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INHIBITION OF SELECTIVE AND NON-SELECTIVE SICLOOXYIGENASE ON ANSIOLITIC EFFECTS INDUCED DIAZEPAM IN MICE

Doni Anshar Nuari [1*], Cindra Tri Yuniar [2], Ahmad Jaidi[1], Siva Hamdani[1], Genialita Fadhila[1]

[1]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Garut, Jl. Jati No. 42B, Tarogong Kaler, Garut, West Java, 44151, Indonesia
[2] School of Pharmacy, Bandung Institute of Technology
Jl. Ganesa No.10, Lb. Siliwangi, Coblong, Bandung, West Java, 40132, Indonesia

*Corresponding author: Doni Anshar Nuari (doni@uniga.ac.id)

ARTICLE HISTORY

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Abstract
Stress is the source of many sociological, medical, and economic problems. Moreover, stresses are known as the etiology of several diseases. Prostaglandins and all four receptors affect the brain, even thought to affect behavior. Hence, the inactivation of cyclooxygenase (COX) causes a decrease in levels of prostaglandins that contribute to stress development, thus decreasing the anxiolytic effect of diazepam. This study aims to see the effect of selective and non-selective COX inhibitors decreasing the Anxiolytic effect of diazepam using the EPM (Elevated Plus Maze) method in male white mice; animals were grouped to use Tragakan 2%, Diazepam 0.065 mg/kg BB and Tragakan 2% after an hour, Diazepam 0.065 mg/kg BB and then an hour later gives Ketoprofen 0.65 mg/kg BB for non-selective COX Inhibitor effect group, Diazepam 0.065 mg/kg BW, and then an hour later gives Celecoxib 0.65 mg/kg BB for group use of selective Cox-2 Inhibitor, test parameter in this study is the duration in open arm. Results showing decreased duration on open arm group has given diazepam combination ketoprofen or celecoxib are different P value <0.05 than diazepam only. Decline duration was highest shown by animals given celecoxib, so that could be stated gift selective COX-2 inhibitors bring down the effect of anxiolytic diazepam bigger.

Key words: anxiolytic, celecoxib, cox inhibitors, diazepam, elevated plus maze, ketoprofen

Introduction

Stress is a physiological response, a psychological person trying to adapt to and regulate internal and external pressure.[1] One of the drugs used to reduce anxiety is diazepam, a benzodiazepine group with a working mechanism that occupies GABA receptors to produce inhibitory effects that ultimately provide anxiolytic effects.[2] Previous

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research has shown that prostaglandins and the four receptors greatly influence behaviour.[3] Research conducted by Nuriyama shows that prostaglandin can affect behaviour and is reinforced by Matsuoka, which states that rats that eliminated EP receptors experience inhibition of social behaviour and increased impulsive behaviour.[4] The use of COX inhibitors can reduce the production of prostaglandin.[5]

Prostaglandin influences dopaminergic inhibition due to an increase in GABAergic activity, so inhibition of prostaglandin can increase dopamine flow with its receptors.[4] The present study examined the effect of inhibition of cyclooxygenase on stress development by using drugs that inhibit selective COX and selective COX2 against arachidonic acid. This test uses Ketoprofen and Celecoxib by observing the effects of anxiety from diazepam on mice using the EPM (Elevated Plus Maze). In addition, this study aimed to determine the effect of non-selective NSAIDs and selective COX COX2 against anxiolytic effects of diazepam tested by using EPM (Elevated Plus Maze) in male mice (*Mus musculus*).

Methods

Tool
Mouse cages, Elevated Plus Maze (EPM), animal scales, syringe, stopwatch, camera.

Material
Tragacant, Diazepam, Ketoprofen, Celecoxib

Animal
Male white mice of the Swiss-Webster strain were obtained from the Center of link University Institute Teknologi Bandung, weighing approximately 20-30 grams.

Procedure
Preparations for the test include preparing the suspension test and experimental animals.

Preparation of test suspension
The dose of diazepam used was 0.65mg/KgBW, as much as 6.5mg Diazepam was suspended in 100ml of tragacanth 2%. For ketoprofen and celecoxib, the dosage used was 6,5mg/kg BW, and as much as 65mg of ketoprofen and celecoxib were suspended in 100ml tragacanth 2%.

Preparation of Experimental Animals
The enforcement is conducted two days before the testing is carried out in the Elevated Plus Maze (EPM). Every mouse that is tamed and held on position is given orally. The treatment was carried out repeatedly until the mice were shown to show a reduction in the indication of stress. Adjustment is made one hour before testing.

Observation of Changing behavior with the EPM
The testing session consists of placing the animal in the equipment for five minutes and recording the following behavior: the total time spent in the open arm and the total time spent in the closed arm. Every after test, the labyrinth is cleaned with 70% ethanol after each experiment because it avoids or eliminates the bias from the smell of test animals. It is thought that the mice's reluctance to explore the maze's open arms is caused by fear of open and elevated space.

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All Mice were given diazepam orally except the regular group. After an hour, the ordinary and control group only received Tragacanth substance 2 %, and for the testing group, mice were given ketoprofen and celecoxib an hour after being given diazepam. Test with EPM and collect data as the duration in the open arm.

Result

Anxiolytic effects were observed in the regular and control groups to see the anxiolytic effect. In the comparison group to the control group, see the anxiolytic effect of the given preparation.

Table 1. Average time in Open Arm for each group treated

Treatment	**Average *Time in Open Arm* (seconds)**
Tragacanth 2%	2.25 ± 2.06
Diazepam 0.065mg / kg + Tragacanth 2%	265.50 ± 25.74 [a]
Diazepam 0.065mg / kg + Ketoprofen 0.65mg / kg	168.75 ± 41.24 [ab]
Diazepam 0.065mg / kg + Celecoxib 0.65mg / kg	118.25 ± 29.14 [ab]

Ket: a = Significant difference with Tragacanth group 2% (normal group) ($p < 0.05$)

b = Significant difference with the diazepam group. (control group) ($p < 0.05$)

Table 2. Percentage of time in open arms compared to the total duration of observation

Treatment	***Percentage***
Tragacanth 2%	0.78
Diazepam 0.065mg / kg + Tragacanth 2%	88.55
Diazepam 0.065mg / kg + Ketoprofen 0.65mg / kg	57.84
Diazepam 0.065mg / kg + Celecoxib 0.65mg / kg	38.61

Discussion

The effect of inhibition of cyclooxygenase was studied by administering drug lines which had the effect of selective and non-selective inhibition of COX. Ketoprofen was used as a non-selective inhibitor and celecoxib as a selective inhibitor to obtain a clear picture of the role of the arachidonic acid pathway in the anxiolytic effect of diazepam. The 16 animals were divided into four groups; each consisted of 4 mice. The groups were as follows: Group 1 was given Tragacanth 2%, Group 2 was given Diazepam 0.065 mg/kg BW, group 3 was given Ketoprofen 0.65 mg/kg BW, which one hour before diazepam was given 0.065 mg/kg BW, group 4 given Celecoxib 0.65 mg/Kg BW one hour before Diazepam 0.065 mg/kg BW was given. Before testing, the test animal fasted for 8 hours. Adaptation is made one hour before testing in Elevated Plus Maze (EPM). Adaptation is carried out so that the test animal can adjust both the behaviour adjustments to the condition and the state of the environment.

Table 1. show that diazepam increases time in open arms. The results showed a significant difference ($p < 0.05$) between the control group and the comparison group was given diazepam. It shows that diazepam has an anxiolytic effect which is indicated by

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the increase in the duration of the EPM open arm compared to tragacanth 2%. Diazepam increases the effect of GABA without directly activating the GABA receptor or opening the associated chloride channel; with benzodiazepine interactions, the GABA affinity for the receptor will increase.[5] Giving Ketoprofen and Celecoxib at a dose of 6.5 mg/kg BW significantly ($p < 0.05$) showed a decrease in the average time on the open arm compared to the diazepam group. This is thought to occur because of an increase in arachidonic acid levels due to unchanging prostaglandins in which arachidonic acid can inhibit the transmission of GABA neurotransmitters which ultimately results in an excitation effect of.[4,6]

In table 2. the results showed a decrease in the duration of open arms between the group given diazepam and the group given diazepam with ketoprofen or with celecoxib, where the highest percentage reduction was indicated by the group given diazepam with celecoxib; this could indicate that cox-2 selective inhibitors were given more influential in decreasing the anxiolytic effect of diazepam compared to non-selective, in contrast to a study conducted by Gambel which stated that the use of COX-2 inhibitors could be used to treat depression due to psychiatric problems by increasing the time in open arms. However, only using selective inhibitors cox-2 alone while in this study, diazepam was used, which seems to be suppressing the effects of the antidepressant effect of COX-2 on the anxiolytic effect of diazepam; this is related to the inhibition of prostaglandins and an increase in arachidonic acid which produces an excitation effect and increases the dopaminergic effect.[2,4,6,7]

Conclusion

In this study, the anxiolytic effect of 0, 65mg/kg BW Diazepam was significantly inhibited by Ketoprofen 6,5 mg/kg and celecoxib 6, 5 mg/kg. In addition, the results showed that selective COX2 inactivation had a more significant effect reduction than selective COX inactivation.

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