

Pyridoxine effect on the antidepressant action of imipramine in albino mice

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ABSTRACT

الأهداف: تقييم التأثير السلوكي لبيريدوكسين على عمل الإمبرامين كمضاد للكآبة.

الطريقة: شملت الدراسة مجموعة من الفئران وزن ما بين 25-35gms، أجريت تجربتين، الأولى لدراسة التأثير الحاد لبيريدوكسين على زمن التعوق باستخدام اختبار السباحة الإجماعي، حيث تلقت أربعة مجموعات من الفئران جرعات من المحلول الملحي ولبيريدوكسين 65-125-250mg/kg على التوالي. أجري اختبار السباحة الإجماعي FST-30 دقيقة بعد الحقن. التجربة الثانية شملت ست مجموعات من الفئران، حقنت المجموعة الأولى بمحلول ملحي، المجموعة الثانية 10mg/kg إمبرامين، المجموعة الثالثة 65mg/kg بيريدوكسين، المجموعة الرابعة 250mg/kg بيريدوكسين، المجموعة الخامسة 65mg/kg إمبرامين مع بيريدوكسين، أما المجموعة السادسة فقد تلقت 250mg/kg إمبرامين مع بيريدوكسين على التوالي. تم حقن العقاقير قبل بدء الاختبار بـ (24 ساعة، 5 ساعات، وساعة). أجريت هذه الدراسة في مركز بحوث التقنيات الحيوية - الطويشة - قصر بن غشير - ليبيا، في يونيو 2007م.

النتائج: الجرعة الحادة من البيريدوكسين لم تحدث أي تغيير في زمن التعوق عند مقارنتها بالمرجع. المعالجة المزمنة بجرعة (65mg/kg) بيريدوكسين لم تحدث أيضا أي تغيير في زمن التعوق، بينما المعالجة المزمنة بجرعة (250mg/kg) بيريدوكسين أحدثت نقصاً في زمن التعوق. جرعة (10mg/kg) من الإمبرامين أحدثت نقص ملحوظ في زمن التعوق مقارنة بالمرجع. البيريدوكسين لم يحدث تغير في عمل الإمبرامين.

خاتمة: يمكن لعقار لبيريدوكسين لوحده أن يحتوي على تأثير مضاد للكآبة، بينما البيريدوكسين بالتمازج مع الإمبرامين لم يغير عمل الإمبرامين كمضاد للكآبة.

Objective: To evaluate the behavioral effect of pyridoxine on the antidepressant action of imipramine.

Methods: Male Wistar albino mice of weights 25-35gms were used. Two experiments were carried out; the first on the acute effect of pyridoxine on the duration of immobility, and the second on the sub-chronic effects of pyridoxine alone and in combination with imipramine. In the first experiment, 4 groups of animals received

saline, 65, 125, and 250mg/kg pyridoxine. Forced swimming test (FST) was performed 30 minutes after drug administration. In the second experiment, 6 groups of mice were used. The first group received saline, the second group received imipramine 10 mg/kg, the third group received pyridoxine 65mg/kg, the fourth group received pyridoxine 250mg/kg, the fifth group received combined treatment of imipramine and pyridoxine 65 mg/kg, while the sixth group received a combined treatment of imipramine and pyridoxine 250 mg/kg. Administration of drugs was at 24, 5, and one hour before the test. This work was carried out in the Biotechnology Research Center, Twisha, Libya, in June 2007.

Results: Acute administration of pyridoxine did not change the duration of immobility compared to the control group. Sub-chronic administration showed that pyridoxine (65mg/kg) did not change the immobility time, while a higher dose of pyridoxine (250mg/kg) decreased the immobility time. Imipramine at 10mg/kg reduces the immobility time significantly. Pyridoxine did not change imipramine action.

Conclusion: Pyridoxine alone may produce an antidepressant effect. Pyridoxine in combination with imipramine did not change the imipramine action.

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Pyridoxine has been tried in a wide variety of disorders including the treatment of depression associated with premenstrual syndrome.¹ Pyridoxine has anti-seizure and neuroprotective actions.² It plays a role in tryptophan metabolism, increasing the production of

5-hydroxytryptamine (5-HT).³ Pyridoxine is essential for the proper metabolism of various neurotransmitters that are considered relevant to the pathophysiology of depression and it has been reported beneficial in ameliorating depressive symptoms as part of major depression. Pyridoxine 150 mg/day in addition to antipsychotic treatment for 4 consecutive weeks improves the depressive symptoms in schizophrenic patients with co-morbid minor depression.⁴ Pyridoxine nutritional status has a significant and selective modulatory impact on central production of both 5-HT and γ aminobutyric acid (GABA) neurotransmitters, which control mental depression, pain perception, and anxiety.⁵ The monoamine theory, proposed in 1965 suggests that mental depression results from functionally deficient monoaminergic (noradrenalin [NA] and/or 5-HT) transmission in the central nervous system (CNS).⁶ The antidepressants were developed in 1956 following clinical observations; they are classified into properly speaking antidepressants and drugs with antidepressant activity. The first-generation antidepressant is primarily the tricyclic, the 2nd generation antidepressant is derived mostly from the tricyclics and the monoamine oxidase inhibitors (MAOI).⁷⁻¹³ Tricyclic antidepressants (TCA) are still the main drugs used for mental depression. Imipramine and amitriptyline are still very often the 2 standards by which others are measured and judged.¹ The forced swimming test (FST) is currently the most widely used animal model for assessing depression related behavior in rodents.^{7,9,10} The FST is a behavioral model developed to predict the efficacy of antidepressants in humans. Many classes of antidepressants decrease the immobility time in FST, including TCA, MAOI, and atypical antidepressants.¹¹ The time that the animals spend in an immobile posture is decreased by acute or chronic treatment with numerous antidepressant compounds.^{7,9,10} The aim of the present study is to evaluate the behavioral effect of the co-administration of pyridoxine on imipramine action as an antidepressant using FST. We hypothesized that the addition of pyridoxine to antidepressant treatment could improve mental depression, and decreasing the dose with the addition of pyridoxine could change the management of antidepressant drugs.

Methods. This work was carried out in, and funded by the Biotechnology Research Center, Twisha, Gasr ben Ghachier, Libya in June, 2007, and was granted ethical approval by the management of the center. Male Wistar albino mice of weights 25-35 gms were housed at room temperature. All drugs were freshly prepared before use and were injected intraperitoneally in a volume dose of 5 ml/kg. Pyridoxine hydrochloride was obtained from B. Braun Melsungen AG, D-34209, Melsungen, Germany,

while imipramine hydrochloride from Novartis Pharma AG, Bassle, Switzerland. Imipramine was prepared in sterilized saline. The acute effect of different doses of pyridoxine hydrochloride on the duration of immobility, using FST, was investigated. The experiment was performed between 9:00 AM to 1:00 PM. Four groups of 10 animals each were used. The first group received saline (control), the second group received 65 mg/kg pyridoxine hydrochloride, the third group received 125 mg/kg pyridoxine hydrochloride, and the fourth group received 250 mg/kg pyridoxine hydrochloride. The drugs were administered intraperitoneally in a dose of 5 ml/kg. Forced swimming test was performed 30 minutes after the drug administration. The sub-chronic effects of pyridoxine hydrochloride alone and in combination with imipramine, on the duration of immobility, were investigated. Six groups of mice each of 7 were administered by the intraperitoneal route. The first group received saline, the second group received imipramine 10 mg/kg, the third group received pyridoxine 65 mg/kg, the fourth group was administered pyridoxine 250 mg/kg, the fifth group received combined treatment of imipramine 10 mg/kg and pyridoxine 65 mg/kg, while the sixth group received combined treatment of imipramine 10 mg/kg and pyridoxine 250 mg/kg. The administration of drugs was at 24, 5, and one hour before the performance of the FST. The FST was based on a previous report by Porsolt et al.⁷ The usual procedure to carryout experiments of FST in mice involves only one 6 minute session, where the activity in the 2 first minutes is ignored.⁸ Mice were placed individually into a cylinder (height, 25cm, diameter, 10cm) containing 15 cm height of water, maintained at 21-23°C, and observed for 6 minutes. A mouse was judged to be immobile when it floats in an upright position and made only small movements to keep its head above the water. The duration of immobility was recorded during the last 4 minutes of the testing period.

Descriptive statistical analysis was applied to the parameters of different samples using SPSS version 14. Kolmogorov-Smirnov maximum deviation test was applied for all groups. If the parameters are normally distributed, treatments were compared using one-way ANOVA test followed by post hoc tests, if non parametric, treatments were compared using Mann-Whitney U-test. The difference was considered to be significant at $p \leq 0.05$.

Results. The acute administration of different doses of pyridoxine (65, 125, 250 mg/kg) did not change the duration of immobility using FST compared to the control treated group ($p=0.539$, $p=0.922$, $p=0.826$). The sub-chronic administration of imipramine at 10

Table 1 - The subchronic effect of pyridoxine on imipramine antidepressant action.

Treatments	Duration of immobility	P-value
Control (saline)	94.857±19.954	
Imipramine (10 mg/kg)	17.285±13.105	0.001
Pyridoxine (65 mg/kg)	54.714±23.092	0.079
Pyridoxine (250mg/kg)	32.571±8.917	0.008
Imipramine + pyridoxine (65 mg/kg)	23.857±16.368	0.003
Imipramine + pyridoxine (250 mg/kg)	6.142±4.939	0.000

mg/kg reduces immobility time, using FST, significantly compared to the control (Table 1). The sub-chronic administration of pyridoxine in a dose of 65 mg/kg produced an insignificant decrease in the immobility time, while a dose of 250 mg/kg produced a significant decrease in the immobility time compared to the control (Table 1). The sub-chronic administration of imipramine combined with pyridoxine hydrochloride in a dose of 65 mg/kg decreased the immobility time significantly compared to the control, also the combined treatment of imipramine and pyridoxine hydrochloride in a dose of 250 mg/kg produced a significant decrease in the immobility time compared to the control (Table 1). The post hoc analysis demonstrated that the group receiving a combined treatment of imipramine 10 mg/kg and pyridoxine 65 mg/kg did not show any changes in the duration of immobility compared to the imipramine alone ($p=0.679$) or pyridoxine (65 mg/kg) ($p=0.173$) treated group. While the combined treatment of imipramine 10 mg/kg and pyridoxine 250 mg/kg did not change the duration of immobility compared to the imipramine alone ($p=0.618$) or pyridoxine (250 mg/kg) ($p=0.241$) treated group. The sub-chronic effect of pyridoxine on the duration of immobility was dose dependent; it was found that pyridoxine 250 mg/kg produced a significant decrease in the duration of immobility more than that produced by 65 mg/kg (insignificantly).

Discussion. The acute administration of different doses of pyridoxine (65, 125, 250 mg/kg) did not change the duration of immobility using FST compared to the control treated group. This may be because pyridoxine needs to be converted into pyridoxal-5-phosphate (PLP) by pyridoxal kinase. The resultant PLP is a coenzyme for many enzyme systems that are involved in amino acid and neurotransmitter metabolism,¹⁴ and important for the synthesis of catecholamine.¹⁵ The synthesis of these neurotransmitters needs time.

In the present study, we demonstrated that pyridoxine exhibits an antidepressant-like effect in mice after sub-chronic administration. Pyridoxine in a dose of 65 mg/kg did not produce an antidepressant like effect although the duration of immobility was decreased insignificantly. This may be because the dose of pyridoxine was a sub-threshold dose. Pyridoxine (250 mg/kg) reduced the duration of immobility in the FST compared with the control via an increase in swimming activity. The neurotransmitters NA, acetylcholine (ACh), dopamine (DA) and the allergy regulator histamine are very important body chemicals that depend on PLP in their metabolism.¹⁴ Also, the brain needs pyridoxine to convert tryptophan into 5-HT, which is considered an important antidepressant neurotransmitter.¹⁵ The major theory of depressive disorder (the monoamine hypothesis) proposes that decreasing the levels of one or more of the brain monoamine neurotransmitters, such as 5-HT, NA, or DA produce such diseases.¹⁶ Vitamin supplements may influence mood, this improvement in mood was associated particularly with improved pyridoxine status.¹² Administration of pyridoxine to elderly depressed persons already on TCAs improved their depression and ability to think.¹⁷ The treatment of normal adult rats with pyridoxine or a B-vitamin mixture led to an increase in the 5-HT content of various brain areas and to a decrease in the number of 5-HT (5-TH₂) receptors, which is responsible for postsynaptic excitation. The results indicate that the pyridoxal phosphate level in brain regions regulates decarboxylation of 5-hydroxytryptophan, the precursor of 5-HT.¹⁸

Imipramine at 10 mg/kg reduces immobility time significantly. The anti-immobility effect of imipramine is through their ability to oppose the neuronal re-uptake of brain monoamines (5-HT, NA, and DA).¹³ In this work, the results show no change in the effect of imipramine when administered in combination with both doses of pyridoxine (65, 250mg/kg) on the duration of immobility using the FST compared with imipramine alone, this may be due to the role of pyridoxal phosphate as a cofactor in the synthesis and metabolism of different types of excitatory and inhibitory neurotransmitters. Imipramine produces its antidepressant action through GABAergic mechanisms, causing release of catecholamine.¹⁹⁻²⁰ Previous studies demonstrated that PLP was rapidly permeable into the brain through the immature blood-brain barrier and then bound directly to GABAA receptors. It is probable that a specific amino group of a functionally crucial lysine that residues on the GABAA receptor reacts in vivo with the carbonyl group of PLP to form Schiff bases; this leads to in vivo modification of the receptor and produces a degeneration of GABAergic neurotransmission.^{21,22} Gamma aminobutyric acid

aminotransferase (GABA-T) is a PLP-dependent enzyme responsible for the degradation of the inhibitory neurotransmitter GABA.²³

Pyridoxine 250 mg/kg alone has the same potency of antidepressant action as imipramine. A study has demonstrated that the level of PLP in mouse brain was partly regulated by the concentration of biogenic amines, such as DA, NA, and 5-HT, without apparent induction of pyridoxal kinase. The production of PLP from pyridoxine by pyridoxal kinase was inhibited by the addition of DA, NA, and 5-HT, but not by that of epinephrine and N-acetyl-serotonin. The mechanism of PLP inhibition by catecholamine was through the combination of DA and NA with PLP by a non-enzymatic reaction, whereas 5-HT was bound only slightly with it.²⁴ Therefore, the combined administration of imipramine with pyridoxine (250 mg/kg) showed less effect on the immobility time compared to the summation of each alone.

In conclusion, pyridoxine alone may produce an antidepressant effect, pyridoxine in combination with imipramine did not change the imipramine effect. Further studies are needed to clarify the efficacy of pyridoxine with its relation to brain neurotransmitters that are involved in mental depression disorders. Much basic research is still needed on the effects of pyridoxine on the antidepressant action of imipramine. The subchronic administration of pyridoxine may be beneficial in the treatment of minor depression.

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