Original Article

Vanillic Acid Prevents Interferon-alpha and Cyclosporine A-induced Depressant-like Behavior in Mice

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Abstract

Background: Interferon-alpha (IFN-α) is a useful therapy for some types of cancers and viral infections. Cyclosporine A (CSA) is an immunosuppressant drug used to reduce the risk of graft rejection. Chronic use of IFN-α and CSA are related to psychological symptoms such as depression. Vanillic acid (VA) is a naturally occurring flavoring substance with antidepressant potential. The aim of this study was to evaluate the effect of VA on depression caused by these two drugs in a mice model. **Materials and Methods:** Male Swiss mice (25–30 g) were used. Depression was induced by IFN-α 1600000 IU/kg, sc for six days, or CSA20 mg/kg, ip for 3 days as VA 25 mg/kg, and pretreatment was ip injected. After evaluating the locomotor activity, depression was assessed by forced swimming test (FST) and sucrose preference (SP) test. **Results:** The selected treatments did not cause significant changes in the locomotor activity. IFN-α significantly increased the immobility time during FST (184.5 ± 12.9 s vs. vehicle 107.1 ± 11.4s) indicating depressive-like effect, and VA pretreatment reversed it (94.8 ± 17.8 s vs. IFN-α), SP increased to 76%. CSA also increased the immobility time during FST (160.3 ± 3.4 s vs. vehicle 113.2 ± 7.6 s; $P < 0.05$), VA pretreatment reduced it (81.8 ± 16.9 s, vs. cyclosporine; $P < 0.001$), and SP increased from 38% to 75%. SP results were in agreement with FST results. **Conclusion:** VA showed useful effect against IFN-α and cyclosporine-induced depression in mice. Further clinical studies regarding VA antidepressant effect in patients receiving IFN-α or CSA are warranted.

Keywords: *Animal test, cyclosporine A, depression, interferon alpha, vanillic acid*

Introduction

Interferon-alpha (IFN-α) is broadly used for treatment of some types of malignancies as well as chronic hepatitis C.[1] Chronic use of IFN-α has been associated with psychological side effects such as depression and sometimes is followed by suicidal ideation and suicidal attempts.[2] According to studies, depression caused by IFN-α injection is reversible with antidepressants.[3] It has been reported that IFN-α stimulates nitric oxide synthase expression and influences the mechanistic target of rapamycin (mTOR) pathway.[4,5] In mammalian cells the mTOR pathway is important for maintaining body homeostasis and also has an important role in development of depression. Therefore, it seems that mTOR pathway is a possible mechanism of IFN-α-induced depression.[4,6]

Cyclosporine A (CSA) has been approved for prevention of graft rejection in kidney, liver, and heart transplants. Other indications of the drug are treatment of rheumatic diseases and some types of autoimmune disorders.[7,8] This drug is known as an inhibitor of calcinurin.[9] It has been also reported that CSA prevents kidney and pancreas transplant rejection through the activation of mTOR pathway.[10] Depression and neurological complications have been reported as side effects of chronic use of the drug and as mentioned above the mTOR pathway is involved in development of depression.[11,12] Also it may be concluded that CSA-induced depression is mediated to somewhat by activation of mTOR pathway.[4,6]

Preventing depression with appropriate and harmless supplements would preclude unnecessary medications, adverse drug reactions, and drug interactions.[13] Vanillic acid (VA) is found in many herbs especially *Angelica sinensis* and is used as a flavoring agent in foods.[14] Studies have shown that VA has analgesic and anti-inflammatory effects in addition to neuroprotective activity and attenuation of cognitive impairment induced by streptozocin or amyloid beta.[15-18] Kim *et al.*[19] reported that the anti-inflammatory effect of VA is mediated by suppressing NF-κB. Also VA and its derivatives have shown

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significant antioxidant activity,[20] and inhibited the harmful effect of oxidative stress on learning and memory in mice and it has been suggested that its chronic use could prevent the progression of Alzheimer's disease.[16] In a recent study conducted by Chuang *et al*.[21] the role of mTOR pathway in antidepressant effect of VA was documented. Based on the possible involvement of mTOR activity in IFN-α and CSA-induced depression, this study was aimed to evaluate the effect of VA on depression caused by these two drugs in a mice model.

Materials and Methods

Chemicals

CSA (Sandimmun, 50 mg/mL; Novartis, Switzerland), VA (Merck, Germany), IFN-α (PDferon, Pooyesh Darou 3 × 106 IU, Iran), and fluoxetine HCl (Sigma-Aldrich, India) were used in this study.

Animals

Male Swiss mice (25–30 g, 6–8 weeks old) were kept in standard conditions of humidity, temperature, and light/dark cycle and had free access to pellet chow and water. Six mice were housed in each cage, all the animal experiments were performed according to guidelines for the care and use of laboratory animals provided by the National Ethical Committee of Iran (Ethics code: IR.MUI.REC. 1399.490). All the efforts in the experiments were made to minimize animal suffering and to reduce the number of animals used in the experiments.

Experimental design

Totally 16 groups consisting six mice per each were applied and randomly received the following treatments: three groups of animals received one single dose of VA (12.5, 25, or 50 mg/kg, intraperitonealy; ip); control group animals received one single-dose normal saline (10 mL/kg, ip);[16] VA25 group animals received VA (25 mg/kg, ip) once daily for 6 days; control group, received normal saline once daily for 6 days (ip); IFN-α group, animals that received IFN-α (1600000 IU/kg) once daily for 6 days subcutaneously (sc); IFN-α vehicle group animals received normal saline once daily for 6 days (sc);[13,22] VA25 + IFN-α group animals received VA (25 mg/kg, ip) and IFN-α once daily for 6 days; fluoxetine +IFN-α group animals received the standard antidepressant drug fluoxetine (15 mg/kg, ip) and IFN-α once daily for 6 days; VA25 group animals received VA (25 mg/kg, ip) once daily for 3 days; control group animals received normal saline once daily for 3 days (ip); CSA group animals received CSA (20 mg/kg, ip) once daily for 3 days; CSA vehicle group animals received 2%v/v EtOH/normal saline (ip) once daily for 3 days;[11] VA25 + CSA group animals received VA (25 mg/kg, ip) and CSA once daily for 3 days; fluoxetine +CSA group animals received fluoxetine (15 mg/kg, ip) and CSA once daily for 3 days.

The tests were performed the day after the last injection (i.e., on day 7 or on day 4) the sucrose preference (SP) was measured for each group, then each animal was first subjected to the locomotor test and finally the forced swimming test (FST).

Locomotor activity test

Locomotor test was conducted to evaluate the possible sedative or stimulant activity of different treatments. In an open-field apparatus (Borj Sanat, I.R. Iran), with white floor that was divided into 15 zones by red beams. Mice were carefully placed in one corner of the field and allowed to explore it for 3 min. The total activity for each mouse was calculated based on the sum of: number of zone crossings (horizontal exploration) that was counted automatically and the number of rearing on hind-legs (vertical exploration) that were counted manually.[11]

Forced swimming test

This test was performed as described previously.[23] Mice were forced to swim in 25°C water in a cylindrical beaker filled with 12 cm of water at 25°C for 6 min. The first 2 min was considered as habituation time and in the last 4 min all movements of animal were recorded using a camera and later the immobility time was measured in different groups and compared. The time spent for swimming behavior and climbing the container walls were also measured. At the end of the experiment, the mice were removed from the water and dried carefully to avoid hypothermia.

Sucrose preference test

Anhedonia was measured by SP test as another depression-related endophenotypes. The test was performed in three days (for the 6 day protocol started day 4 and ended day7; the 3-day protocol started day 1 and ended day 4), the first two days were for habituation. On the first day animals had access to two bottles of sucrose solution (2% w/v) in their cage, and on the second day there was one bottle of sucrose solution and one bottle of water. On the third day, two bottles were placed that contained a certain amount of sucrose solution and tap water that was finally measured after 24 h, and the SP percentage was calculated (sucrose consumption/ water plus sucrose consumption ×100). SP of less than 65% was considered as an index for anhedonia.[23]

Statistical analysis

Results are expressed as mean ± SEM. All results were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's *pot hoc* test. *P*-values less than 0.05 were considered significant. The software programs that were used for data analyzing and making graphs were GraphPad Prizm 8 and Excel 2020, respectively.

Results

Effect of different treatments on locomotor activity in the open field

As seen in Table 1, total activity number in animals that received VA at doses of 12.5 and 25 mg/kg was not different from control animals. VA at a dose of 50 mg/kg increased locomotor activity but it was not significant compared with the control group. IFN-α alone or CSA alone did not alter activity behavior. Pretreatment with VA also showed no change

in total activity count. Similarly, pretreatment with fluoxetine could not produce any significant change in activity behavior.

Effects of vanillic acid alone and vanillic acid pretreatment on interferon alpha depressive-like effects

The possible antidepressant effect of VA after administration was examined in the FST. In this test [Figure 1], mice treated with three different doses of VA (12.5, 25 and 50 mg/kg) showed decreases in their immobility times, (127.5 ± 10.9s, 94.0 ± 8.5 s and 36.1 ± 5.3s, respectively) when compared with control (150.17 ± 4.9s) which was significant ($P < 0.001$) for doses of 25 and 50 mg/kg. Administration of a single dose of VA (50 mg/kg) significantly ($P < 0.001$) increased both swimming and climbing times. The other tested doses (12.5 and 25 mg/kg) could not exert significant changes on these parameters [Table 2].

The effect of VA (25 mg/kg) on IFN-α-induced depressive-like behavior is shown in Figure 2. IFN-α significantly increased the immobility time ($P = 0.023$) when compared with vehicle-treated group (184.5 ± 12.9s vs. 126.1 ± 12.9s) and pretreatment with VA reversed this alteration (94.8 ± 17.8s vs. IFN-α, $P < 0.001$). In another group of animals, fluoxetine served as a positive control and showed similar effects (51.8 ± 9.2s vs. IFN-α, $P < 0.001$). The SP results were in agreement with FST results, SP was 68% for the control group, VA increased SP up to 80%, whereas IFN-α reduced SP to 59%, and pretreatment with VA improved SP up to 76%.

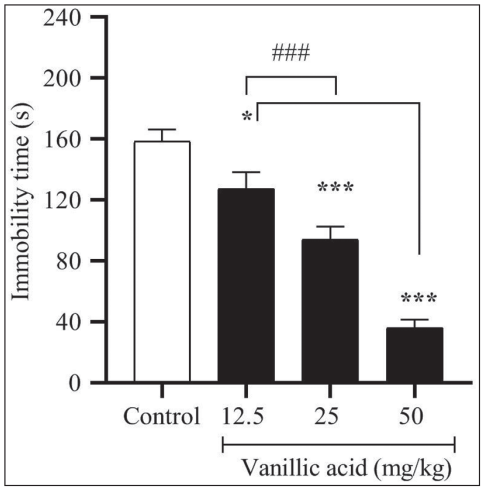

Figure 1: Effect of different doses of vanillic acid on immobility time in FST. Control animals; normal saline (ip). Results are expressed as group ($n = 6$) mean ± SEM and analyzed by ANOVA followed by Tukey's comparison test. $^{***}P < 0.001$ compared with control group and $^{###}P < 0.001$ compared with vanillic acid 12.5 mg/kg

As it is observed in Table 2, administration of VA (25 mg/kg) for 6 days increased swimming time (125.0 ± 17.0 vs. control 57.5 ± 6.9, $P < 0.001$). IFN-α significantly ($P < 0.01$) reduced both swimming and climbing times (compared with vehicle-normal saline treated animals) and pretreatment with either VA or fluoxetine reversed the effect.

Effects of vanillic acid pretreatment on cyclosporine A depressive-like effects

Figure 3 shows the effect of VA on CSA behavior in FST. Again CSA increased the immobility time in comparison with vehicle-treated group (160.3 ± 3.4s vs. 113.2 ± 7.6s, $P = 0.0418$). Pretreatment with VA reversed the effect of CSA on immobility time (81.8 ± 16.9s vs. CSA group, $P < 0.001$) so that this parameter in animals receiving VA and CSA was not different from that of vehicle-treated animals. In this test fluoxetine also as a typical antidepressant reversed the effect of CSA on immobility time. The SP values were in agreement with FST results as SP was very low 38% for CSA group and rose up to 75% following pretreatment with VA.

The effect of VA on CSA swimming and climbing time is summarized in Table 3. As it is seen CSA also reduced swimming time in FST and pretreatment with VA or fluoxetine reversed the effect. Pretreatments with VA or fluoxetine also significantly increased climbing time.

Discussion

In this study for the first time the effect of VA on cyclosporin and IFN-α-induced depression was evaluated and the findings showed promising antidepressant activity against both drugs. VA in a dose-dependent manner reduced immobility time in FST and these data are in agreement with previous reports

Table 1: Effect of VA, IFN-α, and CSA on total activity count in locomotor activity test

Groups ($n = 6$)	Total activity no.	Groups ($n = 6$)	Total activity no.
Control (NS) one dose	166.7 ± 8.5	IFN-α + VA (25 mg/kg), 6 days	156.5 ± 12.2
VA (12.5 mg/kg) one dose	158.8 ± 13.4	IFN-α + FLX, 6 days	158.3 ± 8.2
VA (25 mg/kg) one dose	152.2 ± 7.5	Control (NS) 3 days	166.7 ± 8.5
VA (50 mg/kg) one dose	199.6 ± 21.8	VA (25 mg/kg) 3 days	162.7 ± 4.5
Control (NS) 6 days	152.0 ± 13.2	Vehicle1 3 days	140.2 ± 13.0
VA (25 mg/kg) 6 days	158.7 ± 19.8	CSA 3 days	118.7 ± 6.6
Vehicle (NS) 6 days	151.6 ± 16.1	CSA + VA(25 mg/kg), 3 days	131.8 ± 5.2
IFN-α 6 days	157.9 ± 12.4	CSA + FLX, 3 days	148.5 ± 11.4

IFN-α = interferon-α (1600000 IU/kg), NS = normal saline, VA = vanillic acid, FLX = fluoxetine (15 mg/kg), CSA = cyclosporine A (20 mg/kg), Vehicle1 = (2%v/v EtOH/NS)

Total activity during locomotor test = (horizontal + vertical) exploration

Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's comparison test

Table 2: Effect of different doses of VA and IFN-α pretreatment on swimming and climbing time in FST

Group (n = 6)	Swimming time (s)	Climbing time (s)
Control (NS) one dose	56.8 ± 10.5	24.7 ± 5.2
VA (12.5 mg/kg) one dose	54.5 ± 8.6	56.3 ± 9.9
VA (25 mg/kg) one dose	58.7 ± 6.0	87.3 ± 14.0
VA (50 mg/kg) one dose	106.8 ± 7.9 ***	97.6 ± 11.5 ***
Control (NS) 6 days	57.5 ± 6.9	76.9 ± 7.3
VA (25 mg/kg) 6 days	125.0 ± 17.0***	33.2 ± 5.2**
Vehicle (NS) 6 days	62.0 ± 8.0	73.4 ± 12.1
IFN-α 6 days	28.1 ± 5.3^{vv}	26.1 ± 9.8^{vv}
IFN-α + VA (25mg/kg), 6 days	58.8 ± 4.5#	86.3 ± 19.3#
IFN-α + FLX, 6 days	80.0 ± 9.8##	108.2 ± 10.0##

IFN-α = interferon-α (1600000 IU/kg), NS = normal saline, VA = vanillic acid, FLX = fluoxetine (15 mg/kg)
Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's comparison test
** P <0.01 and *** P < 0.001 compared with the relevant control group (for single dose, or 6 days), ^{vv} P < 0.01 compared with vehicle group, # P < 0.05 and ## P < 0.01 compared with IFN-α group

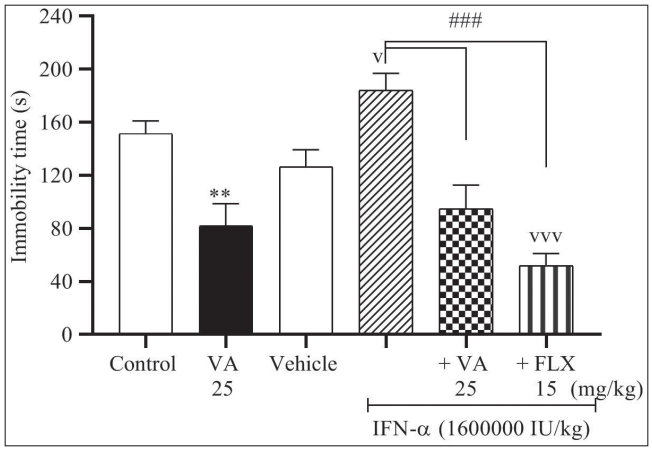

Figure 2: Effect of IFN-α and pretreatment with vanillic acid or fluoxetine on immobility time during FST. Control animals; normal saline (ip), and vehicle; normal saline (sc). Results are expressed as group (n = 6) mean ± SEM and analyzed by ANOVA followed by Tukey's comparison test. *** P < 0.001 compared with control group, ^{v} P < 0.05, ^{vvv} P < 0.001 compared with vehicle, and ### P < 0.001 compared with IFN-α group. IFN-α = interferon-α, VA = vanillic acid, FLX = fluoxetine

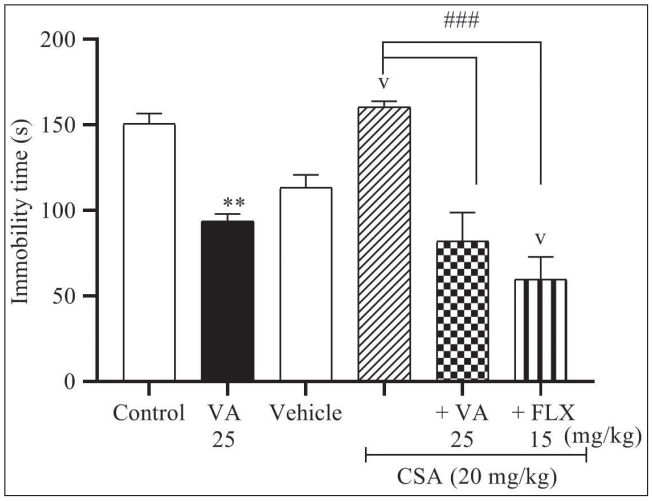

Figure 3: Effect of cyclosporine and pretreatment with vanillic acid or fluoxetine on immobility time in FST. Control animals; normal saline (ip), and vehicle; (2%v/v EtOH/normal saline, ip). Results are expressed as group (n = 6) mean ± SEM and analyzed by ANOVA followed by Tukey's comparison test. * P < 0.05 compared with control group, ^{v} P < 0.05 compared with vehicle group, and ### P < 0.001 compared with cyclosporine group. CSA = cyclosporine A, VA = vanillic acid, FLX = fluoxetine

on antidepressant effects of VA.[21] The FST has been used worldwide as a reliable and sensitive test for evaluation of different classes of antidepressants and also for screening of new compounds in industry. In this test serotonergic drugs tend to increase swimming, whereas tricyclic antidepressants have more effect on climbing.[24] As our results show VA reduces immobility time while increasing both swimming and climbing time behavior indicating that VA affects both serotoninergic and adrenergic neurotransmitter systems.

Locomotor activity test is also a complementary and helpful test for FST as alteration of locomotor activity can deviate the results of FST.[11] In our study the higher dose of VA increased locomotor activity, whereas doses of 12.5 and 25 mg/kg were ineffective and therefore we selected the dose of 25 mg/kg to evaluate its effect on IFN-α or CSA depression. In addition, IFN-α and CSA at applied doses could not change locomotor activity.

In our study, IFN-α showed depressive-like behavior in FST which was in favor of previous reports.[22,25,26] In addition, IFN-α did not alter total activity in locomotor test indicating that the effect of IFN-α on immobility time in FST was mostly due to the animal despair behavior. Clinical reports also indicated that despite the effectiveness of IFN-α in the treatment of chronic hepatitis C virus (HCV), many patients (up to 35%) receiving the drug show depression.[27] IFN-α-induced depression is so common that some researchers emphasized on prophylactic treatment with antidepressant drugs. In a recent clinical study conducted by Shakeel *et al.*,[28] citalopram and escitalopram were compared for their antidepressant effect following IFN-α-induced depression and they emphasized on better tolerability of escitalopram. IFN-α increases production and release of several cytokines that are believed to promote depression and the association between up-regulation of interleukin-1beta (IL-1β), IL-6, and tumor necrosis factor-alpha (TNF-α) and depression have been reported in previous studies.[30] In our study, VA inhibited the depressant effect of IFN-α in mice.

Table 3: Effect of VA and CSA pretreatment on swimming and climbing time in FST

Group (n = 6)	Swimming time (s)	Climbing time (s)
Control (NS) 3 days	56.8 ± 10.5	44.7 ± 5.2
VA 3 days	70.7 ± 6.9	75.8 ± 6.4*
Vehicle 3 days	58.0 ± 5.6	68.8 ± 5.9
CSA 3 days	26.4 ± 2.0^{vv}	61.4 ± 5.0
CSA + VA, 3 days	59.8 ± 5.0$^{\#}$	97.7 ± 8.3$^{\#}$
CSA + FLX, 3 days	84.3 ± 6.3$^{\#\#\#}$	96.2 ± 9.8$^{\#}$

CSA = cyclosporine A (20 mg/kg), NS = normal saline, VA = vanillic acid (25 mg/kg), FLX = fluoxetine (15 mg/kg)
Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's comparison test
*$P < 0.05$ compared with the control group, ^{vv} $P < 0.01$ compared with vehicle group (2%v/v EtOH/NS), $^{\#}$ $P < 0.05$ and $^{\#\#\#}$ $P < 0.001$ compared with CSA group

Calixto-Campos *et al.*[18] reported that VA inhibits cytokine production and NF-KB activation and therefore it might be a possible mechanism for antidepressant activity of VA. Another possible mechanism is mTOR pathway as the effect of IFN-α and VA on this pathway has been reported separately.[4,21]

In the other part of our study, the effect of VA on CSA-induced depression was evaluated and our findings in agreement with Mesripour *et al.*[11] showed that CSA increased immobility time in FST at a dose, which had no effect on locomotion. CSA is a well-known calcineurin inhibitor and previous studies have shown that calcineurin inhibition is involved in neurotransmission and neuronal plasticity.[30] In addition, Yu *et al.*[31] reported that inhibition of calcineurin induces depressive-like behavior via mTOR pathway. Therefore logically one might link CSA-induced depression to mTOR pathway. In our study treatment of mice with VA inhibited depressive behavior caused by CSA. On the contrary, recently Chuang *et al.*[21] notified the role of mTOR signaling in antidepressant activity of VA. Taking these findings together one possible mechanism for antidepressant effects of VA against CSA is their intervention with mTOR activity. Therefore, IFN-α and CSA might share similar pathways in producing depressive-like behavior. Indeed, further studies are needed to find out the definite mechanism.

Conclusion

VA showed acceptable antidepressant effect against IFN-α and CSA-induced depressive behavior in mice at a dose that was comparable with a classical antidepressant (fluoxetine) and showed promising effect so that clinical trials are suggested to evaluate its effect on patients receiving either IFN-α or CSA.

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Conflicts of interest

There are no conflicts of interest.

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