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THE DIFFERENCE BETWEEN KETAMINE AND PROPOFOL ADMINISTRATION TO INDUCE APOPTOSIS NEURODEGENERATION IN MICE CEREBRAL CORTEX

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Abstract:

Background: The ketamine and propofol stimulates neuroapoptosis in the mice brain if administration. The programmed cell death (apoptosis) follows regular growth of the central nervous system. The mechanisms that normalize neurons is submit to apoptosis are poorly understood. Blockade of N-methyl-D-aspartate (NMDA) glutamate receptors generate common apoptotic neurodegeneration in the developing mice brain, suggesting that excitatory neurotransmitter glutamate, acting at NMDA receptors, controls neuronal perseverance.

Objectives: This study aims to study the neurotoxic significance of either ketamine or propofol exposure on mice brain using immunohistochemical marker.

Methods: administration of ketamine or propofol intraperitonially at normal dose to mice and assessed the degree of neuroapoptosis (Programmed cell death) in frontal cortex of the brain areas following either saline or normal drug doses administration (ketamine or propofol). Each drug was administered as only one-time inject in dose range that would be measured anesthetic dose, and the brains were evaluated by immunohistochemical marker methods five hours following drug administration. Neuroapoptosis (Programmed cell death) was identified by Immunohistochemical antibodies to malondialdehyde (MDA). The ketamine or propofol produced a dose-dependent, statistically significant increase in the rate of neuroapoptosis (Programmed cell death).

Conclusion: mild administration of ketamine or propofol can trigger apoptotic neurodegeneration in the developing mice brain frontal cortex.

Key words: NMDA antagonist, Immunohistochemical antibodies to malondialdehyde (MDA).

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Introduction:

The study have reveal that brief treatment of mice to several types of the drugs including ketamine or propofol, those that block NMDA glutamate receptors, activates widespread neurodegeneration (Programmed cell death) in the developed brain (Ikonomidou *et al.*, 1999; 2000; Jevtovic-Todorovic *et al.*, 2003).

The cell death procedure caused by ketamine or propofol drugs has been intended histologically and found by light microscope to classic morphometric parameter of the neuroapoptosis (Programmed cell death) (Dikranian *et al.*, 2001; Olney *et al.*, 2002a).

The drugs (ketamine or propofol) is superior consideration, because widespread and very commonly used in the world, use both emergency and in the anesthetic theater in the hospital, and also be used with increased occurrence as playful drug, irregularly used by pregnant mother. The ketamine or proofol is observed as harmless drug, principally for children, truly, it has been use in recent years with increased frequency in pediatric surgery (Reich & Silvay, 1989; Gilman *et al.*, 1990). The ketamine or propofol are widespread used because simply to administered for anesthetic operation room, and has a slight incidence of cardiorespiratory side effect. The ketamine or propofol used to induce anesthesia in both gynecological and pediatric operation surgery, and very frequently are used to sedate patients suffering procedures such as endotracheal intubation in general anaesthesia in respiratory care unite and fracture reduction in day case operation (Bergman, 1999; Green *et al.*, 2001; Law *et al.*, 2003). The propofol, is a substituted isopropylphenol, non-barbiturate, nonsteroid, sedative-hypnotic drug having minimal analgesic action at subanaesthetic dose. The propofol can be used for induction, as well as maintenance of general anaesthesia. Preanaesthetic medication significantly reduces the induction dose of the propofol. The propofol has been investigated as intravenous anaesthetic in horses (Oku et al., 2006), sheep (Lin et al., 1997), goats (Prassinos et al., 2005), swine (Graham et al., 1998) and buffaloes (Malik, 2008).The propofol delivers rapid induction of anaesthesia, as well as smooth fast recovery after administration by both intermittent bolus or continuous intravenous infusion method which requires as a risk-free and an perfect anaesthetic (Adetunji et al., 2002). Administration of a single dose of propofol briefly reduced arterial pressure, accompanied by a stable heart rate (Cullen and Reynoldson, 1993).

The propofol, is of superior significance of common used and because of general use in combination with ketamine for general anesthetia. Since propofol is a GABAmimetic drug and ketamine an NMDA antagonist, the grouping exposures patient to the analogous double mechanism by which ethanol damaged in the human fetal brain (Ikonomidou *et al.*, 2000).

The ketamine, when administration subcutaneously to infant rats in a sequences of administration separately over 9-hour period, cause well design of apoptotic neurodegeneration (Ikonomidou *et al.*, 1999). We also study the side effects of a single ketamine administration. The neurotoxicity; is defined as the anomalies of the nervous system follow exposure to a chemical or biological or physical cause. The contact to neurotoxicity during development as the blood brain barrier is not well growth, neurogenesis and synaptogenesis were want on at high rates. Ceccatelli S, Bose R, Edoff K, Onishchenko N, Spulber S.

Aim of research:

The effect of ketamine or propofol premedication (single injection) on cerebral cortex changes on frontal cortex mice.

Methods:

Animal treatment and handling

In this study, 45 well female adult Swiss albino mice (Mus musculus) were used, the mice are approximately 5 weeks of age and weigh about 20-25 gram. The mice animals were taken from the (animal house of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University). The mice animals were kept under constant conditions of normal light period (12 hours light/dark cycle), and temperature (24-32 °C). The mice animals had permitted access to usual diet and water. The mice animals were divided into three animal groups with 15 mice animals in each group; the control group (1) and the experimental (drug) groups (one for ketamine (2) and other for propofol (3). The control group of female mice animals was given 0.1 ml of distilled water intramuscular. The experimental groups (2, 3) was given a sub-anaesthetic dose of ketamine (75 - 100 mg/kg, intraperitoneal) and propofol (26 - 30 mg/kg, intraperitoneal). All mice animals, care actions were in accordance with standards approved by the Animal Studies Committees of Diyala University. The mice animals of the experimental groups (2) administered intraperitoneal ketamine hydro-chloride injections (Kanox, ketamine 50 mg/ml preservative; chlorobutanol 5%, batch number 122228E, Duopharma), experimental groups (3) administered intraperitoneal propofol, and the control group (1) administered intramuscular normal saline doses. The animals mice were sacrificed by decapitation after one day of the administration, their brains were removed from the cranium, and coronal paraffin sections of 5μm thickness of the cortex were prepared after fixation in 10% formalin (12). Digital camera (Sony cyber shot) was used for documenting tissue staining and histology.

Anti-MDA antibodies

The antibodies provide from Abcam, (code no. ab6463). They remain rabbit polyclonal antibodies having minor molecule of the synthetic- malondialdehyde, conjugated to the bovine, serum - albumin.

The immune-histochemistry detection, kit was call Expose- Mouse and Rabbit- Specific, HRP/DAB-Detection IHC Kit- from Abcam (code no. ab80436).

Six sections were designated from the sections of the cerebral cortex of mice of each group (1, 2 and 3).

Aperio- Image Scope -version 9, software were used for the assessment of MDA antibodies, immunohistochemical reaction. The image analysis software, include counting the sum of strong-positive pixels to evaluate the immunohistochemical stain.

The list of positive- pixel total algorithm includes parameter, found from the application of this software- to quantify, the quantity of a specific stain existing in a scanned slide image. The parameters when first designated had been pre-configured for brown color, quantification. Pixels- were stained, but do not fall into the positive-color specification, were considered, negative -stained pixels.

Statistical methods

All of the data was analyzed using SPSS statistics ver. 19 (SPSS, Chicago, IL, USA). Before analysis, a test was performed. If the data normally distributed, density of apoptotic cells were analyzed using one-way analysis of variance (ANOVA) and the Dunnett's method for post hoc analysis. If data did not follow a normal distribution, Kruskal-Wallis test were performed and the Mann-Whitney U-test with Bonferroni correction were used. The P value or adjusted P value less than 0.05 were indicate statistical significance.

Result:

The analysis of variance (ANOVA) statistical evaluation of the mean values of MDA immuno-histochemical reactivity in the frontal cortex of mice of group A (the control group) showed non-significant differences.

The evaluation of the counted mean values obtained by the application of the Aprio Image Scope software in the cortex of mice of groups (B, and c) revealed statistically significant variability compared to those of the control group A.

This counting of the mean value of the number of strong positive pixels was highest in group B (14400.1 ± 134.1). The mean values in group C were (7562.6 ± 564.6) and for the groups A 3379.8 ± 185.02 respectively. The p values ≤0.00 for the groups A, B, and C.

The multiple comparison statistical test done for the mean differences between the experimental groups B and C showed significant variability ($p \geq 0.00$), and high significant variability was shown between the groups B and C compared with group A ($p \leq 0.00$).

The ANOVA statistical analysis of the counted mean values obtained for group C compared to that of the group A (the control group) showed significant variability ($p \geq 0.00$). While significant variability was found from the values of comparing the group B with control group A (the control group) ($p \leq 0.00$), and a highly significant variability was found for group B group comparing the group C ($p \leq 0.001$)

Variable	number	Mean S.E	P value
Control	15	3379.8 ± 185.02	
Ketamine	15	14400.1 ± 134.1	*0.000 (significant
Propofol	15	7562.6 ± 564.6	*0.000 (significant)

*P value ≤ 0.05 considered statically significant.

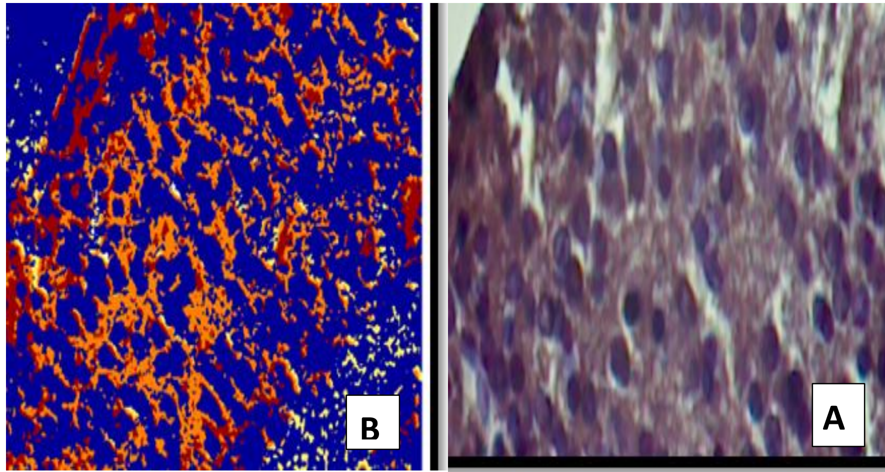


Figure (1): (B) Anti MDA reactivity of cerebral cortex from mice of group. Anti-MDA positive stain is seen in all layer of cerebral frontal cortex.400X. (A): The snap shoot as examined by Aperio positive Pixel Count Algorithm software.

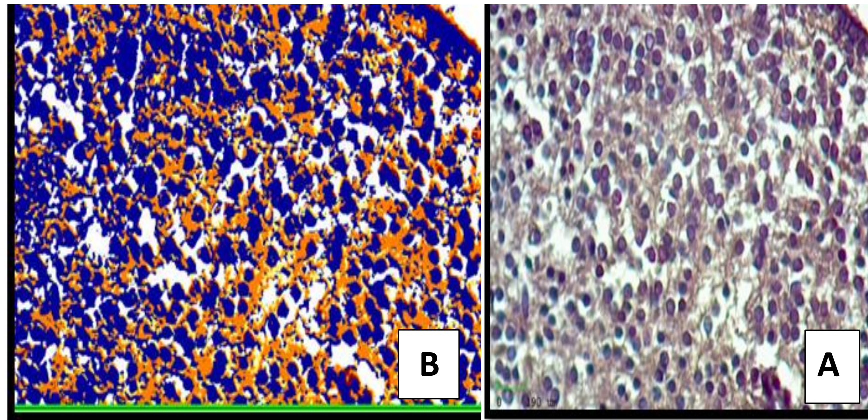


Figure (2): (B) Anti MDA reactivity of cerebral cortex from mice of group. Anti-MDA positive stain is seen in all layer of cerebral frontal cortex.400X. (A): The snap shoot as examined by Aperio positive Pixel Count Algorithm software

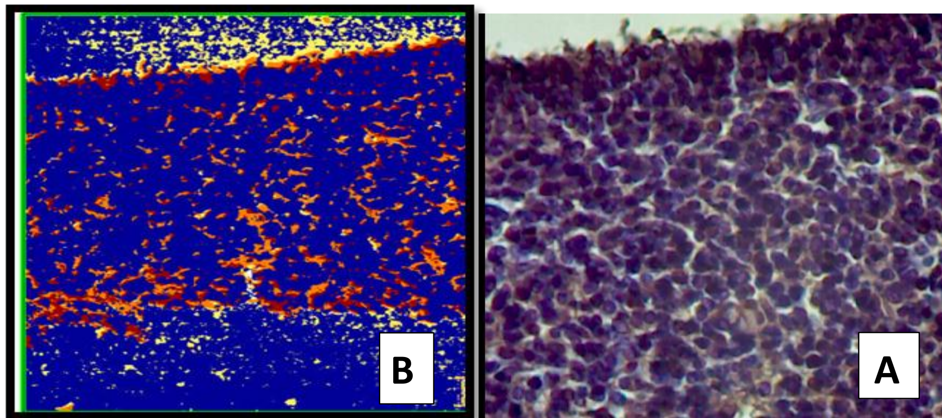
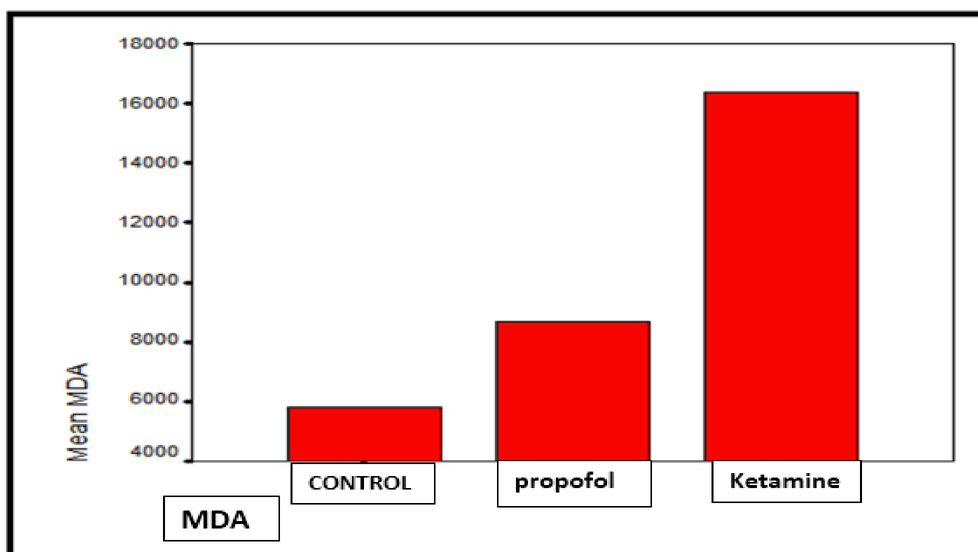


Figure (3): (B) Anti MDA reactivity of cerebral cortex from mice of group. Anti-MDA positive stain is seen in all layer of cerebral frontal cortex.400X. (A): The snap shoot as examined by Aperio positive Pixel Count Algorithm software



Discussion:

The general anesthetics, that performance through GABA and NMDA receptors apoptotic neurodegeneration in the developing animal's brain. The protest that a single injection of ketamine (75 - 100 mg/kg, intraperitoneal) or propofol (26 - 30 mg/kg, intraperitoneal) triggers apoptotic neurodegeneration in the frontal cerebral cortex of mice animals and that these two drugs can cause a neuroapoptotic response to the drug. The technique for noticing the apoptotic response to MDA immunohistochemistry, a method that confirmed reliable for selectively staining neurons were undergo apoptotic degeneration after administration of (ketamine or propofol) neuroapoptosis-promoting drugs (NMDA antagonists, GABAmimetics, sodium channel blockers, ethanol) to the animals mice. In prior studies, (Dikranian *et al*., 2001; Olney *et al*., 2002a), we have assessed by aperio light microscop the neurodegenerative process in every stage of evolution, and demonstration that the affected neurons of cerebral cortex. After becoming MDA positive, progress to advance stage of programmed cell death and degradation over a period.

An apparent contradiction between the ketamine results and the reported of the treatment of infant rats with ketamine triggered neuroapoptosis (Hayashi *et al*. 2002). While it may be important that our present findings are in mice and another were in rats, we suspect that the difference is more likely due to variances in methodology than to a species effect. The neurons that die within this period are degrade rapidly into particulate debris. The debris particle remained visible for many hours, and donate to argyrophylia of the region, but after 24 h they are not distinguishable or countable as dying neurons.

Since ketamine has a short half-life, most sensitive neurons – that are susceptible to die after a short-term, apoptogenic stimulus are possible to be affected by only subanesthetic dose of ketamine or propofol. Although the present study was not intended to address subject, it is possible that the combination of ketamine plus propofol, might postponement the time interval during neuronal activity is repressed, and cause additional neurons to be demolished neurons that would not have been

murdered by ketamine or propofol alone. The ketamine or propofol used in the current experiments related to those used in human anesthesia.

Thus, it is common use for neonates to expose to a relatively high dose of ketamine. The dose of ketamine need to induce anesthesia in mice is >80 mg kg^{-1} s.c. (Green *et al.*, 1981). The doses of ketamine (75 - 100 mg/kg, intraperitoneal) and propofol (26 - 30 mg/kg, intraperitoneal) shown in the present study to induce significant neuroapoptosis, are in the sedation (subanesthetic) range for mice, and would be equivalent to a sedating/subanesthetic dose for an infant human. The use propofol more commonly use than ketamine because ketamine induced psychosis. The result of this study that both ketamine or propofol trigger neuroapoptosis in the developing mice frontal cerebral cortex, and the exposure of the developing brain to the both drugs, in combination results in additive neurotoxicity, suggests the need for a risk/benefit reevaluation. It is usually recognized that rodent data carry an imprecise basis at greatest, and an unrelated basis at worst, to evaluate human risk. For additional satisfactory evaluation of human risk further research, rather in a non-human primate species, will be needed later on (Acworth *et al.*, 2001). There are several restrictions in this study.

- First, long-lasting neuronal damage affect, the neuronal stem cells not investigated.
- Second, according to previous study, the brain cell death increase with time, and persevere after a single exposure.
- Third, cardiopulmonary function not assess, such as arterial blood gas analysis. Decrease cardiopulmonary function might be associated with brain hypoxia and ischemia.

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