行政院國家科學委員會專題研究計畫 成果報告

探討 D-cycloserine 與合併使用 parecoxib 對巴金森氏症之行為障礙與神經發炎現象的效果：前置基礎研究
研究成果報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 99-2410-H-040-008-
執 行 期 間 ：99 年 08 月 01 日至 100 年 10 月 31 日
執 行 單 位 ：中山醫學大學心理學系（所）（臨床組）

計 畫 主 持 人：何應瑞

計畫參與人員：此計畫無其他參與人員

報 告 附 件 ：出席國際會議研究心得報告及發表論文

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附件一

行政院國家科學委員會補助專題研究計畫 ■ 成 果 報 告 □期中進度報告

（計畫名稱）

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計畫參與人員：

成果報告類型(依經費核定清單規定繳交)：■精簡報告　□完整報告

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本研究成果已經被 Chinese Journal of Physiology 接受發表

（附論文接受函）

Behavioral and IL-2 Responses to Diosgenin in Ovariectomized Rats

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Running title: Effects of Diosgenin in Ovariectomized Rats

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Abstract

Neuroimmune system is involved in communication between the endocrine and nervous systems, which may take part in the effects of dioscorea, reversing changes of anxiety-like behavior and interleukin (IL)-2 levels in the brain of ovariectomized (OVX) rats. This study was aimed at evaluating administration of diosgenin, an ingredient of dioscorea, on neuroimmune and behavioral functions in OVX animals. One month after ovariectomy, female Wistar rats were fed daily with diosgenin (0, 10, 50, or 100 mg/kg/day) and the elevated plus-maze and learned helplessness tests were used to measure anxiety-like and depressive behaviors after 23 and 24 days of diosgenin treatment, respectively. In the learned helplessness test, the rats needed to cross from one compartment of the shuttle box to the opposite compartment to avoid or escape the shock. If the rat failed to escape the shock in 10 sec, a "failure" was recorded. Two days after the behavioral tests, the brain was removed to measure levels of IL-2, used as an indicator of neuroimmune function. Anxiety-like behavior in OVX rats was not affected by diosgenin treatment. However, avoidance behavior in the learned helplessness test in OVX rats with high anxiety (HA) levels was improved by treatment with diosgenin at the dosage of 10 mg/kg/day. Interestingly, failure number in the same test was increased when the dosage of diosgenin was increased to 50 mg/kg/day, and this was accompanied by an increase in IL-2 levels in the pituitary gland. In addition, treatment with 100 mg/kg/day of diosgenin resulted in decreased IL-2 levels in the amygdala and prefrontal cortex of OVX rats with low anxiety levels, and in increased IL-2 levels in the amygdala of OVX HA rats. These results show that chronic diosgenin treatment influences IL-2 levels in the brain of OVX rats and affects depressive behavior in OVX HA rats, but not OVX LA rats.

Key words: dioscorea, diosgenin, menopause, ovariectomy, individual difference, IL-2, anxiety, depressive behavior

Introduction

Menopause is associated with a rapid decline in circulating sexual hormones and results in menopausal syndrome, including hot flushes, osteoporosis, and affective disorders, for example, anxiety and depression. Medications containing one or more sexual hormones are used in hormone replacement therapy (HRT) for menopausal syndrome (2). Phytoestrogens, such as isoflavone, are an alternative choice for HRT due to their safety (15, 51). Dioscorea (wild yam), a common source of food and Chinese medicines which contains phytosteroids, e.g., diosgenin and steroidal saponins (13), has long been used to treat menopausal syndrome and has been demonstrated to have anti-osteoporotic (12, 61), anti-diabetic (56), and anti-hypercholesterolemia activities (11). Previously, we showed that dioscorea reverses changes in anxiety-like behavior and interleukin (IL)-2 levels in the brain of ovariectomized (OVX) rats that is used as a menopause animal model (21).

Diosgenin, one of the important bio-active ingredients in dioscorea, is similar to sex hormones in chemical structure and thus long been used as a precursor of steroid hormones, e.g. estrogen, progesterone, testosterone, and cortisol (48). Diosgenin has been shown to improve epidermal functions in OVX mice (53). Furthermore, an in vivo study showed that diosgenin has a similar effect to estrogen on bone density in OVX rats (17), suggesting that diosgenin may have benefits for OVX animals. However, its effects on behavioral and neuroimmune functions in OVX rats are not clear.

Previous studies have shown that IL-2 is involved not only in the regulation of immune function (1), but also in stress responses and communication between the endocrine and nervous systems (34). IL-2 is also involved in anxiety behavior (24, 36, 38) and the secretion of corticotrophin releasing hormone (CRH) from the hypothalamus and amygdala (44, 45). Stress, a risk factor for inducing anxiety and depression, activates the hypothalamic-pituitary-adrenocortical (HPA) axis, and IL-2 in the pituitary gland may play an important role, as it also elicits activation of the HPA axis through the release of CRH and adrenocorticotrophic hormone (34). The aim of this study was therefore to clarify the effect of diosgenin on IL-2 levels in the brain and on anxiety and depressive behaviors in OVX rats.

Materials and Methods

Animals

Three-month-old female Wistar rats (264 ± 2 g; n = 93; National Laboratory Animal Center, ROC) were housed in groups of five in acrylic cages (35 × 56 × 19 cm) in an animal room with a 12 h light-dark cycle (lights on at 07:00 h) with food and water provided *ad libitum*. Each animal was handled for 15 min/day on the 2 days preceding each experiment. All experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care Committee of the Chung Shan Medical University (IACUC approval No.: 273).

General Procedure

Four weeks after ovariectomy, an elevated plus-maze test (5 min) was performed for evaluating anxiety-like behavior (8, 18, 19, 41), then the animals were given diosgenin (0, 10, 50, or 100 mg/kg/day) orally for 27 days. On day 23 of diosgenin treatment, the elevated plus-maze test was again performed, followed by a 2-day session (days 24 and 25) of the learned helplessness test in a shuttle box for evaluating depressive behavior (6, 18, 27). Two days after the behavioral tests (day 28), the brain was removed to measure IL-2 concentrations in different regions using an enzyme-linked immunosorbent assay (ELISA). Furthermore, anxiety levels after ovariectomy were taken into account, based on our previous findings showing the important role of anxiety levels in behavioral and molecular responses to pharmacological manipulations (18, 21, 39, 60). All the procedures of behavioral tests were the same as described in our reports (21, 27, 52, 58-60).

Ovariectomy

The procedure used for ovariectomy was the same as that in our previous report (12, 21). Briefly, the rat was anesthetized by intramuscular (IM) injection of ketamine (100 mg/kg) and the ovaries retracted and removed. Immediately following surgery, each rat was injected with Penicillin-G procaine (0.2 ml, 20,000 IU, IM). The sham-operated group underwent the same surgical procedure except for the removal of the ovaries.

Behavioral tests

Elevated Plus-maze Test: The construction of the elevated plus-maze and the testing procedures were the same as in our previous reports (21, 52, 59, 60). The following measures were analyzed from videotapes: (1) open arm time and (2) enclosed arm time: the time spent by a rat in an open or enclosed arm, respectively, and (3) arm activity: the number of times that a rat crossed a virtual line which divided an arm into a proximal and a distal half. The elevated plus-maze test was performed twice in this study, once 4 weeks after ovariectomy and the other after 23 days of diosgenin treatment. The open arm time in the first elevated plus-maze test was used to screen individual anxiety levels and to divide the rats into high and low anxiety groups (approximately 50% of each, with high open arm time as low anxiety and low open arm time as high anxiety) and to set up treatment groups that were matched for comparable numbers of low anxiety and high anxiety rats.

Learned Helplessness Test: The learned helplessness test was performed in a shuttle box (AccuSan, USA), as in our previous reports (27, 58). A computer controlled the shuttle box to deliver conditioned stimuli (CS) (3 sec of a 75 db tone and 250 lx of light) and unconditioned stimuli (US) (10 sec of electric foot shock at 0.5 mA). The learned helplessness task was a two-day session: on day 1, the rats received 40 trials (1 min/trial) of inescapable CS-US pairings, with a mean interval of 60 sec (range 50–70 sec). The day 2 session consisted of 16 trials, in which the rats had to cross from one compartment to another to escape the shock, which was preceded by a 3 sec CS. If the rat crossed to the opposite compartment during the CS, an "avoidance" was recorded, the CS ceased, no foot shock was applied, and another trial was initiated. If the rat escaped during the shock, an "escape" was recorded. If the rat failed to escape, the shock was terminated after 10 sec, the test was recorded as a "failure", and another trial initiated.

Measurement of IL-2 Levels

Two days after the learned helplessness test, the rats were euthanatized by exposure to CO_2 vapor and their brains removed after cardiac perfusion. The amygdala, prefrontal cortex, non-prefrontal cortex, striatum,

hippocampus, and pituitary gland were dissected out on an ice-bath plate, then their IL-2 content was measured using ELISA kits (CytoSets^{TM}, BioSourse, CA, USA), as described in our previous reports (21, 27, 59).

Diosgenin Administration

Diosgenin (0, 10, 50, or 100 mg/kg), purchased from Sigma (USA), was wrapped in a ball of toast (about 1 g) and fed daily to each rat. The sham-operated group was fed the same amount of toast without diosgenin, as we previously showed that dioscorea does not affect anxiety and depressive behavior in sham-operated rats (21).

Data Analysis

As in our previous studies (18, 19, 35, 60), OVX rats were ranked using the open arm time in the first elevated plus-maze test and assigned to two sub-groups with high anxiety levels (38 animals with a shorter open arm time; HA rats) or low anxiety levels (36 animals with a longer open arm time; LA rats). The group results are presented for the effects of diosgenin on behavior and IL-2 levels. Statistical testing was performed for comparisons within or between groups using *t*-tests for paired or unpaired data. Effects of ovariectomy and diosgenin were analyzed by repeated measures or one-way analysis of variance (ANOVA), followed by least-significant difference (LSD). All results are expressed as the mean ± SEM. The level of significance was defined as $P < 0.05$.

Results

Changes in Body Weight

Ovariectomy significantly increased body weight. ANOVA with repeated measures revealed significant main effects of time ($F(4,364) = 139.60$, $P < 0.001$) and surgery ($F(1,91) = 10296.80$, $P < 0.001$) and a significant time×surgery interaction ($F(4,364) = 27.90$, $P < 0.001$). A simple *t*-test with a Bonferroni post hoc test showed that, starting one week after ovariectomy, the body weight of OVX rats was higher than that of sham-operated rats (all *P* values < 0.05) (Fig. 1A). Diosgenin treatment (10, 50, or 100 mg/kg/day) did not affect the change in body weight in OVX rats, ANOVA with repeated measures revealed a time effect ($F(4,280) = 129.40$, $P < 0.001$), but no dosage effect or time×dosage interaction (Fig. 1B).

Behavior after Ovariectomy

Four weeks after ovariectomy, the enclosed arm time, open arm time, and total arm activity in the first elevated plus-maze test were not different between OVX and sham-operated rats. However, enclosed arm activity was increased ($df = 91$, $t = -2.88$, $P < 0.01$) and open arm activity decreased ($df = 91$, $t = 2.08$, $P < 0.05$) in the OVX rats, compared to the sham-operated rats (Table 1).

Based on the open arm time in the first elevated plus-maze test, the OVX rats were divided into the HA and LA sub-groups, with the following profiles (Table 1). The open arm time and open arm activity for the OVX HA rats were significantly lower than those in OVX LA rats ($df = 72$, $t \geq 9.40$, both *P* values < 0.001) and sham-operated rats ($df = 55$, $t \geq 4.89$, both *P* values < 0.001). However, OVX HA rats showed a higher enclosed arm time and enclosed arm activity than OVX LA rats ($df = 72$, $t \geq 3.41$, both *P* values < 0.001) and sham-operated rats ($df = 55$, $t \geq 3.78$, *P* values < 0.001). Total arm activity was not different between sham-operated and OVX rats. However, total arm activity of OVX HA rats was lower than that in OVX LA rats ($df = 72$, $t = 2.06$, $P < 0.05$), but not different from that in sham-operated rats. Interestingly, all the above behaviors in OVX LA rats were similar to those in sham-operated rats.

Behavior after Diosgenin Treatment

Elevated plus-maze test: A paired *t*-test showed that diosgenin treatment (10, 50, or 100 mg/kg/day) did not affect behavior in the elevated plus-maze test in OVX rats, irrespective of their anxiety levels before treatment (Table 2).

Learned Helplessness Behavior: As shown in Table 3, ovariectomy did not affect behavior in the learned helplessness test, as the avoidance number (1.5 ± 0.3 vs. 1.0 ± 0.3), escape number (12.1 ± 0.7 vs. 12.8 ± 1.0), and failure number (2.2 ± 0.7 vs. 2.2 ± 1.0) in OVX rats receiving vehicle treatment were not different from those in sham-operated rats. However, ANOVA with the LSD post hoc test revealed that 24 days of treatment with 10 mg/kg/day of diosgenin resulted in a significant increase in avoidance number ($F(4,47) = 2.18$, $P < 0.01$) and a significant decrease in escape number ($F(4,47) = 1.84$, $P < 0.05$) in the learned helplessness test in OVX HA rats, compared to that in sham-operated rats. Interestingly, at the dosage of 50 mg/kg/day, diosgenin increased the failure number, compared to HA rats receiving vehicle treatment ($F(3,37) = 2.37$, $P < 0.05$). Learned helplessness behavior in OVX LA rats was not affected by diosgenin treatment.

IL-2 Levels

As shown in Table 4, ANOVA with the LSD post hoc test revealed that IL-2 levels in the hippocampus of sham-operated (4.27 ± 0.27 pg/μg protein) and OVX rats receiving vehicle treatment (4.00 ± 0.40 pg/μg protein) were higher than those in other brain areas (all P values < 0.05). IL-2 levels in the pituitary gland of OVX rats receiving vehicle treatment (1.50 ± 0.15 pg/μg protein) were significantly higher than those in the pituitary gland in sham-operated rats (1.07 ± 0.08 pg/μg protein) (df = 29, $t = -2.40$, $P < 0.05$), while IL-2 levels in other brain areas were unaffected by ovariectomy.

Diosgenin at the dosage of 100 mg/kg/day resulted in a trend to a decrease in IL-2 levels in the amygdala and prefrontal cortex of OVX LA rats, compared to OVX LA rats receiving vehicle treatment ($F(3,25) = 1.58$, both P values < 0.07). In contrast, the same dosage of diosgenin increased IL-2 levels in the amygdala of OVX HA rats ($F(3,32) = 1.55$, $P = 0.05$) compared to OVX HA rats receiving vehicle treatment. Except in rats receiving 50 mg/kg/day of diosgenin, IL-2 levels in the pituitary gland of OVX LA rats were significantly higher than those in sham-operated rats ($F(4,42) = 2.20$, $P < 0.05$). In contrast, IL-2 levels in the pituitary gland of OVX HA rats were not different from that in sham-operated rats, with the exception of rats receiving 50 mg/kg/day of diosgenin, which had higher IL-2 levels compared to sham-operated rats ($F(4,40) = 1.80$, $P < 0.05$).

Discussion

Four weeks of diosgenin treatment of OVX rats influenced learned helplessness behavior and IL-2 levels in the brain, and these changes were related to the anxiety levels of the animals and showed a reversed U-shape dose response. Four weeks after ovariectomy, the body weights of the OVX rats were significantly higher than those of sham-operated rats. Ovariectomy caused significantly increased anxiety-like behavior in the elevated plus-maze test in a proportion of the rats, with an increased enclosed arm time and a decreased open arm time. The changes in body weight and anxiety-like behavior in OVX rats were not affected by 4 weeks of administration of diosgenin at the dosages of 10, 50, and 100 mg/kg/day. However, the avoidance behavior of OVX HA rats in the learned helplessness test was improved by diosgenin treatment at the dosage of 10 mg/kg/day. Interestingly, failure number was increased when the dosage of diosgenin was increased to 50 mg/kg/day, which was accompanied by an increase in IL-2 levels in the pituitary gland. In addition, 100 mg/kg/day of diosgenin decreased IL-2 levels in the amygdala and prefrontal cortex of OVX LA rats, but increased IL-2 levels in the amygdala of OVX HA rats. Furthermore, low dose diosgenin (10 mg/kg/day) resulted in improved depressive behavior, but a higher dose (50 mg/kg/day) led to a deterioration. These results show that 4 weeks of administration of diosgenin affects behavioral and neuroimmune functions in OVX rats. Moreover, individual differences in anxiety level should be taken into account when measuring behavioral and molecular responses to pharmacological manipulation.

Menopausal state was evidenced in the present study by the increase in body weight in the OVX rats (7, 50). Consistent with our previous finding (21), ovariectomy caused an increase in anxiety-like behavior in a proportion of the rats in the elevated plus-maze test, with an increased enclosed arm time and a decreased open arm time, which is compatible with clinical observations that 50% of menopausal women present anxiety disorders (40, 55). We have previously demonstrated that 4 weeks of administration of dioscorea decreases anxiety levels and depressive behavior in OVX rats, but does not affect the body weight gain caused by ovariectomy (21). In the present study, anxiety levels and body weight gain after ovariectomy were not changed by diosgenin treatment, whereas the behavior of OVX HA rats in the learned helplessness test was affected.

The learned helplessness test is widely used to measure depressive behavior, in which failure number is an indicator of despair behavior. The learned helplessness test consisted of 2 sessions. In the day 1 session, animals received 40 trials of inescapable CS (e.g., tone and/or illumination)-US (e.g., shock) pairings. In the day 2 session, 16 trials of escapable CS-US pairings were used, as in a typical active avoidance test (27). Our previous study showed that animals present a high percentage (20-40%) of avoidances in a typical active avoidance test (18). In the current study, both sham-operated and OVX rats showed a lower level (around 6%) of avoidances, which might be imply a disability in coping behavior to stressful CS-US pairings. Thus, the increased number of avoidances after treatment with 10 mg/kg/day of diosgenin may indicate an improvement in learning or coping with stress. IL-2, an immunoregulatory cytokine, has been known to be expressed not only in the immune system but also in the pituitary gland and be a regulator of pituitary growth and hormone secretion (3), for example, stimulating adrenocorticotropic hormone release from the pituitary gland (47). Moreover, IL-2 is involved in psychological and physical stress and in compromising the immune activity as well (16). Changes of IL-2 expression in the hypothalamic-pituitary-gonadal system are responsible for reproductive dysfunction in repeated cold stress (54). Based on the fact that increased IL-2 levels was observed in the pituitary gland of OVX LA rats, we suggest that the increased IL-2 levels in the pituitary gland after ovariectomy may participate in dysfunction of the neuroimmune system and may thus be involved in stress responses and depressive disorders during menopause. To our knowledge, these are the first data describing changes of IL-2 level in the pituitary gland after ovariectomy. Additional studies are needed to evaluate the underling mechanisms.

Classical fear conditioning caused by pairings of CS and US is a typical stress paradigm (49). Re-exposure to the CS after the CS-US pairings can cause anxiety-like or fear-like behavior and has been shown to result in endocrine and immunological effects (30). Thus, the rats would experience psychological stress when they had to re-enter the shuttle box and receive the CS in the day 2 session of the learned helplessness test. Foot shocks in the escapable CS-US pairings result in acute physical and psychological stress, which also cause fear and/or anxiety (49). We have previously demonstrated that IL-2 in the amygdala and prefrontal cortex is involved in responses to acute stress accompanied by stressful psychological experiences (27). Although the current study revealed that treatment of diosgenin increases IL-2 levels in some brain areas of OVX rats without influencing the anxiety-like behavior, it has to be noted that the function of IL-2 in the emotional behavior may have area-specificity (39). IL-2 protein and mRNA levels in the striatum are related to anxiety levels in rats (36, 38, 39). Microglia are thought to be a principal source of cytokines (25), and astrocytes (31) and tissue infiltrating immune cells (26) can also produce IL-2 in the brain. Further, it has been reported that diosgenin affects gene transcription and production of inflammatory cytokines in macrophages (22). Some cytokines secreted by immune cells in the blood can actively pass through the blood-brain-barrier (4). Thus, diosgenin treatment may affect IL-2 levels in the brain of OVX rats through influencing the function of glial cells and immune cells. In addition, it has been reported that HRT can modulate the secretion of cytokines from blood mononuclear cells (9) and prevents the augmentation of IL-2 in the blood of women after menopause (23). Therefore, diosgenin treatment may have a potential for regulating psychoneuroimmunological function during the menopause.

Moreover, diosgenin and dioscorea showed a different pattern of effects on IL-2 levels, with dioscorea restoring the reduction of IL-2 levels in the prefrontal cortex of OVX LA rats and increasing IL-2 levels in the non-prefrontal cortex of OVX HA rats (21), while diosgenin decreased IL-2 levels in the prefrontal cortex of OVX LA rats with no effect on IL-2 levels in the non-prefrontal cortex of OVX HA rats. Although dioscorea extract has been reported to contain diosgenin and has molecular and behavioral activities, enhancing anti-oxidative and cognitive functions in aging mice (13), our studies indicate that there are disparities between the effects of dioscorea and diosgenin on behavior of OVX rats. Our previous study has shown that chronic administration of dioscorea decreases despair behavior of OVX rats in the forced swim test and suppresses anxiety-like behavior of OVX HA rats (21). The current study showed that anxiety-like behavior in OVX rats was not affected by diosgenin treatment. However, the behavioral performance in the learned helplessness test in OVX HA rats was improved by treatment with diosgenin at the dosage of 10 mg/kg/day but was impaired when the dosage of diosgenin was increased to 50 mg/kg/day. Diosgenin is an important bio-active ingredient in dioscorea, however, to our knowledge, no paper has reported studies directly evaluating the differences of the function between dioscorea and diosgenin. Thus, further studies are needed to determine if other constituents in dioscorea contribute to the discrepancy.

Stress, a risk factor for inducing affective disorders, has been implicated in IL-2 production. Moreover, the pituitary gland, having important neuroendocrine functions in the HPA axis, plays a critical role in the regulation of responses to stress, in which cytokines are known to be involved (27, 62). Physical stress, such as restraint and electrical shock, and psychological stress caused by conditioned aversive stimulus can activate the HPA axis and facilitate the release of glucocorticoids into the blood, which can reduce plasma IL-2 levels (57). In animals, repeated restraint stress (43) and psychological stress of behavioral conditioning (14) reduce IL-2 production by lymphocytes and splenocytes. Chronic stress in caregivers of dementia patients results in increased cortisol

levels in the saliva and decreased IL-2 levels in the blood (5). Although all the above reports show the relationships between stress and IL-2 levels, they mainly focused on effects in the periphery. IL-2 levels measured in the present study reflex concentrations in the brain tissue itself, as blood was removed from the brain by cardiac perfusion. This study showed that different dosages of diosgenin had different effects on behavior in OVX HA rats in the learned helplessness test and on IL-2 levels in brain areas that may participate in emotional processes, which may suggest that OVX HA rats may be more susceptible to stress in the learned helplessness test and also be more sensitive to diosgenin treatment.

Chronic irregular mild foot shock in mice has been reported to cause an increase in IL-2 levels in brain tissue, which is correlated with activation of the HPA axis (10). Anti-depressants are able to reverse the change in IL-2 levels in the serum of rats subjected to chronic mild stress (29). Interestingly, menopause caused by both ovariectomy and ageing also results in increased levels of inflammatory cytokines in the blood (32), and levels can be normalized by HRT (33). Furthermore, peripheral administration of IL-2 increases locomotor activity and monoamine turnover in the hypothalamus and prefrontal cortex (42). These results suggest that IL-2 may affect neuronal activity and therefore be involved in emotional behavior. Diosgenin can also regulate immune and neuroendocrine functions in OVX rats by reducing the weight of the spleen and adrenal gland (7). Thus, our current findings showing responses of depressed behavior and IL-2 levels in the pituitary gland of OVX rats are in line with the view that IL-2 in neuroendocrine tissues may be involved in pathophysiological changes in neuroimmune function and stress responses in OVX animals (34). Moreover, diosgenin may be an alternative to HRT.

Interestingly, diosgenin at the dosage of 10 mg/kg/day improved learned helplessness behavior in OVX HA rats by increasing the avoidance number, while a higher dose of diosgenin (50 mg/kg/day) worsened despair behavior by increasing the failure number. Thus, diosgenin may have a reversed U-shaped dose-response curve, which is consistent with our previous report showing that a low dose of dioscorea increases, but a high dose decreases, cytokine expression in the brain (21). Dual effects of diosgenin have also been observed at the cellular level in a human erythroleukemia cell line, in which low dose diosgenin increases differentiation, but high dose diosgenin causes apoptosis (28). By using the body surface area normalization method (46), the effective dose, 10 mg/kg/day, of diosgenin in regulating learned helplessness behavior in rats can be translated to human equivalent dose, 1.62 mg/kg/day. However, concerning the characteristic of reversed U-shaped dose-response of diosgenin, further studies are needed to determine the effective dose window for treating menopausal symptoms. In contrast to the responses in OVX HA rats, learned helplessness behavior in OVX LA rats was not affected by diosgenin treatment. Furthermore, the effects of diosgenin on IL-2 levels were not the same in OVX LA and HA rats, as 100 mg/kg/day of diosgenin decreased IL-2 levels in the amygdala and prefrontal cortex of OVX LA rats, but increased IL-2 levels in the amygdala of OVX HA rats. These findings are consistent with our previous studies showing that behavioral (20) and molecular (60) responses to drug treatment are related to anxiety levels in rats. Moreover, in agreement with our previous reports, individual differences in responses to ovariectomy and diosgenin treatment were also seen in terms of behavior (18), molecular expression (36, 39), and responses to pharmacological manipulation (35, 37, 38).

In conclusion, 4 weeks of administration of diosgenin results in a reversed U-shape dose response in terms of behavioral and neuroimmune functions of OVX animals. Diosgenin may be an alternative to HRT. In addition, consistent with our previous reports, the present data suggest that individual differences in anxiety levels should be taken into account when measuring behavioral and molecular responses to drug treatment (21, 39, 60).

Acknowledgements

This work was supported by grants from the National Science Council of the ROC (NSC 98-2410-H-040-003; NSC 99-2410-H-040-008), Chung Shan Medical University Hospital (CSH-2010-C-020) and the German Research Foundation (DFG PA 818/4-1). All authors declare that there is no actual or potential conflict of interest in relation to this article

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Table 1. Effects of ovariectomy on behavior in the elevated plus-maze test performed 4 weeks after surgery.

	Sham	**OVX**		
		Total	Sub-group	
	(n = 19)	(n = 74)	LA rats (n = 36)	HA rats (n = 38)
Enclosed arm time (sec)	149.8 ± 15.3	173.2 ± 6.7	125.9 ± 5.4	217.9 ± 6.1 ***###
Open arm time (sec)	99.3 ± 12.5	79.8 ± 5.5	119.2 ± 4.8	42.6 ± 4.3 ***###
Enclosed arm activity (no.)	16.6 ± 1.3	21.2 ± 0.7 **	18.8 ± 0.8	23.5 ± 1.1 ***###
Open arm activity (no.)	12.2 ± 1.8	8.9 ± 0.7 *	13.2 ± 0.7	4.8 ± 0.6 ***###
Total arm activity (no.)	28.8 ± 2.2	30.1 ± 0.9	32.1 ± 1.2	28.3 ± 1.3 #

LA: low anxiety; HA: high anxiety. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared to sham-operated rats.

# $P < 0.05$, ### $P < 0.001$, compared to OVX LA rats. Data are expressed as the mean ± SEM.

Table 2. Effects of diosgenin on the behavior of OVX rats in the elevated plus-maze test.

		Sham	**OVX**							
			LA rats				HA rats			
		0 mg/kg/day (n = 19)	0 mg/kg/day (n = 10)	10 mg/kg/day (n = 10)	50 mg/kg/day (n = 8)	100 mg/kg/day (n = 8)	0 mg/kg/day (n = 11)	10 mg/kg/day (n = 9)	50 mg/kg/day (n = 9)	100 mg/kg/day (n = 9)
Enclosed arm time (sec)	Before	149.8 ± 15.3	132.8 ± 6.9	129.7 ± 11.1	117.3 ± 12.2	121.2 ± 14.6	230.2 ± 11.9	222.4 ± 12.9	212.6 ± 10.8	203.5 ± 13.1
	After	130.2 ± 17.5	142.1 ± 29.8	129.7 ± 25.9	144.5 ± 23.2	90.1 ± 13.0	190.1 ± 30.5	197.9 ± 28.0	208.0 ± 25.1	185.9 ± 26.3
Open arm time (sec)	Before	99.3 ± 12.5	119.6 ± 9.1	113.1 ± 9.9	118.3 ± 9.0	127.1 ± 11.5	33.6 ± 8.2	34.3 ± 9.5	50.1 ± 8.5	54.2 ± 7.3
	After	105.8 ± 16.6	103.6 ± 28.2	96.1 ± 20.8	92.7 ± 18.8	145.1 ± 17.3	73.4 ± 28.5	70.2 ± 26.0	43.2 ± 19.0	69.8 ± 24.8
Enclosed arm activity (no.)	Before	16.6 ± 1.3	19.9 ± 1.8	18.8 ± 1.3	17.5 ± 1.6	18.9 ± 2.0	24.7 ± 2.6	23.4 ± 1.6	21.9 ± 1.6	23.8 ± 2.8
	After	16.9 ± 1.5	21.4 ± 2.9	18.1 ± 1.8	20.4 ± 1.9	20.1 ± 2.0	21.5 ± 2.3	22.9 ± 2.1	25.2 ± 3.1	20.7 ± 1.7
Open arm activity (no.)	Before	12.2 ± 1.8	14.0 ± 1.8	11.5 ± 1.2	13.6 ± 1.0	14.0 ± 1.1	4.5 ± 1.2	4.0 ± 1.2	5.1 ± 1.3	5.7 ± 1.0
	After	12.1 ± 1.9	8.6 ± 2.5	9.4 ± 2.3	11.4 ± 2.1	13.5 ± 2.9	4.7 ± 1.9	4.7 ± 1.5	4.6 ± 1.9	6.2 ± 1.6
Total arm activity (no.)	Before	28.8 ± 2.2	33.9 ± 3.4	30.3 ± 1.5	31.1 ± 2.2	32.9 ± 2.2	29.3 ± 3.1	27.4 ± 2.5	27.0 ± 2.3	29.4 ± 2.9
	After	29.1 ± 2.4	30.0 ± 3.2	27.5 ± 2.3	31.8 ± 3.3	33.6 ± 2.4	26.2 ± 3.1	27.6 ± 1.4	29.8 ± 2.4	26.9 ± 1.7

LA: low anxiety; HA: high anxiety. “Before” and “After” are respective to the 23 days of diosgenin treatment. The data are expressed as the mean ± SEM.

Table 3. Effects of diosgenin on behavior of OVX rats in the learned helplessness test.

	Sham	**OVX**							
		LA rats				HA rats			
	0 mg/kg/day (n=19)	0 mg/kg/day (n=10)	10 mg/kg/day (n=10)	50 mg/kg/day (n=8)	100 mg/kg/day (n=8)	0 mg/kg/day (n=11)	10 mg/kg/day (n=9)	50 mg/kg/day (n=9)	100 mg/kg/day (n=9)
Avoidance (no.)	1.0 ± 0.3	1.1 ± 0.3	1.2 ± 0.4	0.9 ± 0.4	2.0 ± 0.7	1.8 ± 0.4	3.0 ± 1.2 **	1.6 ± 0.5	1.6 ± 0.4
Escape (no.)	12.8 ± 1.0	11.2 ± 1.4	11.2 ± 1.3	13.3 ± 1.0	12.9 ± 0.8	12.9 ± 0.5	9.0 ± 1.6 *##	10.1 ± 1.5	13.0 ± 0.7
Failure (no.)	2.2 ± 1.0	3.4 ± 1.4	3.6 ± 1.4	1.6 ± 1.1	1.0 ± 0.5	1.1 ± 0.4	3.9 ± 1.5	4.3 ± 1.6 #	1.3 ± 0.6

LA: low anxiety; HA: high anxiety. * $P < 0.05$, ** $P < 0.01$, compared to the sham-operated group. # $P < 0.05$, ## $P < 0.01$, compared to OVX HA rats receiving vehicle treatment. Data are expressed as the mean ± SEM.

Table 4. Effects of diosgenin on IL-2 levels in the brain of OVX rats.

	Sham	**OVX**							
		LA rats				HA rats			
	0 mg/kg/day (n=15)	0 mg/kg/day (n=7)	10 mg/kg/day (n=7)	50 mg/kg/day (n=7)	100 mg/kg/day (n=5)	0 mg/kg/day (n=10)	10 mg/kg/day (n=8)	50 mg/kg/day (n=7)	100 mg/kg/day (n=8)
Amygdala	1.00 ± 0.04	1.16 ± 0.10	0.96 ± 0.04	1.01 ± 0.08	0.93 ± 0.09 #	0.92 ± 0.1	1.10 ± 0.07	1.06 ± 0.10	1.15 ± 0.04 #
Prefrontal cortex	1.24 ± 0.05	1.27 ± 0.12	1.21 ± 0.08	1.32 ± 0.12	0.97 ± 0.09 *#	1.18 ± 0.07	1.34 ± 0.09	1.15 ± 0.08	1.34 ± 0.06
Non-prefrontal	2.42 ± 0.10	2.40 ± 0.22	2.17 ± 0.16	2.80 ± 0.12	1.99 ± 0.23	2.35 ± 0.22	2.72 ± 0.24	2.40 ± 0.17	2.81 ± 0.16
Striatum	1.50 ± 0.09	1.62 ± 0.17	1.49 ± 0.17	1.62 ± 0.15	1.18 ± 0.09	1.65 ± 0.23	1.69 ± 0.21	1.57 ± 0.33	1.92 ± 0.23
Hippocampus	4.27 ± 0.27	3.78 ± 0.61	3.69 ± 0.43	4.23 ± 0.65	3.75 ± 0.33	4.15 ± 0.45	5.17 ± 0.54	3.65 ± 1.03	4.84 ± 0.36
Pituitary gland	1.07 ± 0.08	1.56 ± 0.21 *	1.61 ± 0.23 *	1.54 ± 0.18	1.65 ± 0.29 *	1.45 ± 0.21	1.33 ± 0.19	1.76 ± 0.18 *	1.23 ± 0.24

LA: low anxiety; HA: high anxiety. * $P < 0.05$, compared to the sham-operated group; # $P < 0.07$, compared to rats in the same anxiety category receiving vehicle treatment. Data are expressed as the mean ± SEM. IL-2 levels are expressed as pg/μg protein.

Fig. 1.

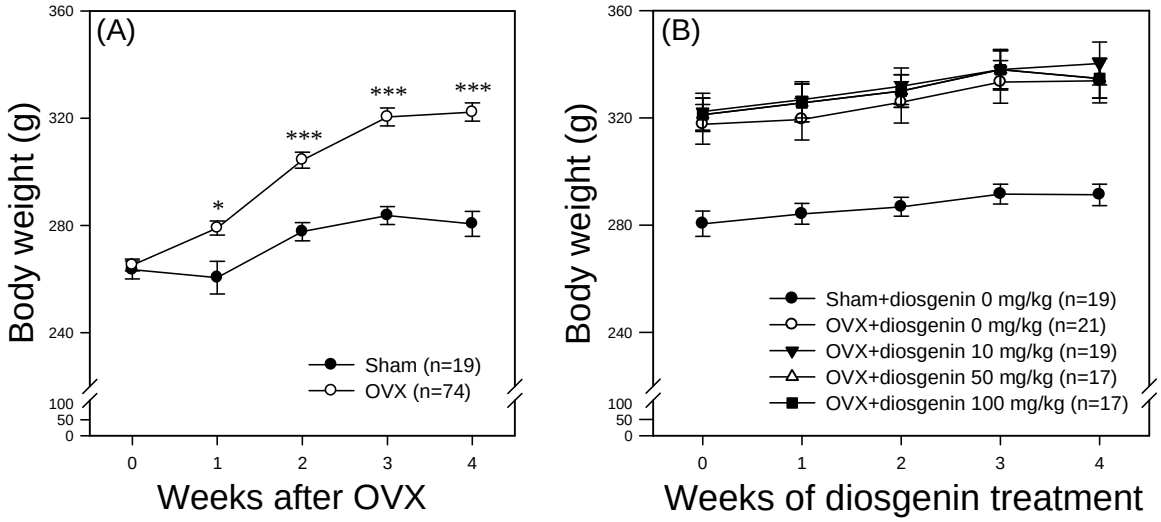

Fig. 1. Effects of ovariectomy and diosgenin on body weight. (A) Time course of body weight changes in sham-operated and OVX rats. (B) Time course of body weight changes in sham-operated and OVX rats with and without diosgenin treatment. * $P < 0.05$, *** $P < 0.001$, compared to sham-operated rats. Data are expressed as the mean ± SEM.

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Dear Dr. Ho,

Now I am so pleased to inform you that your manuscript (**CJP#100011**) entitled "Behavioral and IL-2 Responses to Diosgenin in Ovariectomized Rats" is accepted for the publication in ***The Chinese Journal of Physiology*** (CJP). We will mail you the galley proof as soon as it is ready.
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附件二

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估 ■達成目標 □ 未達成目標（請說明，以 100 字為限） □ 實驗失敗 □ 因故實驗中斷 □ 其他原因 說明：
2. 研究成果在學術期刊發表或申請專利等情形： 論文：■已發表 □未發表之文稿 □撰寫中 □無 專利：□已獲得 ■申請中 □無 技轉：□已技轉 □洽談中 □無 其他：（以 100 字為限）
3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）（以 500 字為限） 本計畫經費支持之部分研究成果已經被 Chinese Journal of Physiology 接受發表（附論文接受函），部分研究成果正在申請專利中。

附件三

國科會補助計畫衍生研發成果推廣資料表

日期：100 年 10 月 24 日

<table>
<tr><td>國科會補助計畫</td><td colspan="3">計畫名稱：探討 D-cycloserine 與合併使用 parecoxib 對巴金森氏症之行為障礙與神經發炎現象的效果：前置基礎研究
計畫主持人：何應瑞
計畫編號：NSC 99-2410-H-040 -008　領域：生物醫學</td></tr>
<tr><td rowspan="2">研發成果名稱</td><td colspan="3">（中文）使用薯蕷皂素來改善與停經期症候群有關聯的認知缺陷</td></tr>
<tr><td colspan="3">（英文） improvement of cognitive deficit associated with menopausal syndrome with diosgenin</td></tr>
<tr><td>成果歸屬機構</td><td>中山醫學大學</td><td>發明人
(創作人)</td><td>何應瑞</td></tr>
<tr><td rowspan="2">技術說明</td><td colspan="3">（中文）
本發明揭示薯蕷皂素可被用來改善與停經期症候群有關聯的認知缺陷(諸如學習、記憶以及辨識能力的下降)</td></tr>
<tr><td colspan="3">（英文）
Disclosed herein is diosgenin can be used in the improvement of cognitive deficit associated with menopausal syndrome, such as the decrease in the abilities of learning, memory and recognition.</td></tr>
<tr><td>產業別</td><td colspan="3">生物醫學</td></tr>
<tr><td>技術/產品應用範圍</td><td colspan="3">醫療、製藥業、保健食品業</td></tr>
<tr><td>技術移轉可行性及預期效益</td><td colspan="3">用以改善停經症候群有關之認知缺陷</td></tr>
</table>

註：**本項研發成果若尚未申請專利，請勿揭露可申請專利之主要內容。**

附件四

國科會補助專題研究計畫項下出席國際學術會議心得報告

日期：100 年 10 月 24 日

計畫編號	NSC 99-2410-H-040 -008		
計畫名稱	探討 D-cycloserine 與合併使用 parecoxib 對巴金森氏症之行為障礙與神經發炎現象的效果：前置基礎研究		
出國人員姓名	何應瑞	服務機構及職稱	中山醫學大學 心理學系
會議時間	100 年 7 月 4 日至 100 年 7 月 8 日	會議地點	**Istanbul, Turkey**
會議名稱	(中文)第 12 屆歐洲心理會議 (英文) The 12th European Congress of Psychology		
發表論文題目	1. **Ho YJ***. D-cycloserine suppresses MPTP-induced deficits of behavioral and neurological functions. The 12th European Congress of Psychology. Jul. 04-08, 2011. **Istanbul, Turkey**. 2. Huang WN, MC Ho, **YJ Ho***. A 5-arm Maze Study investigating Visuo-spatial Attention in MPTP-induced Animal Model of Parkinson's Disease. The 12th European Congress of Psychology. Jul. 04-08, 2011. **Istanbul, Turkey**		

一、參加會議經過

於 100 年 7 月 4 日至 8 日出席於土耳其伊斯坦堡所舉行之「第 12 屆歐洲心理學會議」。

二、與會心得

會議中發表 2 篇章海報論文（如下），並且與其他國家學者共同討論最新之心理神經病理學發現。會議中特別留意到今年亞洲國家之研究團隊出席相當踴躍，並且著重發表生物醫學、生物神經科學之研究成果。本人發表 2 篇研究成果：一篇口頭報告，題目為：D-cycloserine suppresses MPTP-induced deficits of behavioral and neurological functions。一篇海報論文，題目為：A 5-arm Maze Study investigating Visuo-spatial Attention in MPTP-induced Animal Model of Parkinson's Disease

三、建議

建議爾後多鼓勵國內相同領域之學者多共同出席國際會議，可以形成一種學術氛圍並吸引注意。

五、攜回資料名稱及內容

攜回資料名稱及內容（與會手冊封面、論文暨海報發表時程等影本）會議手冊等資料

附件四

國科會補助專題研究計畫項下出席國際學術會議心得報告

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Acceptance Letter

第 12 屆歐洲心理學年會　接受函

口頭報告

----- Original Message -----
From: ECP 2011
To: yjho@csmu.edu.tw
Sent: Sunday, February 27, 2011 1:27 AM
Subject: ECP 2011 // Abstract Notification

Dear **Ying-Jui Ho**,

We are pleased to inform you that your abstract titled *"**D-cycloserine suppresses MPTP-induced deficits of behavioral and neurological functions**"* has been accepted for **"Oral Presentation"** (15 min. presentation + 5 min. Q&A) *in the* **12th European Congress of Psychology.**

Please note that you need to complete your registration by **April 1st, 2011** in order for your abstract to be included in the Final Program. You could also benefit from early registration until **March 15th, 2011. Please visit the congress website regularly for updated information on the Scientific Programme and date of your presentation.**

Please do not hesitate to contact us if you have any questions or need any clarifications.
We t*hank you for your contribution to the* **12th European Congress of Psychology and look forward to seeing you in Istanbul.**

Best Regards,

TOPKON CONGRESS SERVICES
Zuhtu Pasa Mah. Rifat Bey Sokak No:24 Kalamis, Kadikoy / Istanbul
Phone : +90 216 330 90 20 Pbx | Fax : +90 216 330 90 05-06-07-08 |
Email: ecp2011@topkon.com | http://www.topkon.com

D-cycloserine suppresses MPTP-induced deficits of behavioral and neurological functions

Ying-Jui Ho

School of Psychology, Chung Shan Medical University Hospital, Chung Shan Medical University, Taiwan, ROC;

2010 歐洲心理學年會 土耳其伊斯坦堡 2010.07.04-08. oral

Abstract

Glutamatergic dysfunction has been implicated in the neurodegeneration seen in Parkinson's disease (PD). D-cycloserine (DCS; 30, 100, or 200 mg/kg/day, i.p.), a partial agonist of the N-methyl-D-aspartate (NMDA) receptor, was used to evaluate the role of NMDA receptors in neuronal and behavioral changes in a MPTP-induced PD rat model. A transient disturbance of motor function was observed after MPTP lesion. This impairment spontaneously recovered to control levels 6 days after MPTP lesioning and DCS treatment facilitated recovery. MPTP lesioning also caused deficits in working memory and anxiety-like behavior. Object recognition was disrupted in MPTP-lesioned rats, and interleukin-2 levels in the brain areas were increased, both effects being restored by DCS treatment. Furthermore, MPTP lesion-induced dopaminergic degeneration, microglial activation, and cell loss in the hippocampal CA1 area were all improved by DCS treatment. These results suggest that DCS may have clinical potential in the treatment of dementia associated with PD.

Keywords: Parkinson's disease; NMDA receptor; D-cycloserine; dementia; neuroinflammation; cognition

國科會補助計畫衍生研發成果推廣資料表

日期:2011/10/27

國科會補助計畫	計畫名稱: 探討D-cycloserine與合併使用parecoxib對巴金森氏症之行為障礙與神經發炎現象的效果：前置基礎研究		
	計畫主持人: 何應瑞		
	計畫編號: 99-2410-H-040-008- 學門領域: 生物心理學		
研發成果名稱	(中文) 使用薯蕷皂素來改善與停經期症候群有關聯的認知缺陷		
	(英文) improvement of cognitive deficit associated with menopausal syndrome with diosgenin		
成果歸屬機構	中山醫學大學	發明人(創作人)	何應瑞
技術說明	(中文) [使用薯蕷皂素來改善與停經期症候群有關聯的認知缺陷]：本發明揭示薯蕷皂素可被用來改善與停經期症候群有關聯的認知缺陷(諸如學習、記憶以及辨識能力的下降)。申請專利範圍： 1. 一種用於改善一具有心理症狀之停經期或停經後期女性的學習能力的組成物，其包含有一有效量之薯蕷皂素。 2. 如申請專利範圍第1項的組成物，其中該停經期或停經後期女性具有焦慮症狀。 3. 如申請專利範圍第1項的組成物，其中該薯蕷皂素的有效量是落在0.014 g/天至3.5 g/天之範圍內。 4. 如申請專利範圍第1項的組成物，它可被製造成有如一醫藥品或食品產品的形式。 5. 一種用於改善一停經期或停經後期女性的辨識和/或記憶能力的組成物，其包含有一有效量之薯蕷皂素。 6. 如申請專利範圍第5項的組成物，其中該薯蕷皂素的有效量是落在0.07 g/天至7 g/天之範圍內。 7. 如申請專利範圍第5項的組成物，它可被製造成有如一醫藥品或食品產品的形式。 8. 如申請專利範圍第4或7項的組成物，其中該醫藥品是呈一供口服投藥的劑型。 9. 如申請專利範圍第4或7項的組成物，其中該醫藥品是呈一供非經腸道投藥的劑型。 10. 薯蕷皂素用於製備一用來改善一具有心理症狀之停經期或停經後期女性的學習能力之醫藥品或食品產品的用途。 11. 如申請專利範圍第10項的用途，其中該停經期或停經後期女性具有焦慮症狀。 12. 如申請專利範圍第10項的用途，其中該薯蕷皂素是以一範圍落在0.14 g/天至0.7 g/天內的有效量而被給予該停經期或停經後期女性。 13. 薯蕷皂素用於製備一用來改善一停經期或停經後期女性的辨識和/或記憶能力之醫藥品或食品產品的用途。 14. 如申請專利範圍第13項的用途，其中該薯蕷皂素是以一範圍落在0.7 g/天至1.4 g/天內的有效量而被給予該停經期或停經後期女性。 15. 如申請專利範圍第10或13項的用途，其中該醫藥品是呈一供口服投藥的劑型。 16. 如申請專利範圍第10或13項的用途，其中該醫藥品是呈一供非經腸道投藥的劑型。		
	(英文) Disclosed herein is diosgenin can be used in the improvement of cognitive deficit associated with menopausal syndrome, such as the decrease in the abilities of learning, memory and recognition.		
產業別	研究發展服務業		
技術/產品應用範圍	醫療、製藥業、保健食品業		
技術移轉可行性及預期效益	用以改善停經症候群有關之認知缺陷		

註：本項研發成果若尚未申請專利，請勿揭露可申請專利之主要內容。

99 年度專題研究計畫研究成果彙整表

計畫主持人：何應瑞	計畫編號：99-2410-H-040-008-
計畫名稱：探討 D-cycloserine 與合併使用 parecoxib 對巴金森氏症之行為障礙與神經發炎現象的效果：前置基礎研究	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	1	1	100%	篇	1. 莊婷、謝明鴻、陳福士、吳富英、何應瑞*。D-cycloserine 加強暴露療法治療恐懼症之文獻回顧。台灣醫學，2011 in press.
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%		
	專利	申請中件數	1	1	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	2	2	100%	篇	1. Ho YJ*, SY Tai, CR Pawlak, AL Wang, CW Cheng, MH Hsieh. Behavioral and IL-2 Responses to Diosgenin in Ovariectomized Rats. Chin J Physiol (Apr. 12, 2011, in press) (SCI).

							Effects of chronic resistive airway loading on behavioral changes in rats. Chin J Physiol (Aug. 22, 2011, in press) (SCI).
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	2	2	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)		無					

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：■已發表 □未發表之文稿 □撰寫中 □無

專利：□已獲得 ■申請中 □無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限）

研究成果證實薯蕷皂苷可以改善停經症候群之認知功能障礙，此成果具有醫療及保健功效，具有可專利性，目前正在申請專利中。