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Original Research

Effect of ketamine anesthesia on cognitive function and immune function in young rats

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Abstract: The aim of the current study was to explore the effect of the ketamine on the immune function and cognitive function in young rats. The young rats (40) rats were randomly divided into two groups where each group contains 20 rats, such as Group I: normal control; Group II: Ketamine treated group. All group rats received the intravenous injection of treatment for three times and the hippocampal neuronal apoptosis and the immune parameters such as IL-2, IL-4 and IL-10 and whole brain IL-1β level were estimated. The cognitive ability effect of the young rats was also tested using the Morris water maze test. In Morris water maze test, it has been found, as the time increases, the latency of the control group and ketamine treated groups rats were gradually decreased, with a significant difference. The latency rate of the control group was unchanged significantly ($P<0.05$), but after 3 days, the latency has been decreased significantly. The hippocampal neuronal apoptosis of the control group and ketamine treated group rats were found to be 13.5×5.8 % and (2.1×1.4) %, respectively. The level of the serum IL-4 and IL-10 were also found significantly ($P<0.05$) higher in the ketamine group as compared to the control group rats. The level of the IL-2 was found to be almost similar in both normal control and ketamine group rats. Markedly, the level of the whole brain IL-1β was found to be significantly higher in the ketamine treated group in comparison to the control group rats. On the basis of the above fact, it has been conclude that the ketamine might be able to inhibit the cognitive function as well as immune function.

Key words: Ketamine, Immune function, Morris water maze test.

Introduction

Several evidences have confirmed that the intravenous anesthetics has endowed with excellent analgesic effect, with less or no significant side effects on the respiratory system, (1). It has been also found and evidenced by the numerous reports that, the general anesthesia can cause the mental illness, cognitive impairment and other toxic and adverse effects. Therefore, the mechanism of action of the general anesthetic is still in debate and the proper mechanism by which these agents affects the immune and central nervous system is continue to attract attention from researchers (2). Concerning the above, in the present study, we intended to elucidate the effects of Ketamine on the immune function and cognitive function.

Materials and Methods

Chemicals

The chemicals utilized in the present experiment were procured from Sigma Aldrich (U.S.A.).

Experimental study

The animal used in the present study was obtained from the institutional animal facility. The study has been approved by a research ethics committee of the institute. Briefly, the animals were divided into two groups (n=20) as following: group I: normal control and group II: ketamine treated group. Each rat of all group had received the adaptive breeding for 7 days in the animal house room. The control group rats received the intraperitoneal injection (i.p.) of 1 mL saline (0.9%) on every 2 h, continuous for 3 times. Whereas, the group II rats received the 1 mL i.p. injection of ketamine (80 mg/kg) on every 2 h, continuous for 3 times in a day. Moreover, the all rats of test group received the 1 mL of predetermined doses of Ketamine (99%, Sigma Aldrich, USA), and if the dose was less than 1 mL, thenit was suspended in saline to make the required volume. The control group receives saline. The half of the rats of each group was sacrificed after the 15 min of administration of ketamine, and rest of the rats were forwarded to Morris water maze test after 3 weeks. All abandoned or died rats in halfway were supplemented by modeling pain.

Detection of immune parameters

The 5 mL capacity of sterile heparin-filled syringe (disposable) was used for obtaining the blood by percutaneous puncture at the maximal impulse by injecting into theEP tube. The blood sample was kept at 4°C for 30 min and after that the sample was centrifuged at 300 rpm at low temperature for 10 min. The separated serum was stored at -80°C for the estimation of immune parameters. The immune parameters selected for investigation wereIL-4, IL-2 and IL-10 using the ELISA method.

Brain tissue specimen collection, processing and indicators test

After collecting the blood sample, rest of the rats were randomly preferred. The heart was protruded out-

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side via thoracotomy. Theperfusion needle was placed and fixed in the ascending aorta form the ventricle (left) and after that right auricle was slash. The normal saline was used for the washing of the perfusion needle until the effluent of the right atrium was clear and the needle was fixed in the 4% paraformaldehyde phosphate buffer saline. The hippocampal was successfully removed from the brain tissue when the organ and the body tissues were hard. Terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) method was used for the estimation of neuronal apoptosis. The TUNEL method showed the brown particles in the nucleus and the six horizons were uniformly selected and the average optical density was estimated. The following formula was used for the estimation of apoptosis index and positive intensity.

$$Apoptosis\ Index\ (AI) = Area\% \times MOD \times 100$$

Where Area% showed the percentage of total positive nucleus area in total nucleus area. MOD represents the average gray level. The rest of the rats cerebral were quickly obtained by sterile opening cranium, and brain tissues were mixed together in saline (ice normal) using the Homogenizer. The 10% of brain homogenate was centrifuged at 3000 rpm (15 min) at 4°C and the supernatant was kept at 80°C for further estimation. The whole brain tissue 1L-1β level was estimated using the ELISA.

Morris water maze test

The Morris water maze test was used for the estimation of the behavior of the rats. The Morris water maze has four quadrants (round tanks), where the black platform was fixed at the fourth quadrant, positioned 1 cm under water. The animals were put into the water of a randomly selected quadrant and the swim activities of the rats were recorded using a camera and time was recorded for the rats to find the latent platform. After completion, all platforms were removed and the rats were again put into the water from the same starting point and the time of the crossing the platform were recorded.

Statistical analysis

Data were expressed as mean±SD values and analyzed with SPSS 13.0 software. After the variance test, the difference between two groups was compared with single factor analysis of variance. P<0.05 was considered as statistical significant difference.

Results

Effect of ketamine on the serum immune parameters

As shown in fig. 1, a no significant difference has been found in between the normal control and ketamine treated group rats in terms of immune serum parameters, such as IL-2. The other investigated parameters such as IL-4 and IL-10 of the ketamine treated group was found to be significantly (P<0.05) higher as compared to the normal control group rats (fig. 2, 3). Moreover, the level of IL-1β has found to be significantly lowered (P<0.05) in ketamine treated group rats in comparison to normal control group (fig. 4).

Hippocampal neurons apoptosis

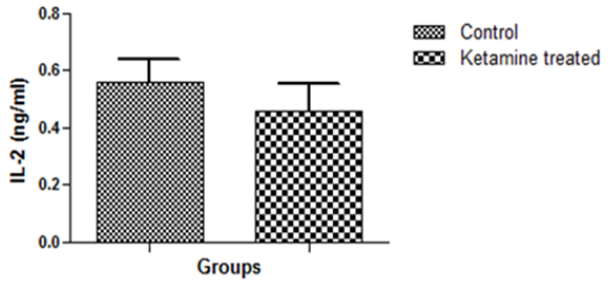

Figure 1. Effect of normal control and ketamine treated rats effect on the immune cell IL-2. Compared with the control group, P <0.05.

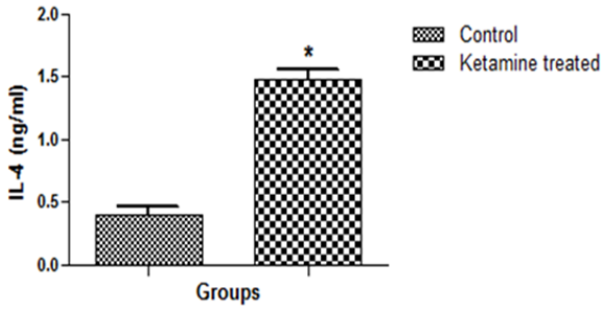

Figure 2. Effect of normal control and ketamine treated rats effect on the immune cell IL-4. Compared with the control group, P <0.05.

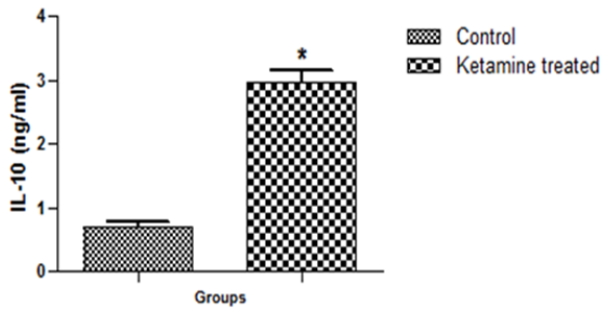

Figure 3. Effect of normal control and ketamine treated rats effect on the immune cell IL-10. Compared with the control group, P <0.05.

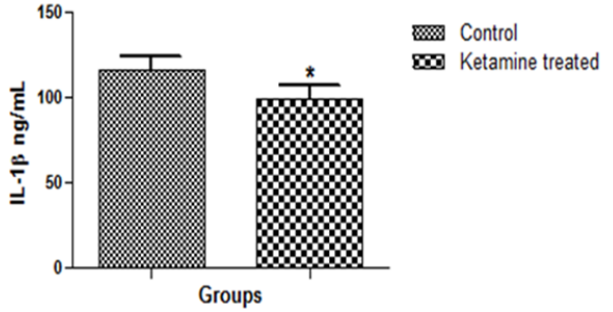

Figure 4. Effect of normal control and ketamine treated rats effect on the IL-1β. Compared with the control group, P <0.05.

The experimental group showed (13.5×5.8) % apoptosis of the hippocampal neurons, which was found significantly (P<0.05) higher as compared to the control

group (2.1×1.4) %.

Rat behavior observations

In this experiment, as the time increases, the latency of the control group and ketamine treated groups rats were gradually decreased, with a significant difference. The latency rate of the control group was unchanged significantly (P<0.05), but after 3 days, the latency has been decreased significantly. As shown in fig. 5, it has been found that there is no significant difference between the control and ketamine treated group at day 1. However, as the day proceeds, the latency of the control group was found to be decreased, while the, rats belongs to the ketamine treated group exhibit significant upsurge in the activity, i.e on day 2 and day 3 (P<0.05). Moreover, in terms time taken to cross the circle, the normal control group rat was found to be unchanged at end of the study, while, the ketamine treated group rats showed significant difference in comparison normal control (figure 6).

Discussion

Recently, postoperative cognitive dysfunction has received considerable attention. Several investigations have shown that, the use of the narcotics drugs which is closely related to the indulgent the dysfunction. It has been found that, for long time memory, the hippocampal neurons is believed to play an important role. Therefore, the injury to these neurons can affect the memory function and capability of learning. In that scenario, the postsynaptic membrane receptors was also implicated in the cognitive functionas a imperative information center, and that the pathogenesis of Alzheimer's patient is connected with the reduced appearance of receptors (3,4,5,6,7,8,9). During the anesthesia, the changes in the immune system have attracted the researcher's consideration. Anesthetic such as ketamine was concerned in the regulation of the central nervous system via suppression the postsynaptic membrane receptors, but the change of the immune function and cognitive function mechanism also still unclear during the anesthesia. Thus in the current study, we have used ketamine as a anesthetic to the rats and then the effect of the drug has been determined on the cerebral expansion and the immune system.

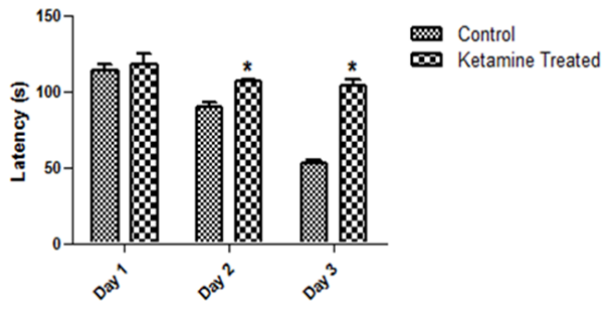

Figure 5. Effect of normal control and ketamine treated rats effect on the latency. Compared with the control group, P <0.05.

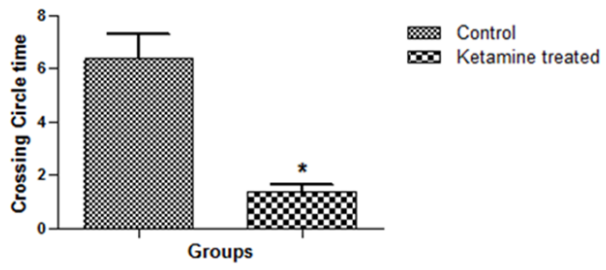

Figure 6. Effect of normal control and ketamine treated rats effect on the crossing circle time. Compared with the control group, P <0.05.

In the current study, we have utilized the classic neurological method (Morris water maze) for the estimation of the memory mechanism (10). On the cognitive function in the rats, Iitially, we intended to explore the latency at the period of three days. Results confirmed that, there is no significant difference was found between the ketamine and control treated group. The latency rate of the control group was unchanged significantly (P<0.05), but after 3 days, the latency has been decreased significantly. As shown in fig. 5, it has been found that there is no significant difference between the control and ketamine treated group at day 1. However, as the day proceeds, the latency of the control group was found to be decreased, while the, rats belongs to the ketamine treated group exhibit significant upsurge in the activity, i.e on day 2 and day 3 (P<0.05). Moreover, in terms time taken to cross the circle, the normal control group rat was found to be unchanged at end of the study, while, the ketamine treated group rats showed significant difference in comparison normal control (figure 6).

Various studies have confirmed that the ketamine anesthesia abusers have a long term memory losswhich is related to the damage of hippocampal neurons. This has prompted the neuronal apoptosis and results in the dysfunction of cognitive ability. The spatial learning memory ability of ketamine treated young rats confirmed the shorter latency as compared to the normal group rats, and the times of cross the flat is also considerably enhanced, which confirmed that the ketamine anesthesia can inhibit the effect on the cognitive functions. The ketamine group also showed the significant lower apoptosis rate by the study of the hippocampal neuronal apoptosis, which is accordance with the change in the memory ability and spatial learning in rats. These results have confirmed that the ketamine can inhibit the anesthesia on the rat cognitive lies of the hippocampal neuronal apoptosis. It has been evident that, on increase of hippocampal neuronal apoptosis, the memory dysfunction and long-term learning are become more apparent (11,12,13,14,15). Several investigation have confirmed that ketamine have significant effect on the cerebral activity of in animals and humans. The cognitive effect of the ketamine may be related with the ability to inhibit the cerebral metabolic rate of the oxygen which causes reduction of excitatory amino acid glutamate neurotransmitter release. It also causes reduction of intracranial pressure, reducing neurotoxicity induced pathological damage and interference with glutamate pathways. The pharmacological effect of ketamine preventsprotein denaturation, lipid peroxidation and secondary damage of neuronal cells via reducing the formation of free radicals and release of various inflammatory mediators (16,17,18).

The immune system is act as major defense barrier to combat infections through coordinate action of its com-

ponents. Whereas, the patients or experimental animals subjected to anaesthesia and surgical procedures affected with numerous immunological imbalances. These alterations are hard to identify, if they are induced by anaesthetic drugs or by stress caused by surgical intervention. Particular;y the stress caused by surgical procedures will either regulate or suppress the immune system of the subjects. It has been evident that, T cells actively take part in generation and modulation of the immune system. Therefore, the immune system continuously works to maintain the normal homeostasis mediated via Th1 and Th2 cells. These cells plays crucial role, and once there is imbalance in Th subsets (Th1/Th2), it will result in dysfunction of the immune system. (19). The effector function of leukocytes is mediated via short-acting molecules known as cytokines. The expression of the cytokine is highly controlled and its activation is necessary for their synthesis to exert their biological activity. Interleukin-2 (IL-2) is belongs to the family of cytokines produced by activated T-cells (CD4+). It deemed to plays an important role in the expression of cell-mediated immunity by stimulating the proliferation of T-suppressor lymphocytes (CD8) and natural killer cells (NK cells). On the other hand, IL-10, an another cytokine plays an anti-inflammatory role by suppression of T-cell activity, especially the activity of cytotoxic T cells and TH1 cells, and thus leads to a reduction of IL-2 synthesis.

The current study confirmed that the ketamine treated group rats have very non-significant effect on the IL-2 immune secretion. Whereas, the level of serum IL-10 and IL-4 were found to be considerably increased. The level of the whole brain IL-1β was also found to be significantly reduced in the ketamine treated group. IL-1β is a cytokines produced by a variety of cell infection and in inflammatory state, which have a wide range of the physiological effects. It frequently called that the central stress-mediated factors. Thus, in the presence of systemic stress response, central IL-1β may show high expression (20).

In summary, the results of the study indicated that, ketamine might able to reduce the cognitive function in young rats and has toxic effect on hippocampal neurons. The result confirmed that the ketamine can inhibit neurotoxicity and shelter the brain tissue. Ketamine also reduces the immune function in young rats. Moreover, there are still a few gaps between the human clinical trials and animal investigation.

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