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Research Paper

Possible mechanism of the antidepressant effect of 3,6′-disinapoyl sucrose from *Polygala tenuifolia* Willd

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Abstract

Objective The present study was designed to observe the effects of 3,6′-disinapoyl sucrose (DISS), an active oligosaccharide ester component obtained from the roots of *Polygala tenuifolia* Willd., on behavioral and biochemical aspects of depression induced by chronic mild stress (CMS) in rats. It is the first exploration of the possible association between DISS's antidepressant-like effects and biochemical markers of depression, and involved measuring monoamine oxidase (MAO) activity, cortisol levels, superoxide dismutase (SOD) activity and malondialdehyde (MDA) levels.

Methods Rats were exposed to stressor once daily for consecutive 5 weeks. DISS and a positive control drug, fluoxetine, were administered via gastric intubation to once daily for consecutive 3 weeks from the third week.

Key findings The results showed that rats subjected to CMS exhibit a reduction in sucrose intake. Conversely, brain MAO-A and MAO-B activity, plasma cortisol levels, and MDA levels were increased, while SOD activity was decreased following CMS exposures. DISS significantly inhibited MAO-A and MAO-B activity and blocked plasma elevated cortisol level, an indicator of the hypothalamic–pituitary–adrenal (HPA) axis. In addition, DISS increases SOD activity, inhibits lipid peroxidation, and lessens production of MDA.

Conclusion These results suggest that DISS may possess potent and rapid antidepressant properties, which are mediated via MAO, the HPA axis and oxidative systems. These antidepressant actions make DISS a potentially valuable drug for the treatment of depression.

Keywords 3,6′-disinapoyl sucrose; chronic mild stress; depression; monoamine oxidase

Introduction

Depression is a common disorder with high lifetime rates. It is a major cause of disability and causes death both by suicide and due to raised rates of physical disorders. It is often associated with alteration of neurochemical markers and some antidepressants achieve their therapeutic effects through reversing these changes. The activity of monoamine oxidase (MAO) has been suggested to be a trait-dependent indicator of vulnerability to psychopathology.[1] MAO exists in two forms: A and B. Inhibition of MAO-A activity is believed to cause antidepressant effects, while coherence inhibition of MAO-B activity is believed to slow the progression of neurodegenerative processes such as Parkinson's disease.[2]

Hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis is one of the key biological abnormalities described in major depressive disorder, occurring in 30–50% of depressed subjects.[3] Elevated cortisol levels have been the most widely used peripheral marker of stress responses and have become a well-established index of HPA axis activation in psychophysiology research in humans.[4] Clinical studies have shown that some antidepressant drugs, such as amitriptyline and fluoxetine, cause significant declines in cortisol concentrations and bring about initial recovery of the HPA axis.[5]

Some studies have also shown that oxidative stress plays an important role in the pathophysiology of neuropsychiatric disorders.[6] Superoxide dismutase (SOD) and malondialdehyde (MDA), a product of lipid peroxidation, are considered possible biochemical markers of mental disorder.[7] Treatment with antidepressant drugs significantly decreases levels of SOD and MDA in depressive patients.[6,8]

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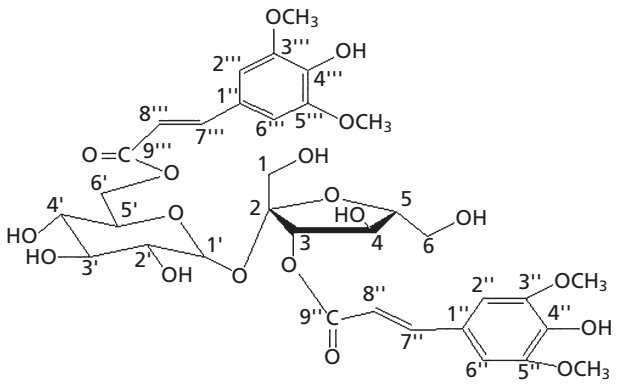
Some active compounds found in wild plants have been shown to have antidepressant effects in animals.[9,10]Disinapoyl sucrose (DISS) is the active oligosaccharide ester component found in the root of *Polygala tenuifolia* Willd., the latter recorded as 'YuanZhi' in the pharmacopoeia of the People's Republic of China. The root has been used in traditional medicine as, among other things, an expectorant, tonic, tranquillizer and antipsychotic agent. Our laboratory has demonstrated that DISS administration significantly decreases immobility time in the tail-suspension and forced-swim tests in mice, and also has notable antidepressant effects in pharmacological depression models. Previous study has suggested that an increase in 5-hydroxytryptamine and norepinephrine in the central nervous system might be one of the possible mechanisms.[11] A subsequent study showed that DISS can alleviate stress-induced behavioral abnormalities and corresponding gene changes in the HPA axis in rats.[12]

To our knowledge, no data are currently available about the behavioral effects of oral treatment with DISS in chronic mils stress (CMS) animals, or about simultaneous changes in biochemical and neurochemical markers. Consequently, the aim of the present study was to examine the effects of treatment with DISS in the CMS rat and to determine if pretreatment values of biochemical and neurochemical markers (MAO-A and MAO-B activity in brain, cortisol and MDA levels and SOD activity in serum) might predict the therapeutic response to DISS.

Materials and Methods

Plant material and preparation

The roots of *P. tenuifolia* were purchased from Traditional Chinese Medicinal (TCM) pharmacy, Chinese People's Liberation Army (PLA) General Hospital (Beijing, China); a voucher specimen (NU-80617) was deposited in the herbarium there. DISS was isolated from the roots of *P. tenuifolia* as described previously.[13] Its chemical structure is shown in Figure 1.



3,6′-disinapoyl sucrose (DISS)

Figure 1 The chemical structure of 3, 6′-disinapoyl sucrose (DISS).

Chemical reagents

Fluoxetine was purchased from Lilly (USA), purity >98%. The assay kits for SOD, MDA and MAO-B were purchased from the Jiancheng Bioengineering Institute (Nanjing, Jiangsu Province, China). Radioimmunoassay kits for CORT concentrations were purchased from the Institute of Biological Technology (Beijing, China). All other reagents used were of analytical grade.

Animals

Male Sprague–Dawley (SD) rats from Department of Experimental Animals, Chinese PLA General Hospital, Beijing, China, weighing 180–220 g at the beginning of the experiment, were used. Seventy-two rats were divided into different groups in different cages. All rats were acclimated to the surroundings for 1 week before the experiment and housed individually under controlled conditions of temperature (22 ± 2°C), humidity (55 ± 10%) and light (12 h light : 12 h dark cycle; lights on at 7 a.m.), and were given food and water *ad libitum*. All animals used in this study were cared for and treated humanely according to the *Guide for the Care and Use of Laboratory Animals of Shanghai Institute of Materials*. The experimental design received the approval of the Animal Ethical Committee of Chinese PLA General Hospital. The experiments in the present study were designed to minimize the number of animals used and their suffering.

Chronic mild stress procedure

The CMS procedure was conducted according to a modification of the method of Willner *et al*.[14] Initially, the animals were given 1% sucrose solution for a 48-h period in their home cages following food and water deprivation for 18 h. Subsequently, they were given sucrose for 1 h per day on six consecutive days. Sucrose intake was measured at the end of the training in order to group the rats. The rats in stressed groups were then subjected to CMS for 5 weeks: one session (2 h) of paired caging, one session (3 h) with cage tilted at a 45° angle, one session (18 h) of food and water deprivation, one session (15 min) of shaking, one session (1 h) of exposure to an empty bottle, one session (21 h) in a wet cage (200 ml water in 100 g sawdust bedding) and one session (36 h) of continuous light. The normal rats were housed under identical conditions in a separate room, and had no contact with the stressed animals.

Sucrose preference test

Formal test was carried out every week (1–5 weeks) after stress. After 18 h of food and water deprivation, 1% sucrose solution and fresh water were provided simultaneously for 1 h at 11:00 am every Tuesday for 5 weeks. The test was carried out on CMS and unstressed rats treated with vehicle or drug. Sucrose intake was measured by weighing the bottles containing the sucrose solution before and at the end of each test, respectively.

Drug administration

The experiment divided rats into six groups: unstressed control group (no stress and no drug), CMS+vehicle group (CMS plus pure water), fluoxetine group (CMS plus

fluoxetine), and DISS I, II and III groups (CMS plus DISS at doses of 5, 10 and 20 mg/kg, respectively). Dose selection for DISS was based on our previous study. The repeated drug treatment of CMS animals was performed once daily from 3 to 5 weeks.

Blood collection and biochemical assays

Following the behavioral test, rats were sacrificed by decapitation between 10:00 and 11:00 am. All animals were decapitated quickly to obtain venous blood. Blood was collected in pre-iced tubes and centrifuged at 3000 rpm at 4°C for 20 min. The separated serum samples were stored at −80°C until the assay of cortisol concentration could be performed. The whole brain tissues were removed rapidly on the ice-plate. The tissues were washed with cold saline, blotted dry and stored at −80°C until assay.

Cortisol assay

Serum cortisol was assayed using a radioimmunoassay method following the manufacturer's instructions (Sino-UK Institute of Biological Technology, Beijing, China).

Monoamine oxidase assay

Rat brain mitochondrial fractions were prepared following a procedure described previously.[15] MAO-A activity was assessed spectrophotometrically as described previously.[16] MAO-B activity was assayed according to the kit manufacturer's instructions.

Superoxide dismutase and malondialdehyde assays

Serum was analysed for SOD and MDA values, according to the method described by Ohkawa *et al*.[17] and Misra and Fridovich[18] following the kit instructions.

Statistical analysis

All data presented were as mean ± SEM. The data was analysed by two-way ANOVA and tests of significant differences were determined by Dunnett's post-hoc tests at $P < 0.05$.

Results

Effects on sucrose preference test

Sucrose consumption was measured every week during the experimental period (Figure 2). Before stress, there was no significant difference among the six groups ($P > 0.05$). After 3 weeks, sucrose consumption in stressed rats was significantly lower than that in the unstressed control group ($F(1, 26) = 9.25$, $P < 0.01$). Five weeks later the sucrose intake was reduced to 5.6 g in the CMS+vehicle animals ($F(1, 26) = 6.73$, $P < 0.01$), as compared to 12.2 g in the unstressed control animals. After 2 weeks' drug treatment with exposure to stressor, treatment with DISS and fluoxetine caused a gradual recovery of the sucrose intake, especially in the DISS (20 mg/kg) group.

Effects on cortisol levels

The CMS procedure evoked a significant increase in serum CORT levels of unstressed control group rats ($F(1, 14) = 8.72$, $P < 0.01$) (Table 1). DISS significantly reduced the stress-induced increase in serum CORT levels at three dosages, and the highest dosage showed the best effect ($F(1, 14) = 22.11$, $P < 0.01$).

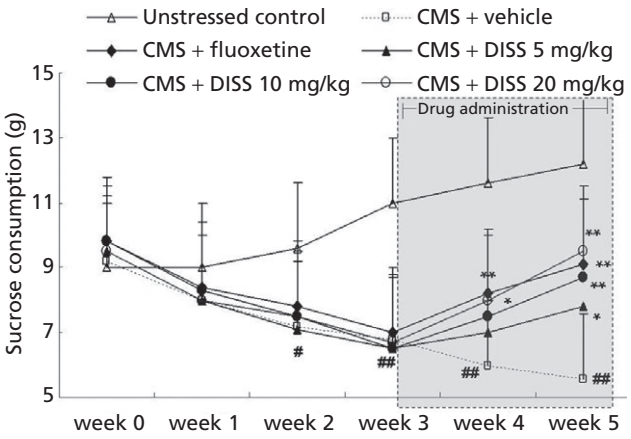


Figure 2 Effects of DISS and fluoxetine on sucrose intake in control and chronic mild stress rats (mean ± SEM, $n = 12$). Chronic treatment with DISS (5–20 mg/kg, i.g.) was given during the last 2 weeks of the 5-week chronic mild stress procedure. $^{\#\#}P < 0.01$; $^{\#}P < 0.05$ compared with unstressed control group; $^{**}P < 0.01$; $^{*}P < 0.05$ compared with CMS+vehicle group, according to ANOVA and Dunnett's post hoc test.

Effects on monoamine oxidase activity

As Table 1 shows, DISS treatment at doses of 10 and 20 mg/kg dose-dependently inhibits MAO-A activity, by 31.8% and 32.9%, respectively, and inhibits MAO-B by 25.5% and 35.6%, respectively, compared with the CMS+vehicle group. DISS at 20 mg/kg and fluoxetine were capable of reversing MAO-A and MAO-B activity to values near to their respective normal values.

Effects on superoxide dismutase activity and malondialdehyde level

The serum total SOD activity and MDA level were measured at the end of the experiment (Figure 3). Exposure to stressor for 5 weeks induced significant decreases in total SOD activity ($F(1, 14) = 9.11$, $P < 0.01$) and significant increases in MDA level ($F(1, 14) = 5.81$, $P < 0.01$) compared to the unstressed control group. DISS treatment dose-dependently increases the total SOD activity and decreases the MDA level, but fluoxetine treatment did not show significant effects on these two variables.

Discussion

The behavioral despair models such as the forced swim test (FST) and tail suspension test (TST) are widely used to screen new antidepressant drugs.[19,20] These models use only a short time stress, however, which does not reflect the circumstances of patients with depression. The unpredictable CMS model was established by Willner *et al*. for the purpose of inducing anhedonia-like behavioral changes in experimental animals. Anhedonia has also been observed to be a core symptom of human major depression.[14] Anhedonia has been defined as

Table 1 Effects of DISS on serum cortisol and brain MAO-A and MAO-B concentrations in rats exposed to CMS (mean ± SEM, $n = 8$)

Group	Dose (mg/kg)	Cortisol concentrations (ng/ml)	MAO Concentrations (U/mg protein)	
			A	B
Unstressed control		339.9 ± 10.19	8.123 ± 0.761	5.345 ± 0.567
CMS+vehicle		471.56 ± 10.70##	13.222 ± 1.231##	9.671 ± 1.023##
CMS+fluoxetine	10	364.90 ± 12.13**	8.876 ± 0.810**	5.657 ± 0.867**
CMS+DISS	5	435.47 ± 22.38**	9.613 ± 1.002*	8.775 ± 0.678
	10	400.74 ± 22.38**	9.012 ± 1.001**	7.189 ± 0.531*
	20	396.79 ± 16.74**	8.867 ± 0.871**	6.234 ± 0.511**

Statistical significance: $^{\#\#}P < 0.01$compared with unstressed control group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with CMS+vehicle group, according to ANOVA and Dunnett's post-hoc test.

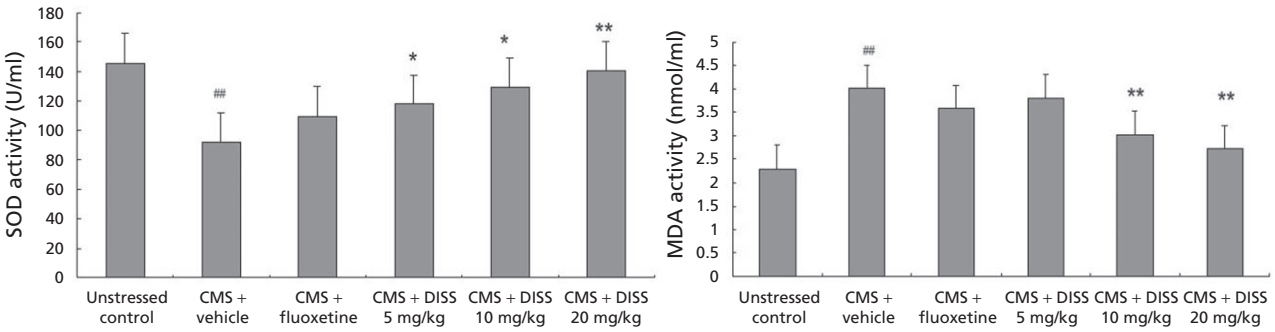


Figure 3 Effects of DISS and fluoxetine on serum SOD and MDA levels in the control and chronic mild stress rats. Data were expressed as means ± SEM. $^{\#\#}P < 0.01$; compared with unstressed control group; $^{**}P < 0.01$; $^{*}P < 0.05$ compared with CMS+vehicle group, according to ANOVA post hoc Dunnett's post-hoc test.

decreased responsiveness to rewards, which can be measured by decreased preference for sucrose solution.[14] The CMS-induced preference of behavioral change has been used as a model to study depression, which involves the presentation of a series of varied and unpredictable environmental stressors such as food and water deprivation, wet cages and light–dark reversal. Under such exposures, animals usually exhibit a persistent reduction in responsiveness to pleasurable stimuli, measured by a decrease in their consumption of 1% sucrose solution.[21] Decreases in sucrose consumption produced by the CMS procedure are shown to be reversed by chronic treatment with either tricyclic antidepressants or selective serotonin reuptake inhibitors (SSRIs).[14] The CMS model of depression in animals is accepted as a valuable method for predicting the potential antidepressant action of compounds in humans.[22,23]

Two weeks' treatment with DISS derived from *P. tenuifolia* produces an antidepressant-like effect on depression-model rats. After 2 weeks' drug treatment with exposure to stressor, there was a dose–effect relationship between DISS and behavioral changes (sucrose intake). A recent study has demonstrated that DISS treatment reduces immobility in the forced-swim and tail-suspension tests in mice.[24] These behavioral data appear to confirm the antidepressant activity of the bioactive compound (DISS) from *P. tenuifolia*.

Dysregulation of the HPA axis system plays an important role in the pathophysiology of depression.[25,26] Normalization of axis hyperactivity precedes the response to pharmacotherapy. In the present study, CMS produced an increase in serum cortisol levels. DISS significantly decreased cortisol levels in CMS-treated animals, suggesting that DISS may be an effective therapeutic option to treat HPA-axis dysfunction in depressive disorders. It was noted that MAO is likewise responsive to the abnormal HPA axis status in depressed patients. MAO activity and cortisol levels have been considered predictors of a favorable clinical response to antidepressant treatment.[4] MAOs play an important role in the pathogenesis of psychiatric disorders, including clinical depression and anxiety.[2,27] The present study shows that CMS markedly stimulates the activities of MAO-A and MAO-B in rat brains, and that the stimulatory action is suppressed by DISS treatment, therefore our study may confirm the previous reports investigating the temporal relationship between changes in cortisol concentration and MAO activity in the CMS model of depression.[23]

Changes in SOD activity and MDA levels in major depression have been reported in some clinical studies.[8,28] Enhanced oxidative stress has been observed in the chronic stress-induced rat depression model, mainly expressed as a significant increase in MDA levels and a significant decrease in SOD activity.[29] Our data supported these findings. Treatment with DISS seems to significantly reverse these parameters. Although there was no direct evidence of the effect of DISS on oxidative stress, our previous study showed that extracts of *P. tenuifolia* and its active compound, DISS, could increase

the activity of antioxidant enzymes, such as SOD, CAT and GPX, and inhibit lipid peroxidation in senescence-accelerated mice.[30] Our present results further support these findings. We observed that DISS treatment dose-dependently reversed the changes in SOD activity and MDA levels in CMS-induced depression model rats. However, fluoxetine, an SSRI, effectively treats a wide spectrum of mood disorders[31] and also protects against the adverse effects of different types of stressors.[32] A previous study indicated that the antioxidant potential of fluoxetine probably contributes to its effects on SOD and MDA in the liver,[33] but in this experiment fluoxetine did not show significant antioxidative stress effect on SOD and MDA in serum.

In conclusion, our results suggest that the effects of CMS on the biochemical and neurochemical markers of depression might be related to MAO, the HPA axis and oxidative systems. Moreover, DISS, as an active compound, not only displays a behavioral profile consistent with an antidepressant-like action on CMS-induced depression model rats but also blocks brain MAO-A and MAO-B activity and reverses the increases in serum cortisol levels induced by CMS. These pharmacological actions could make DISS a potentially valuable drug for the treatment of depression.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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