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Effect of Midazolam on The Oxidative Stress and Activation of Nrf2 Mice Neural Cell Culture

Alqathafi Ali Alqathafi Sultan*, Hidayat Sujuti, Karyono Mintaroem

Post Masters's Program in Biomedical Sciences, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia

Abstract : Midazolam is a short-acting benzodiazepine in adults with an elimination half-life of 1.5-2.5 hours. In the elderly, as well as young children and adolescents, the elimination half-life is longer. Midazolam is metabolized into an active metabolite alphal-hydroxymidazolam. Age-related deficits, rental and liver status affect the pharmacokinetic factors of midazolam as well as its active metabolite. Research purposes: To determine whether midazolam improve the MDA in the brains of mice injected by midazolam and to determine whether midazolam increase activation of NRF2 in the brain of mice that injected by midazolam. Materials and Method: This study is experimental laboratory research using randomized design. The experiments applied simple random sampling. The treatment for the dose provision of NRF2 in every mice that received different concentration. Oneway ANOVA and Kruskal Wallis test will perform for the analysis. Result: when MDA high, so NRF2 not color brown it means Midazolam supported NRF2 when MDA high. But when MDA low, it mean NRF2 still brown color and clear. There is different result between control group and another group that significant in level 10%. Midazolam change theMDA in neuronal culture cell of mice and change the activation of NRF2 in neuronal culture cell of mice. So hypothesis in this research accepted. So midazolam can change MDA in neuronal culture cell of mice and change NRF2 activation in neuronal culture cell of mice.
Keywords : Midalozam, mice, NRF2, MDA.

Introduction

Midazolam is a short-acting benzodiazepine in adults with an elimination half-life of 1.5-2.5 hours. In the elderly, as well as young children and adolescents, the elimination half-life is longer[1]. Midazolam is metabolised into an active metabolite alpha1-hydroxymidazolam. Age-related deficits, renal and liver status affect the pharmacokinetic factors of midazolam as well as its active metabolite. However, the active metabolite of midazolam is minor and contributes to only 10 percent of biological activity of midazolam. Midazolam is poorly absorbed orally, with only 50% of the drug reaching the bloodstream[2]. Midazolam is metabolised by cytochrome P450 (CYP) enzymes and by glucuronide conjugation.

The therapeutic as well as adverse effects of midazolam are due to its effects on the $GABA_A$ receptors; midazolam does not activate $GABA_A$ receptors directly but, as with other benzodiazepines, it enhances the effect of the neurotransmitter GABA on the $GABA_A$ receptors that resulting in neural inhibition. Almost all of the properties can be explained by the actions of benzodiazepines on $GABA_A$ receptors. This results in the following pharmacological properties being produced: sedation, hypnotic, anxiolytic, anterograde amnesia, muscle relaxation and anti-convulsant. It is reported that midazolam activate JNK[53].

Meanwhile, in controversy it is reported that ROS suppressing by midazolam during clinical and surgical procedures, which has been shown to exert ROS-suppressing and apoptosis-modulating pharmacological activities in various cellular systems. This effects of midazolam that combined by ROS in neuronal cells will decrease the level of stress[4].

The cells of the body also has a defense system against free radicals, using the protein calledNRF2 (nuclear factor erythroid 2-related factor 2).Transcription factor NRF2 will protect body cells from damage. This system works optimally when stimulated by substances phytonutrients and fruits. Nuclear factor erythroid 2 related 2p45 (NRF2) is a transcription factor, a mediator oxidation stress response by regulating the genes of more than 100 antioxidant and cytoprotective enzyme.

In this research, we will elucidate the controversy whether midazolam increase or decrease ROS by measuring the MDA, and also we will examine the rote of NRF2 in decreased ROS in neural cells culture treatment by midazolam.

Material and methods

Research Design

This study consists of experimental study. The experimental study whereas the functional study is *in-vitro* study. Midazolam in culture cell will be divided in 4 groups as follows:

1. Control group (C)
2. Group 1 (M1): Midazolam dose 10 mm
3. Group 2 (M2): Midazolam dose30 mm
4. Group 3 (M3): Midazolam dose100 mm

With treatment of time:

1. After 30 min
2. After 2 h
3. After 24 h

The research will be done in 3 biological replicates.

Population

Population of this study was neural cell from newborn mice.

Sample

Neural cell from newborn mice from the Animal Laboratory at Brawjiaya University, Malang.

Variables of Study

Independent Variables: midalozam

Dependent Variables : level of NRF2 and MDA in neural cell

Research Procedures

The research procedures and data analysis on the result obtained to determine the appropiate modum for neural cell culture from newborn mice. Sample used in this research of neural cell culture.

Result and Discussion

This graph below showed the MDA for each group that consist of negative control, first group, second group and third group for 30 min. From Figure 1, it showed that the highest MDA is negative control and the lowest is second groups for 30 min:

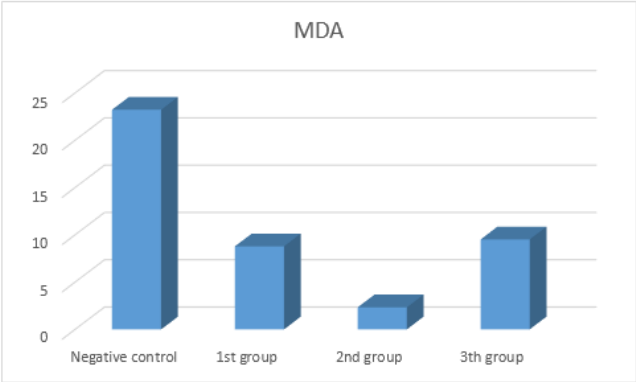

Figure1. MDA Result for 30 min

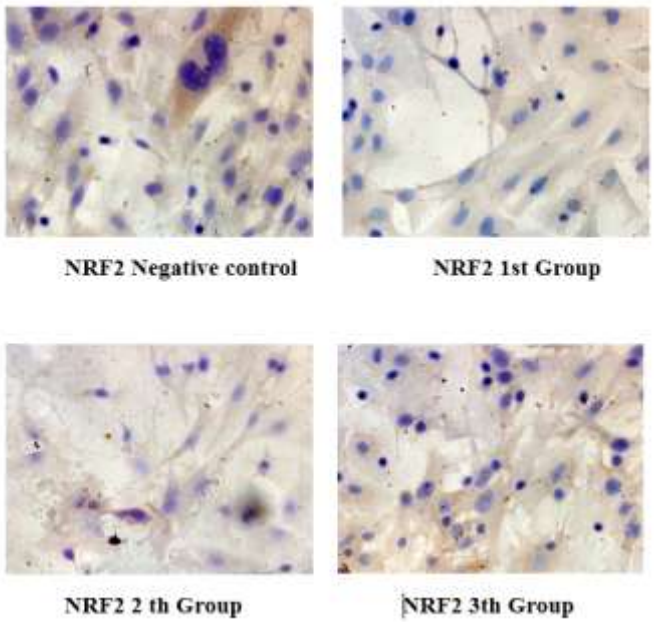

Figure2. NRF2 Result for 30 min

From Figure 2, it shows that when MDA high (in negative control group), so NRF2 not color brown it means midazolam supported NRF2 when MDA high. But when MDA low, it mean NRF2 still brown color and clear (in group 2).

This graph below showed the MDA for each group that consist of negative control, first group, second group and third group for 2 h. From Figure 3 showed that the highest MDA is negative control and the lowest is first groups for 2 h:

Figure 3. MDA Result for 2 h

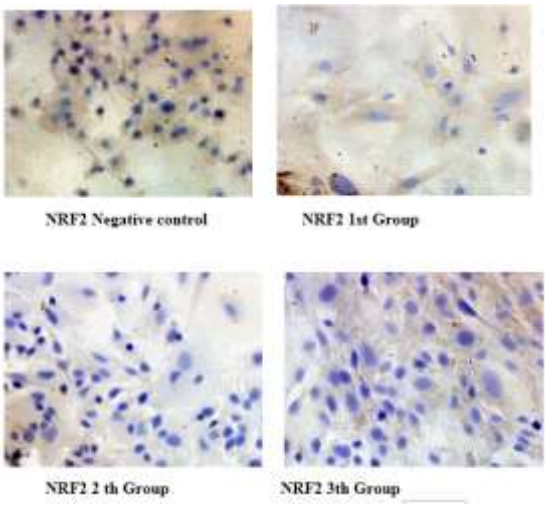

Figure 4. NRF2 Result for 2 h

From Figure 4, it shows that when MDA high (in negative control group), so NRF2 not color brown it means midazolam supported NRF2 when MDA high. But when MDA low, it mean NRF2 still brown color and clear (in group 1).

This graph below showed the MDA for each group that consist of negative control, first group, second group and third group for 24 h. From Figure 5 showed the highest MDA is second groups and the lowest is first groups for 24 h:

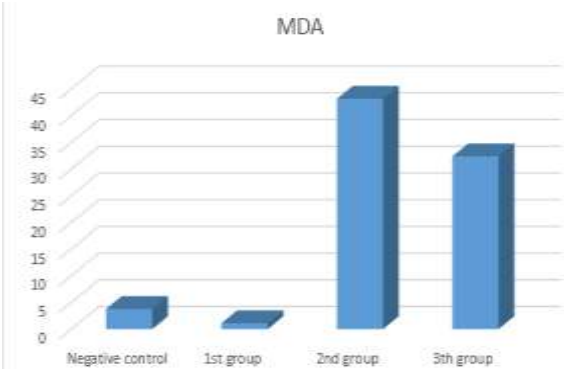

Figure 5. MDA Result for 24 h

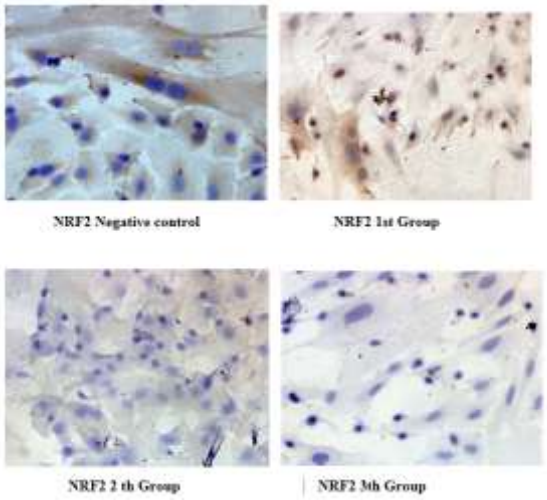

Figure 6. NRF2 Result for 24 h

From Figure 6, shows that when MDA high (in group 3), so NRF2 not color brown it means midazolam suppressed NRF2 when MDA high. But when MDA low, it mean NRF2 still brown color and clear (in group 1).

Anova

The table below showed the ANOVA result :

Table 1. Oneway ANOVA Result (MBA)

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	,098	2	,049	2,736	,080
Within Groups	,594	33	,018		
Total	,692	35			

From the Table 1 showed that the significant value is 0.080<0.10 this mean there is different result between control group and another group that significant in level 10%.

From the result, it can be say that there is positive relationship between MDA and Midazolam because when MDA high, so NRF2 supported with the color brown. This means that midazolam change theMDA in neuronal culture cell of mice that treated with midazolam and midazolam change the activation of NRF2 in neuronal culture cell of mice that treated with midazolam. So hypothesis in this research accepted. This also can see from the ANOVA result that stated the value of significant lower than 10%. So midazolam can change MDA in neuronal culture cell of mice that istreated with midazolam and midazolam can change NRF2 activation in neuronal culture cell of mice that istreated with midazolam.

Midazolam in a concentration range from 5 nM to 100 μM inhibited the neuronal firing rate in a biphasic manner. From 5 nM to 1 μM, midazolam significantly depressed action potential firing. However, the inhibitory action of midazolam did not rise continuously with increasing concentrations. Actually, 500 nM and 1 μM midazolam depressed the neuronal activity only slightly less than the administration of 100 nM midazolam. Though, raising the concentration of midazolam to 25 μM and beyond led to a stronger depression, which was significantly different from the concentration range of 5 nM to 1 μM.

Midazolam need to be indicated in oxidateve stress because midazolam has a potential of about two to three times greater. Midazolam has a benzodiazepine receptor binding affinity by approximately two times greater than diazepam. When compared with other benzodiazepines, midazolam has amnesiac effect much greater than the effects of sedation. so that the patient could be revived when administered midazolam, but still amnestic to events and conversations for a few hours[5].

Midazolam is an anesthetic agent commonly used during clinical and surgical procedures, which has been shown to exert ROS-suppressing and apoptosis-modulating pharmacological activities in various cellular systems. However, the effects of midazolam on oxidative stress in neuronal cells require elucidation. The present study investigated the effects of midazolam on buthionine sulfoximine (BSO) and hydrogen peroxide (H_2O_2)-induced oxidative stress in primary cortical neuronal cells. In addition, the effects of midazolam on middle cerebral artery occlusion (MCAO) in mice and on ethanol-induced neuroapoptosis in the brains of neonatal mice were determined. Subsequently, cell viability was detected using the MTT assay; intracellular reactive oxygen species (ROS) generation was determined using the dichlorodihydrofluorescein diacetate method with confocal microscopy; terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining was conducted to detect apoptotic cells; immunohistochemistry was performed to detect activated caspase; neuronal deficit and infarct volume analyses were conducted; and quantitative polymerase chain reaction and western blotting were performed to detect the expression levels of genes and proteins associated with apoptosis and cell survival pathways.

MDA in high concentration indicates the oxidation process inthe cell membrane. Free radicals and lipid peroxidation is a product of very short half-life and hard examined directly. MDA more stable and a degradation product of lipid peroxidationhave a longer life time, so it can be used as biomarkeroxidative stress

that occurs. Direct measurement of free radicals is very difficult to do, because free radicals do not live long, has a short half-life and disappear in seconds. Various developed as a biological substance biological markers (biomarkers) of oxidative stress. The substance that is already known and widely used as a biological marker of lipid peroxidation and oxidative stress are malondialdehyde (MDA). MDA are abundant in the circulation and is the main products of free radical reactions result with phospholipids, in production constant proportion of lipid peroxidation that occurs, so it is a good indicator to see the speed (rate) of lipid peroxidation in vivo.

NRF-2 is a protein in the cells when in a state of normal, will bind to Keap-1, but when stimulated by the inducer of type-2 including oxidative stress, then it will become active[6]. Active NRF-2 gene would express the cell's defense is one of them antioxidant an internal as Glutathione S-transferases (GSTs)[7] and enzyme Glutamylcysteine Synthase (γ-GCS) that controls the synthesis of GSH[8]. Active endogenous antioxidants result in reduced oxidative stress in the cell membrane is concerned.However, the effect of emphasis by antioxidants externa larger still be read from the low levels of F2-isoprostane were detected in the animals who received the treatment of meat with seasoning maximum dose.

Midazolam is metabolized into an active metabolite, 1-hydroxymidazolam. However, 1-hydroxymidazolam's contribution to the neuronal depressant actions of midazolam is complex and still poorly understood. Within the central nervous system the neocortex seems to be the major target in mediating sedation by benzodiazepines. However, to date only little is known about the potentially differential effects of midazolam and 1-hydroxymidazolam on neuronal activity in this region of the brain. Therefore, in the present study we set out to compare the potency and characteristics of midazolam and 1-hydroxymidazolam to inhibit spontaneous cortical activity. Midazolam depresses cortical activity in a biphasic manner. This is in close agreement with results obtained from previous studies with diazepam. The most convincing hypothesis to explain this biphasic depression is the existence of at least two benzodiazepine binding sites, leading to two separate mechanisms of benzodiazepine action[9].

The first phase of midazolam's action (steady state of depression) is in line with previous studies and this "ceiling effect" could be one of the reasons for the clinical safety of benzodiazepines. There are, however, several possibilities to explain this finding: First, a saturation at the classical benzodiazepine site11 and second, a novel binding site for benzodiazepines at the $GABA_A$ receptor, preventing further depression, as suggested in a previous study[10].

When comparing midazolam with 1-hydroxymidazolam it is evident that 1-hydroxymidazolam is far less potent. Inhibitory actions of 1-hydroxymidazolam could be observed starting at a concentration of 100 nM, corresponding to a plasma concentration of nearly 40 ng/ml. Considering the plasma concentration measured in a previous study, 1-hydroxymidazolam may, therefore, contribute to the effects of midazolam, above all, in cases of deep sedation or in patients with severe renal dysfunction. A summary of clinical and experimental findings concerning effects of midazolam and 1-hydroxymidazolam at different concentrations can be found.

In addition, we detected that midazolam administered at the low concentration of 5 nM caused a significant inhibition of the cortical network activity in cultures from wild-type, but not from α1(H101R)knock-in mice. Therefore it seems reasonable to assume that midazolam at low nanomolar concentrations predominantly acts via α1-containing $GABA_A$ receptors. Thus, despite being a non-$GABA_A$ receptor subtype-selective benzodiazepine, midazolam's sedative actions are largely mediated by α1-containing $GABA_A$ receptors. In contrast to diazepam, at recombinant receptors midazaolam displays a preference for α1-containing $GABA_A$ receptors over α2-containing $GABA_A$ receptors: a recent study showed an approximately two-fold larger potentiation of GABA-induced currents by midazolam at $GABA_A$ receptors containing α1-subunits compared with $GABA_A$ receptors containing α2-subunits, whereas diazepam potentiated both receptor subtypes to a similar extent.

The result of this study stated that there is relationship between MDA and NRF2 and this also supported by previous study done thatstated that midazolam is an anesthetic agent commonly used during clinical and surgical procedures, which has been shown to exert ROS-suppressing and apoptosis-modulating pharmacological activities in various cellular systems[11]. The effects of midazolam on oxidative stress in neuronal cells require elucidation. The effects of midazolam on middle cerebral artery occlusion (MCAO) in mice and on ethanol-induced neuroapoptosis in the brains of neonatal mice were determined.

References

1. Rosenbaum, A., Kain, Z. N., Larsson, P., Lönnqvist, P. A., Wolf, A. R. The place of premedication in pediatric practice. Paediatric Anaesthesia. 2009. 19 (9): 817–28. doi:10.1111/j.1460-9592.2009.03114.
2. Riss, J., Cloyd, J., Gates, J., Collins, S. Benzodiazepines in epilepsy: pharmacology and pharmacokinetics. Acta Neurologica Scandinavica. 2008. 118 (2): 69–86. doi:10.1111/j.1600-0404.2008.01004.
3. Nishina, H., Wada, T., Katada, T. Physiological roles of SAPK/JNK signaling pathway. J Biochem (Tokyo). 2004. 136 (2): 123–6.
4. Wada, T. and Penninger, J. M. Stress kinase MKK7: Savior of cell cycle arrest and cellular senescence. Cell Cycle. 2004. 3 (5): 577–579.
5. Hamilton R. Tarascon Pocket Pharmacopoeia 2015 Deluxe Lab-Coat Edition. Jones & Bartlett Learning. 2015. p. 21. ISBN 9781284057560.
6. Yamamoto, T., Suzuki. T., Kobayashi, A., Wakabayashi, J., Maher, J., Motohashi, H., Yamamoto, M. Physiological significance of reactive cysteine residues of Keap1 in determining Nrf2 activity. Molecular and Cellular Biology. 2008. 28 (8): 2758–70. doi:10.1128/MCB.01704-07.
7. Itoh, K., Chiba, T., Takahashi, S., Ishii, T., Igarashi, K., Katoh, Y., Oyake, T., Hayashi, N., Satoh, K., Hatayama, I., Yamamoto, M., Nabeshima, Y. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun. 1997. 236 (2): 313–22.
8. Wild, M.K., Cambiaggi, A., Brown, M.H., Davies, E.A., Ohno, H., Saito, T., van der Merwe, P.A.Dependence of T cell antigen recognition on the dimensions of an accessory receptor-ligand complex. J Exp Med. 1999. 190 (1): 31–41.
9. Walters, D.M., Cho, H.Y., Kleeberger, S.R. Oxidative stress and antioxidants in the pathogenesis of pulmonary fibrosis: a potential role for Nrf2. Antioxid. Redox Signal. 2008. 10:321–32.
10. Baur, R., Tan, K.R., Luscher, B. P., Gonthier, A., Sigel, E. Covalent modification of $GABA_A$ receptor isoforms by a diazepam analogue provides evidence for a novel benzodiazepine binding site that prevents modulation by these drugs. J. Neurochem. 2008. 106:2353–63. doi: 10.1111/j.1471-4159.2008.05574.x.
11. Liu, J.Y. Feng Guo, Hong-Ling Wu, Ying Wang and Jin-Shan Liu. Midazolam anesthesia protects neuronal cells from oxidative stress-induced death via activation of the JNK-ERK pathway. Spandidos Publication. 2016. 15: 169–79. doi:10.3892/mmr.2016.6031.

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