

Effects of non-steroidal anti-inflammatory drugs on anticonvulsant activity of diazepam in mice

Abstract

As a result of the chronic nature of epilepsy, patients often need concurrent therapy for other diseases. The benzodiazepines are frequently prescribed as anxiolytics, sedatives and hypnotics and these agents may interact with other drugs. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used as anti-inflammatory, analgesic and antipyretic agents. They prescribed and used with diazepam in many clinical cases. In this study, convulsions were chemically induced in Albino mice by given picrotoxin (6mg/kg). The animals were divided into several groups as following: picrotoxin, diazepam (1mg/kg), aspirin (10, 100 & 200mg/kg), diclofenac (10 & 20mg/kg) and Celecoxib 20mg/kg. All drugs were given intra peritoneally 30minute before picrotoxin intake. In combination treatment, the NSAIDs were administrated 3minute before diazepam; the latter was given 30minute before picrotoxin. The following parameters were recorded: onset time in minutes, number of seizures (attacks) in each group and death occurrence within 24hours after treatments. Administration of NSAIDs with diazepam showed that aspirin 200mg/kg, diclofenac (in both doses) and celecoxib potentiated the effect of diazepam ($p \leq 0.001$) while aspirin 10mg/kg showed no effect on diazepam but aspirin 100mg/kg reduced the anticonvulsant's effect of diazepam ($p \leq 0.05$). Thus, these findings may suggest that acute use of NSAIDs, of selective COX-2 inhibitors, but not nonselective COX inhibitors potentiate the effect of diazepam's anticonvulsant effect and this interaction could be of pharmacodynamic type.

Keywords: drug interaction, convulsion, mice, diazepam, non-steroidal anti-inflammatory drugs, picrotoxin

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Abbreviations: DZP, diazepam; NSAIDs, non-steroidal anti-inflammatory drugs; COX, cyclo-oxygenase; ASP, aspirin; PTX, picrotoxin; PGs, prostaglandins; PTZ, pentylenetetrazol; CNS, central nervous system; SEM, standard error of the mean

Introduction

Epilepsy is common neurological disease affecting about 1.0% of the population.¹ It is characterized by recurrent seizure disorders.¹⁻³ Usually, there is no recognizable cause, although it often develops after brain damage such as trauma, tumor growth or neurological diseases. These seizures are resulted from neurons that are abnormally electrical discharged.² Benzodiazepines (BDZs) are the most widely used drugs as anxiolytic, sedative, hypnotic and muscle relaxant as well as they have anticonvulsant activity.^{4,5} Diazepam (DZP) is a common drug that belongs to BDZs class with long action and is often preferred in patients who may require treatment for long periods of time. BDZs produce their actions through potentiating (increase affinity of γ -amino butyric acid (GABA), a major inhibitory neurotransmitter in the central nervous system (CNS) that acts by binding to postsynaptic sub-receptors of GABA_A,^{5,6} which leads to hyper-polarization by influx of chloride ions and decrease neuronal membrane potential and excitability.^{4,6} NSAIDs are widely used for anti-inflammatory, analgesic and antipyretic activities.^{7,8} They produce their effects through inhibition of the cyclo-oxygenase enzyme (COX) which is responsible for converting the arachidonic acid into prostaglandins (PGs); proinflammatory mediators.^{7,9} The COX enzyme exists in two isoforms; namely COX-1 is expressed constitutively in most tissues while COX-2 is inducible during inflammatory response.⁹

Epilepsy is chronic in nature and patients often require an additional therapy for underlying diseases, and thus, drug interaction may occur when the effects of one drug are changed by the prior or concomitant exposure to another drug. Unexpected effect and alterations in pharmacological actions are common risks of drug interactions that may lead, sometimes, to hospital admission.^{10,11} Drug interaction is categorized into pharmacodynamic interactions; in which the response of a drug is changed by another that are taken in the same time. Pharmacokinetic interactions: delivery rate of one drug into its site of action is changed by another which is taken in the same time.^{12,13} NSAIDs as aspirin (ASP) is prescribed and may be used with DZP in several cases as, headache, low back pain and sometimes is taken by patients who suffer from epilepsy.¹⁴

In our previous study,¹⁵ the findings indicated that NSAIDs (COX-1 inhibitors) reduce the hypnotic effect of DZP without a change in the anxiolytic, sedative and muscle relaxant actions of DZP. Furthermore, other studies have indicated that ASP and COX-2 inhibitors potentiate the anticonvulsant action of DZP in pentylenetetrazol (PTZ) treated animals.^{16,17} Thus, the aim of the present study is to further investigate the effects of NSAIDs on the DZP anticonvulsant activity and which one of COX inhibitor is responsible for such an interaction.

Materials and methods

Materials

Chemicals: DZP was obtained from Roche (Sous-Bois, France), diclofenac from Hemofarm (Vrsac, Serbia), ASP from Sanofi-Synthelabo (Paris, France), celecoxib from Pfizer, picotoxin (PTX) from

Colebrook Bucks (England) and normal saline was obtained from Haidylena (Cairo, Egypt).

Animals: Male Albino mice with an average body weight of 25gm were obtained from local animal house of Faculty of Pharmacy, University of Tripoli, Tripoli, Libya. The animals were housed in social groups at room temperature of 22-25°C with 12hours light/dark cycles. Standard animal food and water were freely access to all mice. All the chemicals were injected intra peritoneally (I.P) in a volume of 10ml/kg.¹⁵ The experiments were carried out at Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, University of Tripoli. Ethical approval for all the experiments and animal use was obtained from local ethics committee of the University of Tripoli (2012).

Methods

Effect of different doses of aspirin on diazepam's anticonvulsant activity: Seizures were induced in mice by injecting PTX at a dose of 6mg/kg that was chosen upon pilot experiments and previous study reported by Amabeoku, et al.¹⁸ The mice were divided into six groups (n=10).

- Group I is given only normal saline and served as control group
- Groups II & III were given ASP in two doses of 10 and 100mg/kg, respectively
- Group IV was given only DZP in a dose of 1mg/kg, based upon pilot study (data not shown) and this is in line with previous study¹⁸ to serve as positive control. All the groups were given PTX 30minute after treatments. To study the possibility of interaction between ASP and DZP's anticonvulsant activity
- Groups V & VI were treated with ASP 10 & 100mg/kg, respectively, then both groups were received DZP and PTX. The time intervals between the treatments were taken to be 30minute.¹⁵

Immediately after PTX administration, the mice were individually placed under the glass funnels and observed for 90 minute for the following parameters: onset time of seizures in minutes, episodes number of hind-limb extension/convulsions and occurrence of death.^{19,20} The anticonvulsant activity for these drugs were manifested by prolonging the onset time of seizures or decreasing the number of hind-limb extensions^{19,22} and protection against death. The mortality rate was observed for 24hours after injecting of PTX.²³

The same experiment was carried out with ASP 200mg/kg. Thus, four groups were used (n=8). The first three groups were administrated PTX, 30minute after normal saline, DZP and ASP intake, respectively. The last group was received ASP, DZP and PTX with time intervals of 30minute between all the treatments and observed for the above mentioned parameters.

Effect of diclofenac on diazepam's anticonvulsant activity: A similar design of experiment was carried out as mentioned above for ASP 200mg/kg. In this experiment, diclofenac was used in two different doses (10 & 20mg/kg) and six groups of mice were used for this drug (n=8).

Effect of celecoxib on diazepam's anticonvulsant activity: The same experiment as described with ASP 200mg/kg was repeated using celecoxib 20mg/kg (n=8).¹⁵

Statistical analysis: Data were expressed as mean±standard error of

the mean (SEM). All data were tested for normal distribution by using Kolmogrov-Smirnov test for normality. Then, subjected to Analysis of Variance (one way-ANOVA) followed by post hoc LSD test for multiple comparisons. The Student-t test was used when necessary and * $p\leq 0.05$ was considered statistically significant, ** $p\leq 0.01$ highly significant and *** $p\leq 0.001$ very highly significant different. This was carried out by using Statistical Package for Social Sciences (SPSS Inc, version 17 Chicago, USA).

Results

In Table 1, administration of 6mg/kg of PTX to mice was found to induce convulsive behavior followed by death of 90% of the animals. However, pre-administration of ASP in a dose of 10mg/kg showed about 45% delay in the onset time of episodes and a decrease in the number of episodes and death occurrence by 40% and 50%, respectively. However, with no statistical significant differences were revealed by ANOVA. A higher dose of ASP (100mg/kg) did not produce any significant change in PTX action. The anticonvulsant behavior of DZP is significantly prolonged onset time to episodes by seven-folds ($p\leq 0.001$) and decreased the episodes number by 75% against the PTX treated group. However, co-administration of low dose of ASP (10mg/kg) with DZP was found to be highly significant increased the onset time ($p\leq 0.01$), decreased the episodes number ($p\leq 0.05$) and reduced the mortality rate to 10% against PTX treated group. Furthermore, co-administration of ASP 100mg/kg plus DZP was also found to significantly prolonged the onset time ($p\leq 0.05$) against the PXT treated groups and decreased onset time ($p\leq 0.05$) against DZP treated group. However, significantly increased the number the episodes ($p\leq 0.05$) against the DZP treated groups and reduced the mortality rate to 10%. On the other hand, in order to investigate the time dependant effect, pretreatment of ASP in 10mg/kg at 120 minute showed no effect on onset time and number of episodes against PTX treated group with only reduction of the mortality rate to 50% (data not shown).

In Table 2, pretreatment with ASP at a dose of 200mg/kg significantly decreased the onset time ($p\leq 0.001$) as compared to the DZP plus PTX treated group, also decreased the number of episodes ($p\leq 0.001$) and the mortality rate to 62.5% in comparison with the PTX treated group. However, co-administration of ASP (200mg/kg) and DZP markedly increased onset time to episodes of convulsion ($p\leq 0.001$) against the PTX, DZP plus PTX and ASP (200mg/kg) plus PTX treated groups. For number of episodes, the combined treatment decreased the episodes against the PTX and ASP (200mg/kg) plus PTX treated groups ($p\leq 0.001$ & $p\leq 0.05$), respectively; and completely inhibited the mortality.

In Table 3, pre-administration of diclofenac was found to reduce onset time in comparison with the DZP treated group ($p\leq 0.001$) and increased number of episodes as compared to the DZP treated group ($p\leq 0.01$ & $p\leq 0.05$) for diclofenac of 10 and 20mg/kg, respectively, and reduced the mortality rate to 25%. In addition, the combined treatment was significantly prolonged onset time ($p\leq 0.001$) against the groups of PTX, DZP plus PTX and diclofenac plus PTX and significantly decreased number of episodes ($p\leq 0.001$ & $p\leq 0.01$) as compared to the PTX and diclofenac plus PTX both of 10 and 20mg/kg treated groups, respectively; with a complete inhibition of death.

In Table 4, celecoxib treatment was found to highly significant reduced the onset time ($p\leq 0.01$) against the DZP treated group and significantly reduced the episode attacks against the PXT treated group

($p \leq 0.05$). As well as significantly increased the episodes against the DZP treated group ($p \leq 0.001$) and reduced the mortality rate to about 40%. Combined treatment of celecoxib and DZP was found to highly

increase the onset time ($p \leq 0.001$) against the PTX and celecoxib plus PTX treated groups and reduce the number of episodes ($p \leq 0.001$) against the same groups with complete protection against death.

Table 1 Effect of Aspirin intake on anticonvulsant activity of Diazepam

Treatment (groups)	Onset time(minute)	Episodes(number)	Death(%)
Picrotoxin(control)	8.70±0.58	8:20±1.23	90
Aspirin 10+Picrotoxin	12.56±1.70	5.00±1.38	50
Aspirin 100+Picrotoxin	9.10±0.79	8.20±1.25	100
Diazepam	57.90±13.1***A''B''	2.00±1.01***B''	10
Aspirin 10+Diazepam+Picrotoxin	35.80±9.57*AB	4.40±1.30*B	10
Aspirin 100+Diazepam+Picrotoxin	31.90±9.74*BC	6.82±1.39C	10

Data presented as Mean±SEM, n=10, ANOVA followed by LSD test were used. *Significant difference against the control group, $p \leq 0.05$ and ***Significant difference against the control group, $p \leq 0.001$. A Significant different against aspirin 10+picrotoxin group, $p \leq 0.05$ and A'' Significant difference against aspirin 10+picrotoxin group, $p \leq 0.001$. B Significant difference against aspirin 100+picrotoxin group, $p \leq 0.05$. B'' Significant difference against aspirin 100+picrotoxin group, $p \leq 0.001$. C Significant difference against aspirin DZP group, $p \leq 0.05$

Table 2 Effect of aspirin 200 mg/kg intake on anticonvulsant activity of diazepam

Treatment (groups)	Onset time (minute)	Episodes (number)	Death (%)
Picrotoxin (control)	8.13±0.89	7.88±1.32	87.5
Diazepam+picrotoxin	53.88±13.73***	1.75±0.88***	12.5
Aspirin 200+picrotoxin	15.00±1.88*** C''	3.38±0.49***	62.5
Aspirin 200+diazepam+picrotoxin	90.0±0.00*** C'' B''	0.00±0.00*** B	0

Data presented as Mean±SEM, n=10, ANOVA followed by LSD test were used. ***Significant difference against the control group, $p \leq 0.001$. B Significant difference against aspirin 200+picrotoxin group, $p \leq 0.05$. B'' Significant different against aspirin 200+picrotoxin group, $p \leq 0.001$. C'' Significant difference against diazepam+picrotoxin group, $p \leq 0.001$

Table 3 Effect of Diclofenac intake on anticonvulsant activity of Diazepam

Treatment (groups)	Onset time(minute)	Episodes(number)	Death(%)
Picrotoxin (control)	11.32±1.38	4.16±0.74	37.5
Diazepam+Picrotoxin	57.69±14.05***	1.15±0.49**	0
Diclofenac-10+Picrotoxin	14.38±1.96 C''	3.29±0.47C	25
Diclofenac-20+Picrotoxin	14.00±1.46 C''	3.33±1.23C	25
Diclofenac-10+Diazepam+Picrotoxin	90.00±0.00*** C'' B''	0.00±0.00*** C''B''	0
Diclofenac-20+Diazepam+Picrotoxin	90.00±0.00*** C''B''	0.00±0.00**** C'' B''	0

Data presented as Mean±SEM, N=8 in each group, ANOVA followed by LSD test were used. *Significant difference against the control group, $p \leq 0.05$. **Significant difference against the control group, $p \leq 0.01$. *** Significant difference against the control group, $p \leq 0.001$. B'' Significant difference against diclofenac+picrotoxin, $p \leq 0.001$. C Significant difference against DZP+Picrotoxin, $p \leq 0.05$. C'' Significance different against DZP+Picrotoxin, $p \leq 0.001$

Table 4 Effect of Celecoxib intake on Diazepam anticonvulsant activity

Treatment (groups)	Onset time (minute)	Episodes (number)	Death (%)
Picrotoxin (control)	8.50±0.34	10.17±1.42	100
Diazepam	52.57±14.10**	2.38±1.00***	0
Celecoxib	14.00±1.45 C'	6.75±1.21* C'	37.5
Celecoxib+Diazepam	76.00±9.17*** B''	0.63±0.42*** B''	0

Data presented as Mean±SEM, N=8 in each group, ANOVA test followed by LSD test were used. *Significant difference against the control group, $p \leq 0.05$. **Significant difference against the control group, $p \leq 0.01$.

***Significant different against the control group, $p \leq 0.001$. B'' Significant difference against Celecoxib+picrotoxin, $p \leq 0.001$. C' Significant difference against DZP+Picrotoxin, $p \leq 0.01$

Discussion

Previously, it has been reported that NSAIDs (non-selective COX and COX-1 selective) interact (reduced) with DZP hypnotic activity without effect on the other actions of DZP.¹⁵ In this study, further experimental investigations on the anti-convulsive behavior of DZP were carried out. Thus, picrotoxin is well-known acute seizure model used to evaluate the anti-epileptic drug's activity.²⁴ It produces convulsion via non-competitive antagonism of GABA_A receptor by blocking the chloride ion channels that linked to these receptors in the brain^{12,25} (see also introduction section). Thus, pretreatment of mice with DZP prolonged onset to episodes and decreased number of episodes against the PTX treated group and reduced the percentage rate of death. This is attributed to that DZP blocks PTX induced convulsion through potentiation of GABA neurotransmission activity in CNS.²⁴

On the other hand, inflammatory process plays a crucial role in some diseases such as epilepsy^{7,14} where there is up-regulation of COX enzyme following seizure activity particularly the COX-2 enzyme^{12,26} as COX-2 is highly found in the cerebral cortex and hippocampus; the areas which have a significant role in onset of seizures activity.^{7,16,26} It is also documented that PGs are found in the brain¹⁶ and their levels are elevated through their release from glial cells following seizure induction in animal models.^{27,28} Several studies focused on the role of PGs and NSAIDs that they play in epilepsy. There are some contradictory findings on the role of COX and PGs in epilepsy. While some NSAIDs like ASP lower the convulsive threshold,¹⁶ there are also others like diclofenac increase the latency to onset of PTZ induced seizures in mice.^{29,30}

It should be mentioned that picrotoxin increases the levels of PGs; PGF2 α and PGE2^{31,32} and PGE2 has excitatory effect on the cerebral cortex; the area which has a significant role in onset of seizures activity.^{16,33} It is reported that PGE2 is the product of COX-2,^{16,34} the latter which is rapidly induced and expressed by convulsion.³³ This is explained why celecoxib reduced the episodes because it is selective COX-2 inhibitor, while ASP at 10mg per kg (selective COX-1 inhibitor), ASP at 100mg per kg and diclofenac (non-selective COX inhibitors) had no effect.¹⁵ Thus, PGE2 is not inhibited specially these non-selective COX inhibitors are more potent toward COX-1 enzyme.¹⁶ PGE2 may also enhance the release of glutamate from the nerve terminals and astrocytes which is an excitatory neurotransmitter and leads to decrease the brain GABA levels that results in convulsion.⁷ Aspirin (200mg/kg) reduced the episodes, this might be in high dose, there is a lose for selectivity and inhibits COX-2 in the same potency as COX-1 and may act through GABAergic neurons that enhance the expression of GABA_A protein receptors.⁷ Indeed, this is in line with other studies that used diclofenac, ASP in low and high doses and celecoxib by using PTZ model.^{7,17,35,36}

In the present study, combination treatment of NSAIDs with DZP showed various results. Thus, ASP at 10mg per kg had no effect on DZP while ASP at 100mg per kg significantly reduced the DZP's effect. Pretreatment with ASP 200mg per kg, diclofenac and celecoxib potentiated the DZP's anticonvulsant effect. These findings could be attributed to the fact that ASP 10mg per kg is in low dose (COX-1 selective inhibitor) and ASP 100mg per kg is more likely selective inhibitor towards COX-1;³⁷ the latter which does not play that much role in epilepsy. The potentiation effect of DZP with ASP 200mg per kg, diclofenac and celecoxib is related to that these drugs affect COX-

2 such as celecoxib and diclofenac; the latter which some studies consider it more COX-2 selective³⁸ and ASP in high dose affects both COX enzymes. Inhibition of COX-2 is known to reduce convulsion activity.^{16,37} It is known that GABA receptors have several subunits and the main one is α subunit that has different isoforms ($\alpha 1-\alpha 5$). Each isoform has a specific function; $\alpha 1$ mediates sedative effect and partly anticonvulsant activity, $\alpha 2$ anxiolytic effect and $\alpha 3$ anxiolytic and muscle relaxant effects.⁵ Thus, our previous study indicated that COX-1 selective NSAIDs significantly reduced the hypnotic effect of DZP with no effect on other actions of DZP¹⁵ while the present study indicated that most NSAIDs used in this study potentiated the anticonvulsant effect of DZP.

Interestingly, it should be mentioned that other NSAIDs such as piroxicam, meloxicam, tenoxicam or even others may produce similar anticonvulsant like-behavior as ASP, diclofenac or celecoxib when combined with DZP. This could be due to the involvement of PGs in convulsion as a result of conversion of arachidonic acid to PGs. Thus, the present finding is in line with our previous study about interaction of NSAIDs and DZP's that related to the different pharmacological actions of DZP and different COX isoforms are involved for such interaction and still inconsistency if sleep and convulsion mediate through the same or different (α) GABA subunits or COX has different effects on different (α) GABA subunits. Thus, taken these findings together in our considerations, a possibility of drug interactions activity in humans whenever ASP is used with DZP cannot be excluded.

Conclusion

This study may suggest that acute use of NSAIDs of COX-2 inhibitors but not nonselective COX inhibitors potentiated the effect of diazepam's anticonvulsant effect and this interaction is more likely to be of pharmacodynamic type.

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None.

Conflict of interest

Author declares that there is no conflict of interest.

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