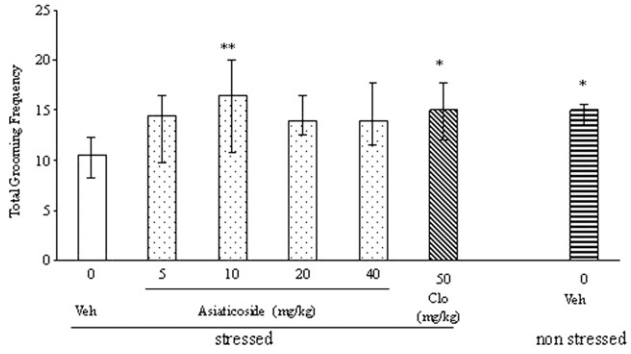

Fig. 3. Effects of asiaticoside (5, 10, 20, 40 mg/kg, PO) and clomipramine (50 mg/kg, PO) on the total frequency of the grooming behavior during the Splash test in mice after the end of the CMS regimen. All of the treatments began after two weeks of stress regimen during the later 4 weeks. Results are expressed as median (interquartile range) of (n=9–10) number of animals. Data are compared by Kruskal–Wallis H followed by Dunn's test when significant differences were detected. *α<0.1, **α<0.05, significantly different when compared to the stressed vehicle [distilled water containing Tween-80 (0.5%), PO].

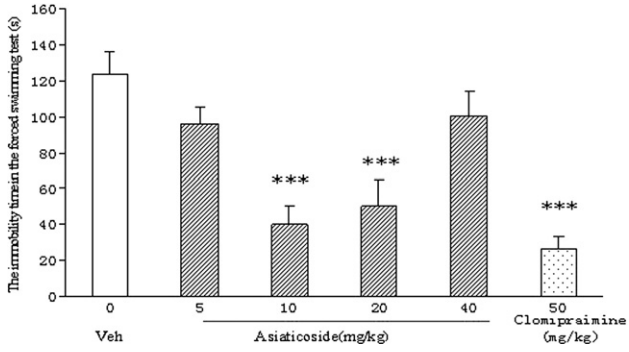

Fig. 5. Effects of Asiaticoside (5, 10, 20, 40 mg/kg, PO) and Clomipramine (50 mg/kg, PO) on the time of immobility during the last 4 min of the 6-min testing period in forced swimming test. Results are expressed as mean±SEM (n=10). The drugs were administered P.O. 60 min prior to testing. ***P<0.001, significantly different when compared to the vehicle [distilled water containing Tween-80 (0.5%), PO]. Comparison was made by ANOVA followed by Dunnett's t-test.

significant differences between the groups (H=21.829, P<0.05). Asiaticoside did not elicit any effect in non-stressed mice (H=7.022, P=0.219). By the Dunn's test, non-stressed mice groomed significantly more than stressed vehicle (α<0.1), asiaticoside (10 mg/kg, α<0.05) and clomipramine (50 mg/kg, α<0.1) significantly augmented the frequency of the grooming behavior in stressed mice in the splash test.

3.3. Open-field test

The results of locomotor activity are shown in Table 2. ANOVA for number of squares entered yield [F (11,106)=0.507, P=0.895]. No significant augmentation or impairment of locomotor activity due to unpredictable chronic mild stress regimen or treatment was detected.

3.4. Tail suspension test in mice

Date are summarized in Fig. 4. ANOVA (df=5,54) indicated significant treatment effects on the immobility time (F=9.032, P<0.001). Further analyses confirmed that asiaticoside (10 mg/kg, P<0.01; 20 mg/kg, P<0.001) and clomipramine (50 mg/kg, P<0.001) significantly decreased the immobility time.

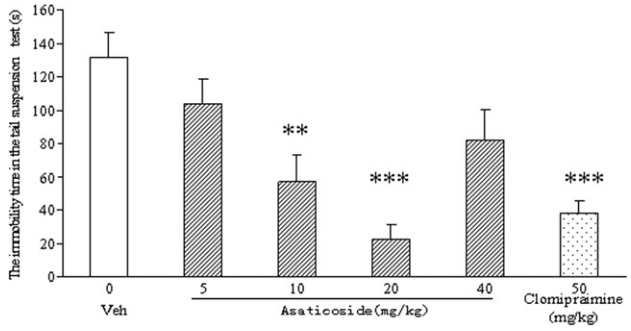

Fig. 4. The effects of asiaticoside (5, 10, 20, 40 mg/kg, PO) and clomipramine (50 mg/kg, PO) on the immobility time during the tail suspension test. Results are expressed as mean±SEM (n=10). The drugs were administered P.O. 60 min prior to testing. **P<0.01, ***P<0.001, significantly different when compared to the vehicle [distilled water containing Tween-80 (0.5%), PO]. Comparison was made by ANOVA followed by Dunnett's t-test.

3.5. Forced swimming test

The time of immobility during the last 4 min of the 6-min testing period are presented in Fig. 5. ANOVA (df=5,54) indicated significant treatment effects on the immobility time (F=11.597, P<0.001). Further analyses confirmed that asiaticoside (10 mg/kg, P<0.001; 20 mg/kg, P<0.001) and clomipramine (50 mg/kg, P<0.001) significantly decreased the immobility time.

4. Discussion

The results of the present study demonstrate that asiaticoside has antidepressant-like effects in unpredictable chronic mild stress (CMS) model, tail suspension test and forced swimming test.

The CMS is generally thought to be the most promising and valuable model to study depression in animals, mimicking several human depressive symptoms (Willner et al., 1987). The results showed that the CMS regimen induced a degradation in the state of the coat and decreased the grooming behavior in the splash test in the stressed vehicle mice, when compared to the non-stressed vehicle mice. The decrease of the grooming behavior in the splash test, were counteracted by asiaticoside and clomipramine. This may be not due to the effects of the treatments on activity, since locomotion remains unchanged. The degradation in the state of coat was counteracted by asiaticoside and clomipramine. The results indicate that asiaticoside may have an antidepressant-like effect. It has been recently shown that the regulation of α_2-adrenergic receptor may be the major mechanism of this model (Ipek and Fazilet, 2005). However, further experiments evaluating the levels of noradrenaline and serotonin in different brain regions are necessary to confirm this hypothesis.

The TST are well established screening paradigms for antidepressants. A variety of antidepressants are known to reduce immobility time in TST (Steru et al., 1985). The results illustrated that asiaticoside has significantly reduced the immobility time.

The FST was designed by Porsolt as a primary screening test for antidepressants and it remains one of the best models for this purpose for several reasons. It is a low-cost, fast and reliable model to test potential antidepressant treatments with a strong predictive validity (Porsolt et al., 1977a,b, 1978). Porsolt et al. proposed this behavioral model for the screening of new antidepressant compounds, concluded that the immobility time observed in the test reflected a state of lowered mood or hopelessness in animals, thus, this animal model is the most widely used tool for preclinical screening of putative antidepressant agents (Cryan et al., 2002, 2005). The FST shows a strong sensitivity to monoamine alterations and is a very specific cluster of stress-induced behaviors that have no direct, empirical relation to depression symptoms in humans, but which are nonetheless exquisitely sensitive to monoaminergic manipulations (Petit-Demouliere et al., 2005). It also provides a useful model to study neurobiological and genetic mechanisms underlying stress and antidepressant responses (Lucki et al., 2001; Nestler et al., 2002). The present study describes the pharmacological evaluation of asiaticoside which showed a clear antidepressant activity in mice evaluated in the FST.

In summary, the results of present study indicate that asiaticoside might induce antidepressant-like effects in unpredictable chronic mild stress (CMS) model, tail suspension test and forced swimming test, but its mechanism is still unclear and required to be further investigated.

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