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Research Article

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Evaluation of the Effect of Benzodiazepines on Cognitive Status in Rats

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ABSTRACT

Benzodiazepines are drugs that are widely used in the treatment of certain emotional disorders. They are the most commonly prescribed psychotropic medications in the treatment of anxiety and sleep disorders. They are effective, tolerated and have few side effects. However, they can lead to addiction and dependence, an alteration of the state of consciousness with behavioural and memory disorder. The aim of this work is to evaluate the relation between the duration of treatment with benzodiazepines and cognitive status in rats. The present study examines the impact of diazepam at a dose of 1 mg/kg on cognition in Wistar rats in period of 3 months using the object recognition test, while the results showed a significant decrease in the percentage recognition of the new object for the treated group during the 12-week period compared to the control group. We conclude that chronic treatment with benzodiazepines is one of the leading causes of cognitive decline. Further study will be conducted in the same direction to determine the mechanism that induces this undesirable effect.

Keywords: Benzodiazepines; Rat; Cognition

INTRODUCTION

Dementia is a disorder of memory and ideation, sufficiently important and prolonged to affect the daily tasks, characterized by alterations in certain cerebral functions, including memory [1]. Age-related diseases include dementia, which is one of the causes of disability and loss of autonomy among the elderly in the world. Alzheimer's disease is the most common form of dementia, and although more than a century ago separates us from its first description, the current level of knowledge about its etiopathogenesis is still limited [2]. However, several substances have been suspected of inducing or influencing the dementia trajectory. Epidemiological studies have suggested a link between benzodiazepine (BZD) consumption and the risk of dementia [3].

BZDs are the most commonly prescribed psychotropic medications in the treatment of anxiety and sleep disorders. They are effective, tolerated and have few side effects. However, they can lead to addiction and dependence, a syndrome associating, to varying degrees, an alteration of the state of consciousness with behavioural and memory disorder [4].

In 2012, a research group/team demonstrated that there is an increased risk (50%) of developing dementia in people over the age of 65 and consuming benzodiazepines. While another study has shown that the use of benzodiazepines over a period of three months or more is associated with an average increase in the risk of developing Alzheimer's disease [5]. Some results contradict each other about the duration of treatment if it affects the cognitive status of the person [6,7]. Our objective is the evaluation of the effect of benzodiazepines on the cognitive status of rats.

MATERIALS AND METHODS

Animals

Twenty-Four Wistar rats (Separated 12 males and 12 females) in the weight range of 190-210 g were used in the study. The animals were belonged to the Department of Pharmacology of the Faculty of Medicine and Pharmacy of Casablanca, Morocco. They were acclimatized at 22°C +/-1 and 50% +/-1 humidity, day night cycle. The animals were fed with standard diet every alternate day and water ad libitum.

All procedures were in strict accordance with the guidelines established by ethics committee for biomedical research of Casablanca.

Treatment

The animals were divided as per their body weight in to three groups (n=8):

- Group 1: Control group
- Group 2: Daizepam 1 mg/kg, p.o. for 6 weeks
- Group 3: Diazepam 1 mg/kg, p.o. for 12 weeks

The experiment was carried out at 8:00 am in an experimental room adjacent to the animal facility the rats were transported before the experiments. The experiments were blinded by using cameras and filters to prevent noise interference. Each rat was handled carefully during handling sessions in order to accustom it to the experimenter for 7 days before starting of the treatment. The animals were weighed daily at the same time.

Object Recognition Test

It is a test that evaluates the memory of discrimination in rodents, based on their curiosities to discover all that is new, so the animal that presents no deficit will be attracted to the new object without any interest to the familiar object. Three groups of twenty-four male and female rats were used in the object recognition test

The device is a square white wooden enclosure illuminated by an 80 W lamp suspended 60 cm from the ground surface. The open area (100 × 100 cm) is delimited by walls 40 cm high to prevent the animal from escaping.

The test takes place in three phases:

- The habituation phase: On the first day, the animal is placed in the center of the device to freely explore the open surface without the presence of objects for 10 minutes. This step is to familiarize the rat with the device so that it is more interested by the objects than the device in the next phases.
- The acquisition phase: On the second day, the animal is again placed in the enclosure with two identical objects for 3 minutes. During this phase, the number of initiatives to explore objects is measured, and clean by ethanol between sessions.
- The test phase: on last day, the animals return to the apparatus for 3 minutes. This time, one of the two objects of the previous phase is exchanged by a new object. The parameters measured during this step are: the time spent exploring the new object and the percentage of recognition [(Time spent exploring the new object/total time) × 100].

Statistical Analysis

Statistical analysis was performed using SPSS 20, one way ANOVA followed by paired comparisons to examine condition differences, Differences were considered significant when $P<0.05$.

RESULTS

Body Weight

We noticed throughout the experimental period a slight increase in weight for the three groups whereas this increase is not significant (F (3.10)=0.8, p>0.05) (Figure 1).

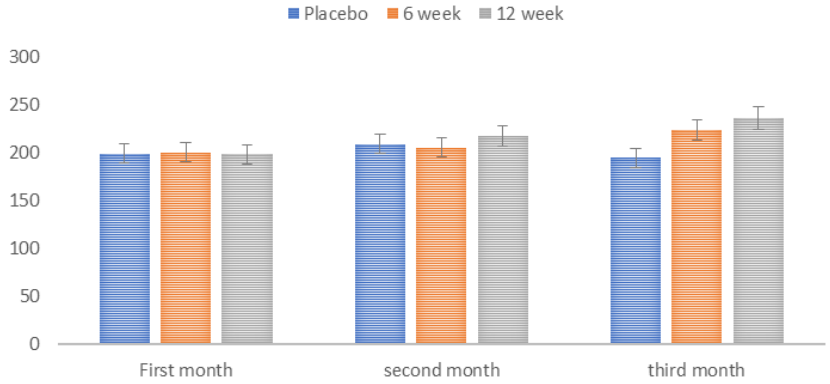

Figure 1: The changes in body weight in rats (Mean ± SD)

Object Recognition Test

During the acquisition phase, the difference between the initiatives to discover the objects was not significant between the three groups (F (20.97)=3.54) (Figure 2).

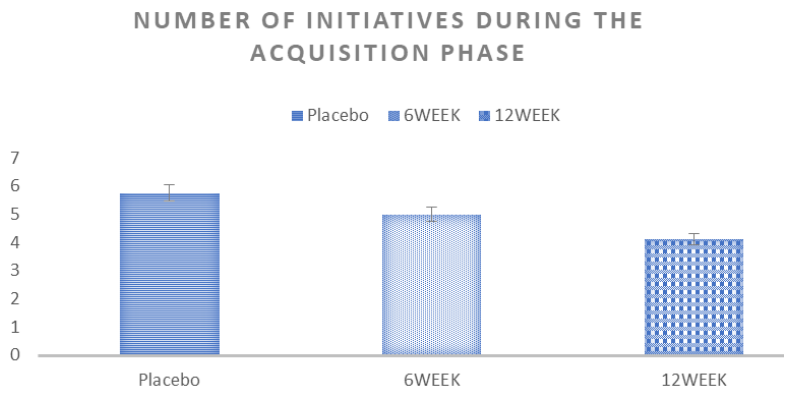

Figure 2: Number of initiatives in control group rats, 6 weeks and 12 weeks. Each column corresponds to the average of 8 animals (Mean ± SD)

For recognition, we noticed that the control group had the highest percentage compared to the groups that had been treated with diazepam. While the group treated for 12 weeks with benzodiazepines has the lowest percentage F (8.27)=0.007 (Figure 3).

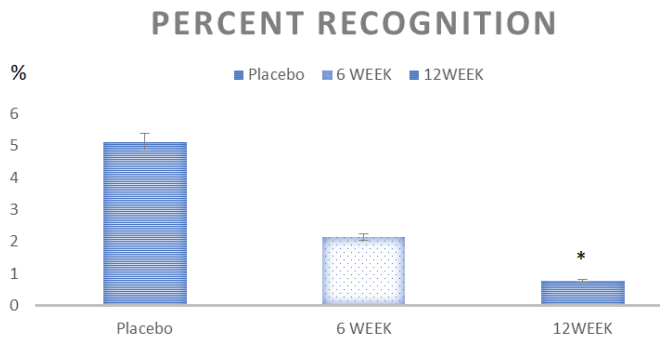

Figure 3: Object recognition test results for the three groups, Control, 6 weeks and 12 weeks. Each column corresponds to the average of 8 animals (Mean ± SD), (*P<0.005)

DISCUSSION

In this work we evaluated the effect of chronic benzodiazepine treatment on cognitive status in rats as an experimental animal model. The result of the present study showed that the administration benzodiazepines (1 mg/kg b.w) significantly reduced the recognition percentage in Object recognition test.

Benzodiazepines act on GABA receptors by potentiating the inhibitory effect of GABA in the central nervous system [8]. Anxiety and insomnia disorders are the main indication of benzodiazepines and are also prescribed for their sedative, myorelaxant effect, and anticonvulsant action [9].

Benzodiazepine (BZD) exposure during brain development can result in persistent modification of brain functions, behavioural alterations and cognitive deficits [10]. Effects of BZDs are specifically mediated by their interaction with BZD receptor binding site, which modulates the efficacy of the major inhibitory neurotransmitter, γ-aminobutyric acid (GABA), at the BZD-sensitive GABAA (BZD/GABAA) receptors.

Many studies indicate the likely implication of benzodiazepines in cognitive decline. This work aims to evaluate the relation between the duration of treatment with benzodiazepines and cognitive status in rats. One of the major complaints about the use of benzodiazepines is their effects on memory, it has been reported in recent studies that benzodiazepine-related amnesia is due to an impairment of the ability to encode new information, while preserving the recall process, but it is difficult to generalize, due to their pharmacokinetic and pharmacodynamic properties [11]. During our study, the percentage of recognition of objects was lower in the group which was treated for 12 weeks. This was determined by their inability to remember the familiar object. This supports our hypothesis that benzodiazepines alter cognitive status. It has been shown in one study that even short-term exposure to benzodiazepines during the early postnatal period can result in complex behavioural changes that can be detected later in life [12].

A study revealed significant reduction of global fluorodeoxyglucose (FDG) uptake after acute (-16.2%) and chronic (-23.2%) administration of diazepam, and uptake levels returned to normal after interrupting the administration of diazepam 12. It was provided that the administration of benzodiazepines induced an increase of the oxidative stress parameters, also several studies reported enhanced TBARS levels and protein carbonyl content, as well as altered enzymatic activity, such as decreased glutathione reductase activity, in the cerebellum and brain stem after diazepam administration [13,14]. It was demonstrated that diazepam (0.5, 1.0, or 2.0 mg/kg, i.p.) may affect the acquisition process and indicate memory impairment [15]. It was reported that one dose of diazepam increased the NF-κB and ERK 1/2 levels and the activation of NF-κB, both in the hippocampus and in the frontal lobe [16].

Lagnaoui et al., during a study to examine the association between benzodiazepine intake and the risk of dementia on 3,777 elderly people were followed for eight years, showed that benzodiazepine use was associated with an increased risk of dementia 1.7 of risk factor (95% confidence interval CI: 1.2–2.4)3. Another study in 2015 directed by Imfeild examined the relation between benzodiazepine use and Alzheimer's disease retrospectively and there was no association between Alzheimer disease and previous benzodiazepine use. However, the risk was twice as high and statistically significant when only the consumption of benzodiazepines during the year preceding the diagnosis of dementia is taken into consideration (RC=2, 2, IC 95%, 1, 91-2, 53) 16. It has been reported in a study that focused on cognitive change in relation to psychotropic drug use among 1,200 subjects 65 and over, and concluded that benzodiazepine consumption was not associated with cognitive decline. But this may also have been due to the depletion of susceptible effect [17]. These all results cited had weaknesses and biases due either to the duration of treatment or the number of samples [17,18].

On the other side, Beirman and his team evaluated cognitive performance in 2 105 elderly patients by exposing them to certain tests and revealed a significant negative effect of cumulative exposure to benzodiazepines (10 mg of Diazepam) on cognitive performance, the study This work takes into account the type, dosage, frequency and duration of use of benzodiazepines [19]. A longitudinal study is to examine the effect of chronic administration of benzodiazepines in a population of 5,195 people aged 65 years and over was significantly associated with decreased cognitive performance (β=-1,79, $p<0,001$), although it was not associated with the acceleration of their decline [20]. It has recently been shown that benzodiazepines increase the risk of cognitive impairment without dementia but do not appear to have an effect on Alzheimer's disease [21].

Indeed, it has been reported in Vincent Richeux's study that a use of benzodiazepines for three months or more is associated with an average increase in the risk of later developing Alzheimer's disease. These results were later confirmed by Billioti's work, while exploring more parameters such as duration of exposure to benzodiazepines (RC=1.43, 95% CI, 1.28-1.60). This association is stronger in case of prolonged consumption (OR=1.74, 95% CI, 1.53-1.98), or in case of consumption of benzodiazepines with a long half-life (OR=1.59, IC 95%, 1.36-1.85) [22]. Similarly studies have reported that the use of benzodiazepines induces mild cognitive impairment amnesic and non-amnesic [23]. Whereas the chronic administration of benzodiazepines also induces a negative regulation of their binding receptors and a reduction in the number of these receptors appears to be correlated with cognitive decline [24,25].

Down regulation of receptors has been reported that the real impact on cognitive decline due to the chronic use of benzodiazepines should be distinguished from the immediate change in the cognitive level induced by the initiation or cessation of benzodiazepine administration, appearance or disappearance of their acute cognitive effects. Rosenberg and his team examined the probable association of the use of psychotropic drugs with cognitive, functional and neuropsychiatric symptoms in 230 patients with Alzheimer's disease. The effect of multiple drugs, including benzodiazepines, was examined by the global deterioration scale, and was associated with a more rapid decline in cognition and an increased severity of dementia [26,27].

CONCLUSION

In addition, benzodiazepines users compared with non-users showed a deterioration rate 2.8 times higher. And regarding the possible recovery of cognitive functions after removal of the long-term use of benzodiazepine. This post-withdrawal recovery may be incomplete with a remaining impairment in some areas of cognition.

At this stage of our experiment, we can estimate that the initial objective, namely that of the set-up if there is an association between the administration of benzodiazepines and the cognitive decline in an animal model. We hope to engage in a research process to highlight the mechanisms that can induce cognitive decline and Alzheimer's disease.

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