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Toxic Effect of Colchicine on Hippocampal Cortical Area

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Background and Objectives: Study of deleterious effect of neurotoxins on the animals' brain is a fascinating research plan. In this project, the damage effect of colchicine on the hippocampal cornu ammonis 1 (CA1) was examined by the studying of the hippocampal tissue.

Materials and Methods: Injections of colchicine (5,25 µg/rat, intra-hippocampal CA1) were performed in cannulated male Wistar rats while being settled in the stereotaxic apparatus. Control group was solely injected saline (1 µl/rat, intra-CA1). Other groups of rats were trained in the conditioning device to receive the colchicine (5 and 25 µg/rat, intra- CA1) prior to the testing; the control group was given saline (1 µl/rat, intra-CA1). At the end of the experiments, the rats were decapitated and their brains were removed for histological studies.

Results: The number of the small pyramidal cells of hippocampal CA1 showed a decrease in the colchicine-received rats than the control group. The novelty behavioral assessment showed a significant difference between the colchicine given rats versus control ($p<0.05$).

Conclusion: Hippocampal CA1 layer plays an important role in the memory and learning processes. Lesion of this region by the aid of neurotoxins (*e.g.* colchicine) may lead us to provide a proper animal model to study the learning dysfunctions in the future. This research may appropriately validate the lesion effect of the toxin in the hippocampal CA1. It may also propose an incidence of the novelty seeking behavior due to the lesion in the hippocampus.

1. Introduction

Colchicine is an alkaloid extracted from certain plants of the Liliaceae family, and is synthetically formed by phenylalanine and tyrosine as the precursors. This drug was used for centuries to treatment of acute gouty arthritis. Also, in 1973 it was administered as an effective treatment for the prevention of attacks of familial Mediterranean fever. The alkaloid has additionally been indicated as an anti-cancer drug.

The antiproliferative action of anticancer colchicine is related to properties of colchicine – tubulin complex (1). This matter attaches to the bottom of microtubules and prevents of elongation of microtubules polymer. The microtubules are the most important of filamentous proteins making up the cytoskeleton. These components are vital and necessary for many activities including cell migration, cell division and polarity.

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By stop of microtubules elongation, the spindle action will disrupt. Due to prevention of microtubules polymerization, the cell division will inhibit. This effect of colchicine may successfully introduce it as a mitotic poison. It disrupts the neuronal axoplasmic transmision via this ability (1).

Colchicine is therefore an agent for disruption of microtubules. So, it is used as a neurotoxin to injury the central nervous system (2). This drug after entering in the brain connects to tubulin which is known as the fundamental structural protein of microtubules. It causes instability and depolymerization of the microtubules and inhibits the axonal conduction. It eventually leads to the death of the nervous cells (2).

Toxicity aspects of the central neuronal system induced by colchicine in animal models are very similar to the symptoms of Alzheimer's disease (3). Systemic administration of colchicine in rats, similarly to those with Alzheimer's disease, causes cognitive deficits. These protests can be determined by recent learning forgetfulness and loss of memories which have already been formed (3). In addition, colchicine injections into the brain cavity significantly impair memory because of reduced level of norepinephrine, dopamine and serotonin in the cerebral cortex, hippocampus and caudal nucleus (3).

By use of colchicine, the granular cell in the rat dentate gyrus can be lost specifically. These cells in adult mice are significantly damaged by colchicine (4). Colchicine at high concentrations has neurotoxic effects on other neurons (5). But, a gap that still feels is providing an effective learning disorder caused by old cortical neurons such as hippocampus cortex. This type of disorders necessarily does not lead to progressive dementia such as neurological diseases (*e.g.* Alzheimer's disease). They rather induce a lower degree level of illness or syndrome which is classified as memory impairment. Furthermore, it is critical to provide a suitable animal model for accurate studying of these mechanisms. Showing colchicine destructive contact on the cortical pyramidal neurons of the hippocampus may make it a good candidate in this context. Therefore, the aim of this research was study of the injurious effect of colchicine in hippocampal CA1. We further investigated the neuronal elimination due to colchicine.

2. Materials and Methods

2.1. Animals

In this study, 48 male Wistar rats (obtained from Pasteur Institute of Iran) were used. The animals were kept at animal care center of School of Basic Sciences of Shahed University under standard conditions. The confine boxes were autoclaved polyethylene cages. The temperature (21-23°C) and light cycle (12h) were controlled. The water and standard food (provided by Pars animal food company, Tehran) were used *ad libitum*. Subjects were 250-300 g at the time of the experiments. 3-4 rats were housed per cage. After surgery, they were individually placed in the cage. The animals were tested only once. All the experimental procedure was affirmed by the local ethical committee at the Shahed University.

2.2. Drugs

Drugs used in this study included colchicine (Merck Co, Germany) and ketamine-xylazine (obtained from Veterinary Organization, Tehran). Ketamine-xylazine were injected intraperitoneally as milligram per weight of the rats. The desired concentrations of colchicine were solved in 0/9% sterile physiological saline to freshly inject into the hippocampal CA1. Amounts of colchicine used in this study are based on pilot and previous studies at this laboratory.

2.3. Surgery and cannula placing in the CA1 area of the rat hippocampus

Animals were anesthetized by a 5 to 2 ratio of ketamine (100 mg/kg) and xylazine (20 mg/kg). Each animal then was placed in the stereotaxic apparatus under zero of the apparatus. After making an incision in his skull and later to the necessary calculations, two guide cannulae (21 G) were inserted in the rat skull based on atlas (v: 3, L: ±1/8-2/2, AP: -3/8). The tips of the cannulae were 1 mm above of the injection area. The guide cannulae placement areas were then fixed by dental self-curing acrylic while the animal was still held in the device. The injection of the drug was made through an injection cannula with the length 1 mm further than the guides (in order to accurately access the hippocampus). The injection volume was one micro-liter for each rat brain. The control group received saline instead of drugs.

2.4. Experimental exercise

The animals were trained in the behavioral box one week after recovery (in order to restore their mental potencies). They were decapitated one week later and their brains were carefully removed. The safe and complete brain specimens were kept in 10% formalin solution for 10 days to follow the pathological study.

To reason the destructive effect of the colchicine, three safe groups of rats were trained in the conditioning device based on a previously designed paradigm (6) with a new perspective. The rats were injected colchicine (5 and 25 µg/rat, intra-hippocampal CA1) prior to the testing; the third group, the control, received saline (1 µl/rat, intra-hippocampal CA1, prior to the testing).

2.5. Histological study

To investigate the histological data, the brain slices (3-4 micro-meters) were obtained with the use of tissue processor. The neuronal staining was performed with Cresyl violet (Merck Co., Germany) and silver nitrate (Merck Co., Germany). Qualitative analysis of microscopic images was done by the Image Tool to compare the scale of unit surface area (100 micrometer) between control and experimental samples. The photo images were taken by photo-videomicroscope (Olympus).

2.6. Staining by Cresyl Violet

The processed slides after paraffin removal and hydration using the decreasing concentrations of ethanol (95%-50%) were stained (0.1% aqueous Cresyl violet). The slides then were rinsed and dehydrated with the increasing concentrations of ethanol (50%-95%). They finally were cleared and glued.

2.7. Golgi staining

The slides later to paraffin removal and hydration using the decreasing concentrations of ethanol (95%-50%) were placed in colors combinations (2% aqueous silver nitrate plus 2% potassium dichromate in addition to 2% mercuric chloride) lasted 72 h. The slides then were rinsed and dehydrated with the increasing concentrations of ethanol (50%-95%). They were finally cleared and glued.

3. Results

The results obtained in this research indicate the toxic effect of the alkaloid, colchicine at the concentrations used in the study (5,25 µg/rat). It was effective in damaging to pyramidal cells of CA1 layer in test animals versus the controls. Based on the quantitative measurement ($p<0.05$) the numbers of pyramidal cells of CA1 layer were significantly lower than the control group (Fig. 1).

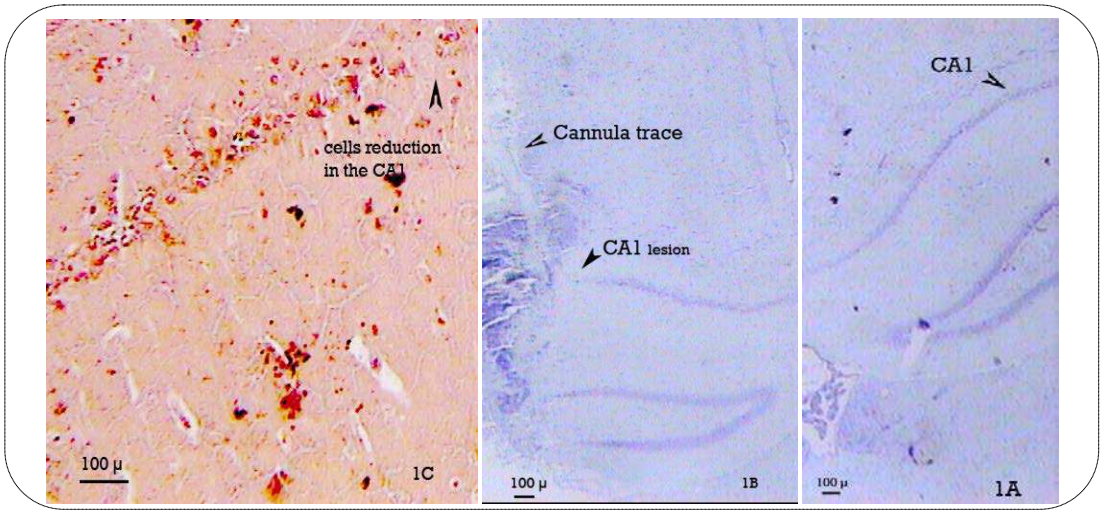

Figure 1. The image shows the microscopic view of the cortical area 1 (CA1) of the rat hippocampus. The thin slices were studied by Cresyl violet or silver nitrate stain in order to detect the position of the layers as properly as possible. A comparison of the layers obtained of the animals exposed to the colchicine (1B-1C) reveal a clear difference between them than those of the control group (1A). This distinction are shown in the images. As seen, the intact CA1 layer (1A) has an entity and its cell population is normal. In fig 1B, in which the trace of cannula for injection of colchicine is evident, the destruction effect of the toxin is obvious. In fig 1C, with use of specific stain, the elimination effect of the alkaloid on the small cells in the layer is clarified under higher magnification.

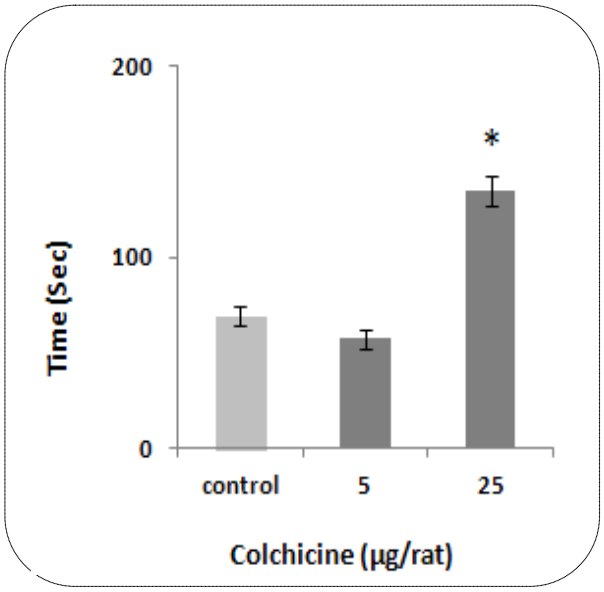

Figure 2. The figure shows the **r**esponse to colchicine or saline (control), intra-CA1. At first, the animals were cannulated at the area by aid of the stereotaxic apparatus. One week later, after recovery, they passed the behavioral training. The experimental animals injected drug or vehicle pre-testing of the place conditioning task. Data are expressed as the score of change in seeking for the novel place and expressed as mean ± S.E.M. A difference between the drug-administered groups versus the vehicle was observed. *Post hoc* analysis by Tukey showed the differences (*$P < 0.05$) versus the control.

The control group which received only saline in the same way showed the layer as in the entity as expected compared with the experimental.

The behavioral data are shown in Fig 2. As can be seen in the figure, use of colchicine resulted in a significant difference in the score of staying in the novel part between the colchicine received rats and the control group ($F_{2,15} = 6.073$, $p<0.05$)

4. Discussion

This study was designed to indicate the harmful effect of colchicine in the CA1 of the rat hippocampus. The toxic effect of the plant derived alkaloid was shown by help of histological study (Fig. 1). The evaluation of the colchicine received animals, furthermore, indicated a significant preferred novelty behavior to the control saline given group.

Hippocampus is a main center for memory consolidation and is responsible for memory processing; it develops the primary memory to become stabilize as the distant remembrance (7). The hippocampus plays especially an important role in the spatial memory (8). Researchers have worked on the techniques to investigate the distinction role of various regions of the hippocampus by microinjecting of neurotoxins in different regions of the dorsal hippocampus (CA1, CA3, dentate gyrus) (9). The above mentioned method was chosen by this team since we know the fact that colchicine can cross the blood-brain barrier and accumulates in the brain (10). This technique is also suitable for the enjoyment of its destructive effect. The present results represent this point. We should notice that the distribution map of colchicine in the brain is not identical at all brain areas and its concentration in the hippocampus, a region that is three- time rather involved in Alzheimer's disease, is much more higher than other brain regions (11). So, it is more likely that the toxic alkaloid provides negative effect on the hippocampal neurons though previous reports had indicated that colchicine caused selective demolition of granule cells (12). In this study has been shown that use of the neurotoxin can destroy the multipolar pyramidal neurons in the CA1 region.

It has been indicated that the central symptoms of colchicine toxicity in animal models is similar to Alzheimer's disease in human being, because, it is similarly associated with oxidative stress, disruption of microtubules, decrease in cholinergic activity, and progressive deterioration of cognitive functions (13). Poisoning of the neurons or damage to neuronal cells by colchicine probably occurs through the production of free radicals and oxidative stress, the way leading to death or necrosis of the pyramidal cells in the hippocampus (14). As it has been evidenced, the relative increase in intracellular glutamate metabolic events increases the production of free radicals, which beat the antioxidant defense and activates oxidative stress. Production of toxic intermediates (such as peroxynitrite and nitric dioxide) can be considered as an outcome of this process of binding (15-18). Thus, central injection of colchicine can enhance the peroxidase activity of cyclooxygenase and oxidative stress, the mechanism that enhance the production of toxic intermediates and causes over production of

nitric oxide. The latter factor is highly toxic for neurons.

Interestingly, colchicine induces the pre-programmed cell death (apoptosis) through a way related with caspase 3 in cultured ongoing granular cell. The results are consistent with findings obtained under in vivo conditions (19).

Colchicine is a known neurotoxin with high affinity toward the tubulin through which the accumulation of mitotic microtubules is stopped, and thereby the axoplasmic transmission is disrupted (20).

We should notify that the neuronal function is dependent on the integrity of functional structural cytoskeleton. Cytoskeleton changes are associated whit neurological disorders that are seen in Alzheimer's disease (21). The dendrites (22) and the axonal membranes (23) are the places enriched of the microtubules. These areas can be considered as places to work on colchicine toxic effect. It would be very interesting if such points are selected as the targets for this purpose and that is the point that the present researchers based on their achievements concluded.

In conclusion, our finding may show the lesion effect of the toxin colchicine in the hippocampal CA1. It may also offer the novelty seeking behavior induction due to the lesion in the cortical hippocampus.

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