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ORIGINAL ARTICLE

The investigation of antidepressant and anxiolytic effects of pregabalin and its mechanisms of action in rats

Pregabalinin sıçanlarda antidepresan ve anksiyolitik etkileri ve mekanizmalarının incelenmesi

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Summary

Objectives: Pregabalin (PGB) is used in drug-resistant epilepsy. Also, it has analgesic effects in painful syndromes. Depression and anxiety are commonly seen in epilepsy and neuropathic pain patients. PGB is often combined with anxiolytics and antidepressants. We aimed to investigate the antidepressant and anxiolytic effects of PGB and compare its effects with those of antidepressant and anxiolytic drugs and their combined use.

Methods: Wistar Albino rats were used, and PGB (5, 10, 20, and 40 mg/kg), amitriptylin (AMT), fluoxetine (FLX), ketamine (KET), and diazepam (DZM), as well as combinations of PGB (20 mg/kg) with AMT, FLX, KET, and DZM, were administered. Elevated plus maze, forced swimming, and locomotor activity tests were performed.

Results: In the elevated plus maze, PGB10, 20, 40, AMT, FLX, and DZM increased open arm time. The PGB20+FLX combination increased compared to PGB20. In forced swimming, PGB doses increased immobility time. AMT, FLX, DZM, and KET decreased compared to control and PGB doses. Other combinations of PGB20 reversed immobility time, except FLX. In locomotor activity, PGB20, AMT, KET, and DZM decreased distance.

Conclusion: PGB had a depressant effect in all doses and a dose-dependently anxiolytic effect. In combinations of PGB with AMT, KET, and DZM, it reversed their antidepressant effects. We assumed FLX could be preferred instead of AMT in patients using PGB. When PGB is used in combination, drug interactions should be considered. These results are also very remarkable in terms of pharmacoeconomics.

Keywords: Anxiety; depression; drug-interactions; pregabalin; rat.

Özet

Amaç: Pregabalin, ilaca dirençli epilepside kullanılmaktadır. Ayrıca ağrılı sendromlarda analjezik etkisi vardır. Epilepsi ve nöropatik ağrısı olan hastalarda depresyon ve anksiyete yaygın şekilde görülmektedir. Pregabalin, anksiyolitik ve antidepresan ilaçlarla sık sık birlikte kullanılmaktadır. Bu çalışmada, pregabalinin antidepresan ve anksiyolitik etkilerinin araştırılması, bu etkilerinin diğer antidepresan ve anksiyolitik ilaçlarla ve kombine kullanımlarla karşılaştırılması amaçlandı.

Gereç ve Yöntem: Çalışmamızda Wistar Albino sıçanlar kullanıldı ve pregabalin (5, 10, 20, 40 mg/kg), amitriptilin, fluoksetin, ketamin ve diazepam, ayrıca bu ilaçların pregabalin 20 mg/kg ile kombinasyonları uygulandı. Yükseltilmiş artı labirent, zorlu yüzdürme ve lökomotor aktivite testleri yapıldı.

Bulgular: Yükseltilmiş artı labirent testinde pregabalin-10, 20, 40, amitriptilin, fluoksetin ve diazepamın açık kolda kalınan süreyi uzattığı tespit edildi. Pregabalin 20+fluoksetin kombinasyonunun, pregabalin 20'ye kıyasla süreyi uzattığı saptandı. Zorlu yüzdürme testinde pregabalin dozlarının hareketsiz kalma süresini uzattığı belirlendi. Amitriptilin, fluoksetin, diazepam ve ketaminin ise kontrole ve pregabalin dozlarına kıyasla süreyi kısalttığı tespit edildi. Pregabalin 20'nin, fluoksetin dışındaki ilaçlarla birlikte kullanıldığında ise hareketsiz kalma süresinin üzerindeki etkisinin tersine döndüğü görüldü. Lökomotor aktivite testinde, pregabalin 20, amitriptilin, ketamin ve diazepamın mesafeyi azalttığı saptandı.

Sonuç: Pregabalinin tüm dozlarında depresan etkisi ve doza bağlı olarak anksiyolitik etkisi tespit edildi. Pregabalinin; amitriptilin, ketamin ve diazepam ile kombine kullanıldığında bu ilaçların antidepresan etkilerini tersine çevirdiği saptandı. Bu nedenle pregabalin kullanan hastalarda amitriptilin yerine fluoksetinin kullanılabileceğini düşünmekteyiz. Pregabalinin diğer ilaçlarla kombine kullanımı ilaç etkileşimi açısından da değerlendirilmelidir. Bu sonuçlar aynı zamanda farmakoekonomi açısından da dikkate değerdir.

Anahtar sözcükler: Pregabalin; depresyon; anksiyete; ilaç etkileşimleri; sıçan.

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Introduction

Pregabalin (PGB), as a gabapentinoid drug, is used in the treatment of epilepsy and neuropathic pain. It is structurally similar to gabapentine and acts via both the GABAergic system and voltage-dependent potassium channels. It is also a ligand of the α2δ-1 subunit of voltage-dependent Ca^{+2}-channels. PGB decreases depolarization-induced calcium influx and the release of excitatory neurotransmitters by binding to the α2δ-1 subunit of voltage-dependent Ca^{+2}-channels such as GABA, but PGB has a higher affinity for this channel than GABA. PGB reveals its antiepileptic, anxiolytic, and analgesic effects by modulating both GABAergic neurotransmission and calcium influx.[1] It is known that depression and anxiety are seen commonly in patients with epilepsy and neuropathic pain. Depression is the most common psychiatric disorder in the world and generally emerges after environmental, genetic, or hormonal changes. It causes psychological disturbances, loss of behavioral activities and cognitive functions, and somatic findings such as fibromyalgia. These may show an alteration among patients.[2] Anxiety is characterized by disruptive feelings of uncertainty, dread, and fearfulness. Anxiety patients also complain of palpitations, tremors, indigestion, numbness or tingling, nervousness, shortness of breath, diaphoresis, and fear.[3]

The World Federation of Societies of Biological Psychiatry suggests that selective serotonin reuptake inhibitors (SSRIs), serotonin-noradrenaline reuptake inhibitors (SNRIs), and PGB are considered first-line agents for the long-term treatment of generalized anxiety disorder. In patients with generalized anxiety and major depressive disorder, studies comparing PGB with SSRI and SNRIs are lacking.[4] As we mentioned above, the comorbidity of depression and anxiety with epilepsy and neuropathic pain requires new medications to be added to the treatment; therefore, that causes a high burden in terms of pharmacoeconomics.

We aimed to compare the antidepressant and anxiolytic effects of PGB in different doses not only with SSRI and SSNRs, but also with other drugs (amitriptylin [AMT], KET, and diazepam [DZM]) that are known for their antidepressant and anxiolytic effects. Also, we aimed to evaluate the drug interactions when these agents are applied as combinations.

Material and Methods

Animals

Albino Wistar rats (250±25 g) were used in our experiments and obtained from the Medical and Surgical Experimental Research Center of X. The animals were kept in our laboratory in a 12-h light/dark cycle and acclimatized 1 week before the experiments. Seven rats were housed in each cage with food and water ad libitum. All experiments were performed between 09:00 and 13:00. The experimental protocol was approved by the Local Ethical Committee of Eskisehir Osmangazi University for Animal Experimentation (501-1/2016).

Drugs and Treatment Groups

PGB, AMT, DZM, fluoxetine (FLX), and KET were purchased from Sigma-Aldrich (St. Louis, USA). Physiological saline solution (0.9% NaCl) was purchased from Eczacıbaşı (Eczacıbaşı Holding Co., İstanbul, Türkiye). All drugs were dissolved in saline and injected intraperitoneally (i.p.). The drug solutions were freshly prepared before use. The experimental animals were randomly divided into 13 groups, and each group had 7 rats: control (saline), PGB 5, 10, 20, 40 mg/kg, AMT 10 mg/kg, and PGB20+AMT, DZM 5 mg/kg, PGB20+DZM, FLX 5 mg/kg, PGB20+FLX, KET 10 mg/kg, PGB20+KET. Behavioral experiments were performed 1 h after intraperitoneal injection.

Behavioral Experiments

Elevated Plus Maze Test

The elevated plus maze test that is used for assessing the anxiolytic effect was performed as Pellow and friends' described in 1985.[5] The rats were tested on a platform 50 cm above the floor, which has 2 open (50×10 cm), 2 close (50×10×50 cm) arms, and a square (10×10 cm) which binds these 4 arms. The rats were observed for 5 min, and the open arm time was recorded.

Forced Swimming Test

The forced swimming test that is used for assessing the antidepressant effect was performed as Porsolt and friends' described.[6] Rats were swum in an 18-cm-diametered plexiglass cylinder. The cylinder was filled with 15 cm of water in 24±0.5. They were swum in the cylinder for 15 min the day before experiments began. On the experiment day, rats were swum for 5 min, and immobility time was recorded.

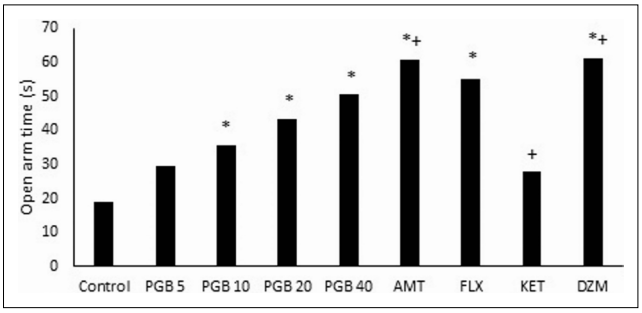

Figure 1. Comparison of open arm time of control, all doses of PGB, and other drug groups in the elevated plus maze test. Data were expressed as medians and percentiles (25–75%).

*P<0.05: compared to control; +P<0.05: compared to PGB20; PGB: Pregabalin; AMT: Amitriptylin; FLX: Fluoxetine; KET: Ketamine; DZM: Diazepam.

Locomotor Activity Test

The automatic-activitymeter system was used (40×40×40 cm plexiglas chamber) (Commat Ltd., Ankara, Türkiye) for assessing locomotor activity. The drugs were administered, and after 60 min, the test was performed for 5 min. The stereotypic movements and distance were recorded.[7]

Statistical Analyses

Statistical analysis was performed using the Statistical Package for the Social Sciences program for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA). Our results did not Show a normal distribution (normality test-Shapiro wilk); therefore, a non-parametric Kruskal–Wallis test was performed. The 0.25–0.75 (Q1-3) percentile and median values of the data were used. A pairwise comparison of single use of PGB and combination use of PGB was evaluated with the Student's t test.

Results

Effects of PGB and its Combinations on Anxiety-Related Behavior in the Elevated Plus Maze Test

PGB 10, 20, 40, AMT, FLX, and DZM significantly increased open arm time in the elevated plus maze test ($p<0.05$), while KET did not change compared to the control ($p>0.05$). Also, KET significantly decreased open arm time compared to PGB20 ($p<0.05$). AMT and DZM groups significantly increased compared to PGB20 (Fig. 1).

All combinations of PGB20 significantly increased open arm time in the elevated plus maze test compared to the control ($p<0.05$). Furthermore, PGB20+FLX significantly increased compared to PGB20 ($p>0.05$) (Fig. 2). The combination of PGB with

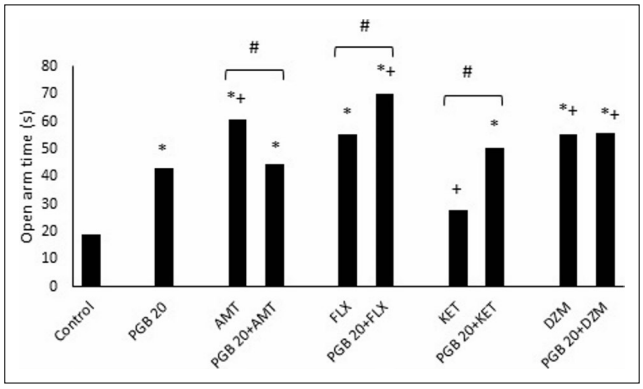

Figure 2. Comparison of open arm times of control, PGB20, and combination groups in the elevated plus maze test. Data were expressed as medians and percentiles (25–75%).

*P<0.05: compared to control; +P<0.05: compared to PGB20; #P<0.05: compared to pairwise comparisons; PGB: Pregabalin; AMT: Amitriptylin; FLX: Fluoxetine; KET: Ketamine; DZM: Diazepam.

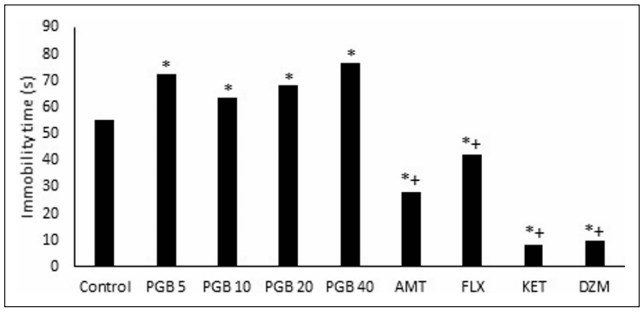

Figure 3. Comparison of immobility time of control, all doses of PGB, and other drug groups in a forced swimming test. Data were expressed as medians and percentiles (25–75%).

*P<0.05: compared to control; +P<0.05: compared to PGB20; PGB: Pregabalin; AMT: Amitriptylin; FLX: Fluoxetine; KET: Ketamine; DZM: Diazepam.

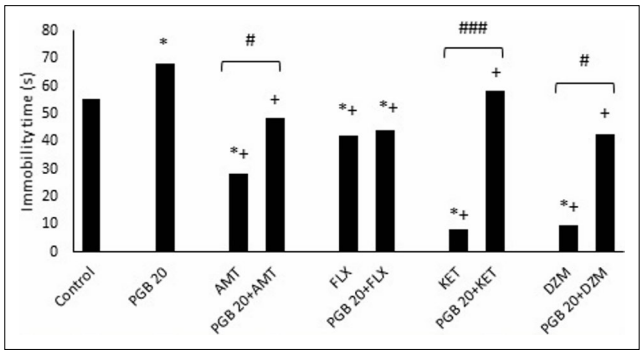

Figure 4. Comparison of immobility time of control, PGB20, and combination groups in a forced swimming test. Data were expressed as medians and percentiles (25–75%).

*P<005: compared to control; +P<0.05 compared to PGB20; #P<0.05: compared to pairwise comparisons; ###P<0.001: compared to pairwise comparisons; PGB: Pregabalin; AMT: Amitriptylin; FLX: Fluoxetine; KET: Ketamine; DZM: Diazepam.

KET increased open arm time compared to the control, while KET did not change the open arm time individually (Fig. 1, 2). The combination with DZM did not show a significant difference in open arm time compared to DZM alone (Fig. 1, 2).

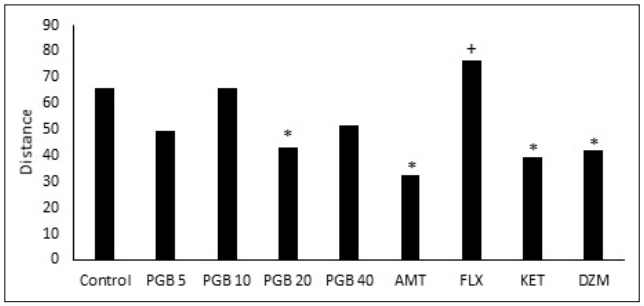

Figure 5. Comparison of distance of control, all doses of PGB, and other drug groups in the locomotor activity test. Data were expressed as medians and percentiles (25–75%).

*P<0.05: compared to control; +P<0.05: compared to PGB20; PGB: Pregabalin; AMT: Amitriptylin; FLX: Fluoxetine; KET: Ketamine; DZM: Diazepam.

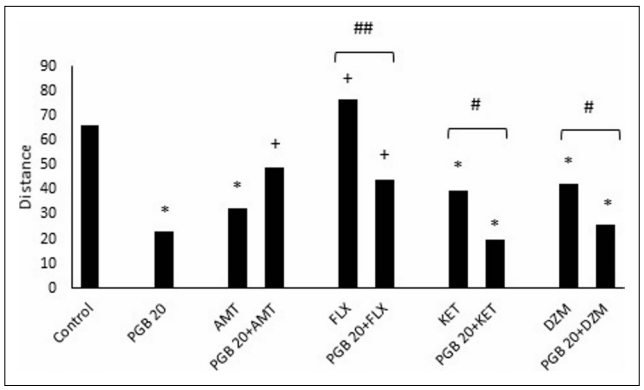

Figure 6. Comparison of the distance of the control, PGB20, and combination groups in the locomotor activity test. Data were expressed as medians and percentiles (25–75%).

*P<0.05: compared to control; +P<0.05: compared to PGB20; #P<0.05: compared to pairwise comparisons; ##P<0.01: compared to pairwise comparisons; PGB: Pregabalin; AMT: Amitriptylin; FLX: Fluoxetine; KET: Ketamine; DZM: Diazepam

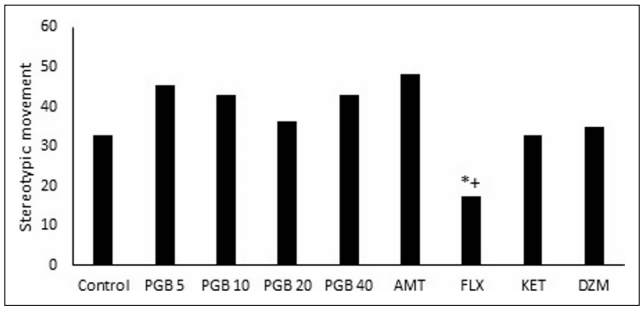

Figure 7. Comparison of stereotypic movement of control, all doses of PGB, and other drug groups in the locomotor activity test. Data were expressed as medians and percentiles (25–75%).

*P<0.05: compared to control; +P<0.05: compared to PGB 0; PGB: Pregabalin; AMT: Amitriptylin; FLX: Fluoxetine; KET: Ketamine; DZM: Diazepam.

Effects of PGB and its Combinations on Depression-Related Behavior in the Forced Swimming Test

All doses of PGB significantly increased immobility time compared to the control, independently of dose (p<0.05). AMT, FLX, DZM, and KET significantly decreased immobility time compared to control and PGB20 (p<0.05) (Fig. 3).

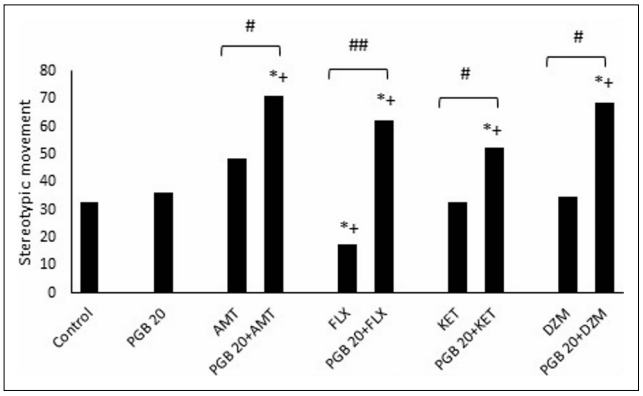

Figure 8. Comparsion of stereotypic movements of control, PGB20, and combination groups in the locomotor activity test. Data were expressed as medians and percentiles (25–75%).

*P<0.05: compared to control; +P<0.05: compared to PGB20; #P<0.05: compared to pairwise comparisons; ##P<0.01: compared to pairwise comparisons; PGB: Pregabalin; AMT: Amitriptylin; FLX: Fluoxetine; KET: Ketamine; DZM: Diazepam.

Combination of FLX, KET, and DZM with PGB20 reversed the depressant effect of PGB20 back to control, while PGB20+FLX did not change immobility time (p>0.05) (Fig. 4).

Effects of PGB and its Combinations in the Locomotor Activity Test

PGB20, AMT, KET, and DZM significantly decreased distance compared to control in the locomotor activity test (p<0.05), while FLX did not change compared to control (p>0.05). But FLX increased compared to PGB20 (p<0.05) (Fig. 5). Otherwise, PGB 5, 10, and 40 did not change distance compared to control (p>0.05).

PGB20, PGB20+KET, and PGB20+DZM significantly decreased distance compared to control (p<0.05), while PGB20+AMT and PGB20+FLX did not change compared to control, but these combinations significantly increased distance compared to PGB20, thus these combinations reversed the effect of PGB20 (p<0.05) (Fig. 6).

Only FLX significantly decreased stereotypic movement compared to both control and PGB20 (p<0.05). The other drugs did not change stereotypic movements individually (p>0.05) (Fig. 7).

All combinations significantly increased stereotypic movement compared to both control and PGB20 (p<0.05) (Fig. 8). The PGB20+FLX combination increased the stereotypic movements compared to FLX.

Discussion

Epilepsy and depressive symptoms are seen together in 80% of epileptic patients. These depressive effects stimulate epileptic symptoms and affect life quality.[8] Similarly, depressive effects are seen in patients with neuropathic pain as well.[9] Furthermore, anxiety is accompanied by these disorders.[2,3]

Some studies reported that PGB, as a gabapentinoid, is more effective than placebo and even a safer option than benzodiazepines for reducing the symptoms of anxiety disorder.[10] The addiction potential of benzodiazepines is also an essential problem. Besides, there are studies reporting that PGB has an effect on schizophrenia-dependent anxiety.[11] Indeed, PGB was recently suggested in the treatment of generalized anxiety disorders by the World Federation of Societies of Biological Psychiatry, but studies comparing PGB with SSRI and SNRIs are lacking.[4] In previous studies, the effects of PGB were contradictory to depression. One of these studies did not support the usefulness of adding PGB in patients with generalized anxiety disorder and unipolar major depression and with an early nonresponse to escitalopram.[12] Another one reported that PGB was not effective in depressive-like symptoms associated with chronic pain but might play an acute antidepressive role given its antinociceptive effect. This acute effect could be caused by acute changes in the seratonergic, dopaminergic, noradrenergic, and GABAergic pathways. According to these results, the acute and chronic effects of PGB on supraspinal systems, which are related to anxiety, pain, and depression pathways, and further explorations are needed to elucidate the implication of anticonvulsants in neuronal circuits related to pain and depressive-like behaviors to find new treatment targets.[9] Based on these findings, we aimed to evaluate the combination of anxiolytic and antidepressant drugs with PGB in terms of drug interactions. Therefore, AMT as a tricyclic antidepressant (TCA), FLX as an SSRI, KET as a dissociative anesthetic, and DZM as a benzodiazepine derivative were chosen for this study. It is known that these drugs have both antidepressant and anxiolytic features,[13] and especially studies on the antidepressant and anxiolytic properties of ketamine (KET) are proceeding.[14] According to our findings, single doses of PGB 5, 10, 20, and 40 mg/kg could have a dose-dependently anxiolytic effect on anxiety. When its effects on depression are evaluated, it could have a dose-independent depressant effect at all doses. PGB has a depressant effect at all doses; therefore, anxiolytic-effected PGB20 was chosen for the combinations. When these drugs were combined with PGB20, we observed that AMT, FLX, and DZM had anxiolytic effects individually, and their combinations with PGB20 also had anxiolytic effects. Although KET did not have an anxiolytic effect individually, its combination with PGB20 was anxiolytic. PGB20 revealed its anxiolytic effect (Fig. 1, 2). The anxiolytic effect did not change when BDZ was used both single and as combination. We assumed that BDZ might have a ceiling effect here. PGB combinations with FLX and KET potentiated anxiolytic effects.

In our study, PGB had a depressant effect at all doses, while AMT, FLX, KET, and DZM had antidepressant effects. However when combined with PGB20, PGB20 neutralized their antidepressant effect (Fig. 3, 4). PGB20 caused a depressant effect when used alone, but the depressant effect decreased to the level of the control group when it was combined with AMT, KET, or DZM (Fig. 4). In this way, AMT, KET, or DZM would contribute to prevent depression in patients using PGB for various reasons. There would not be a difference in the antidepressant effect with FLX or with PGB+FLX. Thus, this would provide convenience in preventing the neuropathic pain or the depression caused by the pain lasting for a long time. In this way, we assumed that FLX would be a better option than AMT as an antidepressant agent for patients using PGB for epilepsy and neuropathic pain in terms of an effective treatment, life quality, and economic burden. Also, the antidepressant effect of KET disappeared when combined with PGB20 (Fig. 3, 4). This combination could be effective in the treatment of anxiety, while it could reduce the effect of the depression treatment.

AMT is a member of TCAs, and the antidepressant effect of it was also observed in the control and PGB groups of our study. AMT tended to have a weaker antidepressant effect than KET and DZM but was more powerful than FLX, but there was no statistically significant difference between these drugs. Combined usage of AMT and PGB decreased the antidepressant effect of AMT (Fig. 3, 4). Experimental studies of AMT presented its anxiolytic effect.[15]

But in some other studies, anxiogenic effects were also observed.[16] In our study, we observed that AMT increased the open arm time in elevated plus maze compared to control, meaning that it had an anxiolytic effect, but its combination with PGB decreased its antidepressant effects, further increasing its anxiolytic effect. The combination of PGB20 with AMT decreased the distance compared to PGB20 (Fig. 5, 6). In all doses of PGB, we did not observe a significant difference in stereotypic movements compared to control (Fig. 7). In our study, we observed that a single-dose injection of AMT did not change stereotypic movements (Fig. 7). A previous study found that the locomotor activity of rats decreased after a single-dose injection of imipramine, which is a member of TCAs.[17] Another study presented that repeated doses of AMT increased locomotor activity and steriotypical effects.[18] In our study, AMT was administered in a single-dose injection, and as the study mentioned above, it decreased distance (Fig. 5). Therefore, its acute injection also did not stimulate dopaminergic release.

FLX is a selective inhibitor of serotonin reuptake. The effects of serotonin on anxiety have been studied by a number of researchers by now, and most of them suggested that increased serotonin levels caused anxiety. In a study based on this hypothesis, rats showed anxiety-like behaviors after the acute FLX injection. Furthermore, they mentioned the contradictory effects of SSRI drugs on different behavioral paradigms.[19] In different studies contrary to this study, it was mentioned that FLX was an anxiolytic drug, but when combined with olanzapine or risperidone, its effects could decrease.[20] We observed in our study that acute FLX injection caused anxiolytic effects in the elevated plus maze test, and when FLX was combined with PGB20, the anxiolytic effect was potentialized (Fig. 1, 2). Therefore, we infer that the combined usage of PGB and FLX would not have a negative effect on the anxiolytic effect of FLX. Also in our study, in the FLX group, the immobility time decreased compared to the control and PGB20, but the effect did not change when they were combined compared to the control. According to a previous study, it was reported that PGB did not inhibit serotonin, dopamine, or noradrenaline re-uptake and was not related to serotonin and dopamine receptors.[21] But FLX acts on the serotonergic pathway. In our study, indeed, PGB did not change the antidepressant effect of FLX because it does not affect these pathways. Most studies are needed to elucidate the underlying possible mechanisms between FLX and PGB. When FLX is evaluated in terms of locomotor activity, it decreases stereotypic movement but increases stereotypic movement compared to both control and PGB20 when combined with PGB20 (Fig. 8). FLX also increased distance (Fig. 5) compared to PGB20. In the combination group, PGB increased the stereotypic activity, which was decreased by FLX.

PGB tends to decrease DA levels in the lateral hippocampus; on the other hand, FLX tends to increase DA levels in the prefrontal cortex. Combining the use of PGB with antidepressant drugs might increase stereotypic movements by increasing DA levels. Thus, this could also induce addiction via DA. In other studies of FLX with 4 different rat strains, locomotor activity decreased in C57BL/6 and 129SvEv rats, increased in DBA/2 rats, and did not change in BALB/c rats. Thus, FLX gives different results according to different subtypes of rats.[22]

KET is a dissociative anesthetic agent; its antidepressant and anxiolytic features have been studied recently. In a previous study, KET 15 mg/kg decreased the immobility time in the forced swimming test while increasing exploratory behaviors and anxiolytic effects in the elevated plus maze test.[23] In our study, the anxiolytic ineffectiveness of KET 10 mg/kg may be caused by its dose or environmental factors such as the season, the type of animal, or the time of the experiment.[24] In a previous KET study and our study, KET 10, 15 mg/kg doses decreased the immobility time without affecting the locomotor activity via the increase of BDNF in the rat hippocampus.[25] The antidepressant effect of KET occurred expeditiously and did not increase the stereotypic movements in our study, (Fig. 7) while distance decreased (Fig. 5).

DZM, as a benzodiazepine derivative, also decreases theta waves in the hippocampus and causes an axiolytic effect.[26] As a hypnosedative drug, addiction and tolerance may occur against DZM treatment, and its anxiolytic effects may decrease in the same doses over time. There are studies supporting this effect.[27] According to these studies, DZM cannot be used in the long term. In another study on DZM, they

observed that diazepam (DZM) increased the levels of intraneuronal metobolite of dopamine "3,4 dihidroxifenlasetic aside" and that increased the neuronal dopamine release. An increase in locomotor activity requires an increase in dopamine levels of dopamine.[28] In our study, the stereotypic movement of DZM was the same as the control. However, the combination of DZM and PGB20 increased the stereotypic movement compared to both control and PGB20 (Fig. 8). Distance decreased compared to control (Fig. 5), but did not change compared to PGB20 (Fig. 6), but stereotypical movement increased in its combination (Fig. 8). This drug affects GABA, so the increase of stereotypic movements and decrease of others are probably occurring via its effects on dopamine. Some existing studies claimed that dopamine decreased the locomotor activity of rats.[29] In a previous study, GABAergic drugs decreased locomotor activities, while a non-GABAergic drug, PGB, did not cause a difference.[30] In our study, DZM, which is a GABAergic drug decreased, distance while PGB20 decreased in the locomotor activity test, contrary to the study above (Fig. 5). The anxiolytic effect of DZM did not show any alteration when it was combined with PGB20. DZM affects the GABA/BDZ/Cl complex, while PGB does not. PGB does not increase GABA levels and does not inhibit the re-uptake.[21] This could be the reason why PGB did not increase the effect of DZM.

Conclusion

Based on our results, drug interactions should be considered when PGBs are used in combinations. This is highly essential in terms of pharmacoeconomics because patients with neuropathic pain and epilepsy treated with SSRI for depression may remain untreated, which could cause an economic burden. PGB was observed as an anxiolytic and depressant drug in our study. As an SSRI drug, FLX should be preferred as an antidepressant drug in the treatment of depression when required.

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References

1. Kaygisiz B, Kilic FS, Senguleroglu N, Baydemir C, Erol K. The antinociceptive effect and mechanisms of action of pregabalin in mice. Pharmacol Rep 2015;67:129–33.[CrossRef]
2. Doğan M. Cognitive therapy approach for depression: Major dimensions and explanations. Anadolu Univ J Soc Sci [Article in Turkish] 2001;1:61–72.
3. Üstün A, Bayar A. An investigation of university student's depression, anxiety and stress levels related to the different variables. J Res Educ Teach 2015;4:384–90.
4. Frampton JE. Pregabalin: A review of its use in adults with generalized anxiety disorder. CNS Drugs 2014;28:835–54.
5. Pellow S, Chopin P, File SE, Briley M. Validation of open: Closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149–67.
6. Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioural despair in rats: A new model sensitive to antidepressant treatments. Eur J Pharmacol 1978;47:379–91. [CrossRef]
7. Gardell LR, Vanover KE, Pounds L, Johnson RW, Barido R, Anderson GT, et al. ACP-103, a 5-hydroxytryptamine 2A receptor inverse agonist, improves the antipsychotic efficacy and side-effect profile of haloperidol and risperidone in experimental models. J Pharmacol Exp Ther 2007;322:862–70. [CrossRef]
8. Miller JM, Kustra RP, Vuong A, Hammer AE, Messenheimer JA. Depressive symptoms in epilepsy: Prevalence, impact, aetiology, biological correlates and effect of treatment with antiepileptic drugs. Drugs 2008;68:1493–509. [CrossRef]
9. Gonzalez-Soler EM, Blasco-Serra A, Alfosea-Cuadrado GM, Igual-Lopez M, Orduna-Valls J, Tornero-Tornero C, et al. Chronic pregabalin treatment ameliorates pain, but not depressive-like behaviors, in a reserpine-induced myalgia model in Rats. Pain Physician 2020;23:E581–90. [CrossRef]
10. Generoso MB, Trevizol AP, Kasper S, Cho HJ, Cordeiro Q, Shiozawa P. Pregabalin for generalized anxiety disorder: An updated systematic review and meta-analysis. Int Clin Psychopharmacol 2017;32:49–55. [CrossRef]
11. Englisch S, Esser A, Enning F, Hohmann S, Schanz H, Zink M. Augmentation with pregabalin in schizophrenia. J Clin Psychopharmacol 2010;30:437–40. [CrossRef]
12. Fountoulakis KN, Karavelas V, Moysidou S, Mavridis D, Pastiadis K, Petalidou N, et al. Efficacy of add-on pregabalin in the treatment of patients with generalized anxiety disorder and unipolar major depression with an early nonresponse to escitalopram: A double-blind placebo-controlled study. Pharmacopsychiatry 2019;52:193–202. [CrossRef]
13. Bandelow B, Michaelis S, Wedekind D. Treatment of anxiety disorders. Dialogues Clin Neurosci 2017;19:93–107. [CrossRef]
14. Kolar D. Addictive potential of novel treatments for refractory depression and anxiety. Neuropsychiatr Dis Treat 2018;14:1513–9. [CrossRef]
15. Lôo H, Malka R, Defrance R, Barrucand D, Benard JY, Niox-Rivière H, et al. Tianeptine and amitriptyline. Controlled double-blind trial in depressed alcoholic patients. Neuropsychobiology 1988;19:79–85. [CrossRef]

16. Enginar N, Hatipoğlu I, Firtina M. Evaluation of the acute effects of amitriptyline and fluoxetine on anxiety using grooming analysis algorithm in rats. Pharmacol Biochem Behav 2008;89:450–5. [CrossRef]
17. Aksöz E, Bilge SS, Kurt M, Kesim Y, Çelik S. The depressant-like effect of sildenafil in the forced swimming test in mice. J Exp Clin Med 2006;23:46–51.
18. Maj J, Wedzony K. Repeated treatment with imipramine or amitriptyline increases the locomotor response of rats to (+)-amphetamine given into the nucleus accumbens. J Pharm Pharmacol 1985;37:362–4. [CrossRef]
19. Silva RC, Brandão ML. Acute and chronic effects of gepirone and fluoxetine in rats tested in the elevated plus-maze: An ethological analysis. Pharmacol Biochem Behav 2000;65:209–16. [CrossRef]
20. Rogóż Z, Skuza G. Anxiolytic-like effects of olanzapine, risperidone and fluoxetine in the elevated plus-maze test in rats. Pharmacol Rep 2011;63:1547–52. [CrossRef]
21. Marks DM, Patkar AA, Masand PS, Pae CU. Does pregabalin have neuropsychotropic effects?: A short perspective. Psychiatry Investig 2009;6:55–8. [CrossRef]
22. Dulawa SC, Holick KA, Gundersen B, Hen R. Effects of chronic fluoxetine in animal models of anxiety and depression. Neuropsychopharmacology 2004;29:1321–30. [CrossRef]
23. Hou L, Qi Y, Sun H, Wang G, Li Q, Wang Y, et al. Applying ketamine to alleviate the PTSD-like effects by regulating the HCN1-related BDNF. Prog Neuropsychopharmacol Biol Psychiatry 2018;86:313–21. [CrossRef]
24. Babar E, Ozgünen T, Melik E, Polat S, Akman H. Effects of ketamine on different types of anxiety/fear and related memory in rats with lesions of the median raphe nucleus. Eur J Pharmacol 2001;431:315–20. [CrossRef]
25. Garcia LS, Comim CM, Valvassori SS, Réus GZ, Barbosa LM, Andreazza AC, et al. Acute administration of ketamine induces antidepressant-like effects in the forced swimming test and increases BDNF levels in the rat hippocampus. Prog Neuropsychopharmacol Biol Psychiatry 2008;32:140–4. [CrossRef]
26. Du Y, Grace AA. Amygdala hyperactivity in MAM model of schizophrenia is normalized by peripubertal diazepam administration. Neuropsychopharmacology 2016;41:2455–62. [CrossRef]
27. Treit D. Evidence that tolerance develops to the anxiolytic effect of diazepam in rats. Pharmacol Biochem Behav 1985;22:383–7. [CrossRef]
28. Rastogi RB, Agarwal RA, Lapierre YD, Singhal RL. Effects of acute diazepam and clobazam on spontaneous locomotor activity and central amine metabolism in rats. Eur J Pharmacol 1977;43:91–8. [CrossRef]
29. Savić MM, Milinković MM, Rallapalli S, Clayton T Sr, Joksimović S, Van Linn M, et al. The differential role of alpha1- and alpha5-containing GABA(A) receptors in mediating diazepam effects on spontaneous locomotor activity and water-maze learning and memory in rats. Int J Neuropsychopharmacol 2009;12:1179–93. [CrossRef]
30. Bortz DM, Grace AA. Medial septum differentially regulates dopamine neuron activity in the rat ventral tegmental area and substantia nigra via distinct pathways. Neuropsychopharmacology 2018;43:2093–100. [CrossRef]