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Injection of colchicine into the dorsal striatum of rat's brain induces epilepsy

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Abstract

Background and Objective: Despite the massive use of costly models, our understanding of epilepsy is insignificant. We aimed to create the cost-beneficial and applicable generalized epilepsy (GE) in rats by injection of identified doses of colchicine (IDC) into the dorsal striatum of rat's brain.

Materials and Methods: 24 male Wistar rats (290-350 g) under deep anesthesia were equipped with guide cannulae at the dorsal striatum (AP: 0.5 mm; L: 3 mm; V: 3.6). The injection cannula attached with the polyethylene tubing to the 5-µL Hamilton syringe guided daily IDC (9-25 µg,) into the dorsal striatum of rat's brain during four consecutive days. The control group only received the saline solution. At the end of the injection, the animal's behavior was investigated. Finally, all the brains were collected in 10% formalin solution and dissected out to include the striatal regions. The samples were cut coronally into 3-4 µm thick slices, embedded in paraffin, and processed by the step section technique. The slices were then stained with Hematoxylin-Eosin method and checked under the light photomicroscope for correct placement of injections as well as the possibility of lesions. The data were compared between vehicle and experimental groups by analysis of variance (ANOVA).

Results: The findings show that GE occurs in animals receiving colchicine without significant devastation effect in the injection site in the brain of rat.

Conclusion: The plant-derived alkaloid, the colchicine, aside depolymerizing of tubulin, may have an inductive effect on epilepsy in the complementary motor striatal region of the rat's brain

Keywords: Generalized epilepsy, Dorsal striatum, Identified doses, Colchicine, Wistar rat

1. Introduction

Epilepsy is a chronic central nervous system disorder that is characterized by recurrent seizures due to excessive discharge of cerebral neurons (epileptic seizures). So far, the most frequently used models of chronic epilepsy and models of acute seizures have been induced by chemoconvulsants, traumatic brain injury, and electrical or sound stimuli. Also, recently spontaneous recurrent seizures of rodents have been generated with chemoconvulsants (1). The animal models, though, have played a vital role in advancing our understanding of epileptogenesis mechanisms (2) but, they generally used with expensive cost and interfered with ethics.

Colchicine has previously been introduced as a selective neurotoxin of dentate granule cells (DGC) when researchers sought to record the extracellular field potentials following intrahippocampal injections of colchicine in the dentate gyrus of the rat, which resulted in granule cell death but, CA1 pyramidal cells unaffected (3). The neurotoxin colchicine inhibits the

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inflammatory responses (4-6), and holds the tubulin polymerization (7). The primary mechanism of action of colchicine is tubulin disruption, which leads to subsequent down regulation of multiple inflammatory pathways and modulation of innate immunity (5). The newly described mechanisms also include various inhibitory effects on macrophages.

The alkaloid substance, colchicine, has formerly been used to induce the limbic epileptic seizure in the rats (8). However, other investigators in the same period challenged this theory and, by reviewing the effects of this substance in the brains of different mammals, described the phenomenon as a rare epiphenomenon of colchicine's action, which is caused by neuronal destruction (9). They identified colchicine as a non-selective neurotoxin that can damage the brain with a non-specific inflammation.

These criticisms can be attributed to the targeted area because it is possible that colchicine is non-toxic in areas other than dentate gyrus. So, we planned to inject it at the identified doses into the dorsal striatum of rat's brain to damage the neuronal conduction in that area likely due to microtubule disassembly. We really aimed to introduce the *nov*. Complementary motor cortical epileptic seizure.

2. Materials and Methods

2.1. Animals

24 Male Wistar rats (290-350 g) were used in this research. They were provided from Pasteur Institute of Iran, Tehran, and housed at the local animal center in two per individual standard PVC cages at constant room temperature (23±2 ºC), and maintained on a 12-h light/dark cycle (light turn on at 07:00 AM). Food and water were available *ad libitum*. All experiments were carried out under the National Institutes of Health Guide for the Care and Use of Laboratory Animals and protocol was confirmed by the local animal ethics committee under the rules governing the graduate research plans.

2.2. Surgery

Rats were anesthetized with ketamine (100 mg) - xylazine (20 mg), and implanted bilaterally with stainless-steel guide cannulae (21-Gauge) at AP: 0.5 mm; L: 3 mm; V: 3.6 mm based on the atlas of Paxinos and Watson (2007) (10).

2.3. Intra-striatal injections

The injections of identified doses of colchicine (IDC) were started seven days after the recovery of the rats that had been cannulated in the dorsal striatal regions. At the time of injection, the substance (9-25 µg), was firstly dissolved in the sterile 0.9% NaCl solution and administered into the dorsal striatum of brain of recovered rat. Each rat received one µL of the volume concentrations of the IDC by using the injection setup. The injection cannula was provided by the aid of dental needle (27-Gauge) and protruded 1 mm beyond the guide and connected to a 5- µL Hamilton syringe by polyethylene tubing. Each animal received the IDC for four successive days. The control group only received the saline solution (1 µL/rat, intra-striatal) during the experimental period.

2.4. Statistical analysis

At the end of each injection, the behavioral signs of experimental animals were recorded. All brain samples were collected and examined histopathologically. They were first cut into the pieces containing the target sites of injections. They were then sectioned coronally into 3-4 µm thick slices, and stained with the hematoxylin-eosin method. Both the histological evidence and the behavioral signs were analyzed by analysis of variance (ANOVA) under $\alpha = 0.05$.

3. Results

Data obtained by monitoring of behavior in animals treated by identified does of colchicine (IDC), intra-dorsal striatal illustrate significant epileptic seizures that look like the generalized epilepsy (GE) (Tables 1-4 (with no lesion effect in the target brain areas (Figure 1). Scores were characterized by the aid of Racine (1972) five-stage scoring (11) in which the generalized motor convulsions, including rearing and falling with forelimb clonus, occurred. Comparison of the results of the study of the effects of IDC (9, 15 and 25 µg/rat, intra-striatal) with control group receiving saline (1 µL/rat, intra-striatal) by the ANOVA showed the meaningful percentage of occurrence of GE attacks (critical phase 5) than the control. According to this study, the rats receiving IDC had the most epileptic seizures on the second day. The findings are based on the protocol that each dose of the alkaloid is injected into the dorsal striatum of six rats and that each rat receives one dose of the substance in that area for four consecutive days. All the data are presented according to average achievements in the Tables 1-4. Also, tissue images of dorsal striatal regions both of control and colchicine-treated rats were analyzed and showed no meaningful neuronal cell damage (Figure 1)

Table 1: Results of intra-striatal injection of saline (control group)

Saline 1 µL					
Day 4	Day 3	Day 2	Day 1	Weight (g)	No of Rat
-	-	-	-	300	1
-	-	-	-	280	2
-	-	-	-	290	3
-	-	-	-	300	4
-	-	-	-	300	5
-	-	-	-	304	6

Saline (1 µL/rat) was injected once daily for four consecutive days into the dorsal striatum of six rats. Epileptic attack did not occur in this group (n= 6) receiving the saline 0.9% intra-striatal.

Table 2: The Generalized Epilepsy (GE) attacks in the colchicine (9 µg)-treated group.

Dose 9 µg					
Day 4	Day 3	Day 2	Day 1	Weight (g)	No of Rat
Stage 5 (35 sec)	Stage 5 (35 sec)	Stage 5 (50 sec)	Stage 5 (43 sec)	300	1
-	-	-	-	283	2
-	-	Stage 5 (90 sec), recurred within 5 h	-	296	3
-	-	Stage 5 (41 sec), recurred within 5 h	-	321	4
-	-	-	Stage 5 (41 sec), recurred within 2 h	350	5
-	-	Stage 5 (10 sec), recurred within 2 h	-	304	6

An identified dose of colchicine (9 µg) was administered into the dorsal striatum of six rats once daily for four consecutive days. In this group (n= 6), the critical phase 5 of Racine's 5 stages took place. This phenomenon lasted from 10 sec to 90 sec and the epileptic seizures recurred in rats within 2 to 5 h.

Table 3: The epileptic attack in the colchicine (15 µg)-treated group.

Dose 15 µg					
Day 4	Day 3	Day 2	Day 1	Weight (g)	No of Rat
-	-	-	Stage 5 (17 sec)	303	1
-	Stage 5 (15 sec)	-	-	298	2
-	-	-	Stage 5 (17 sec), recurred within 2 h	296	3
-	-	Stage 5, recurred within 2 h	Stage 3	291	4
-	Stage 5 (60 sec)	-	-	310	5
-	-	-	Stage 3	333	6

The identified dose of colchicine (15 µg, intra-striatal) was given once daily for four consecutive days in this group (n=6). The GE occurred at a lower frequency than the lower dose (9 µg) of the alkaloid (*cf.* Table 2). According to the data, both a milder stage (phase 3) was observed and the phase 5 lasted from 15 sec to 60 sec. Moreover, the attack recurred in the rats within 2 h.

Table 4: The epileptic attack in the colchicine (25 µg)-treated group.

Dose 25 µg					
Day 4	Day 3	Day 2	Day 1	Weight (g)	No of Rat
-	-	-	-	290	1
-	-	-	Stage 5 (15 sec)	292	2
-	-	Stage 5 (25 sec)	Stage 5 (50 sec)	307	3
-	-	-	-	258	4
-	-	-	-	297	5
-	-	-	Stage 3, (51 sec)	267	6

Colchicine (25 µg/rat, intra-striatal) was administered once daily for four consecutive days in this group of rats (n= 6). The epileptic attack occurred at lower frequencies than the lower doses (*cf.* Tables 2-3). The attack did not repeat in this group.

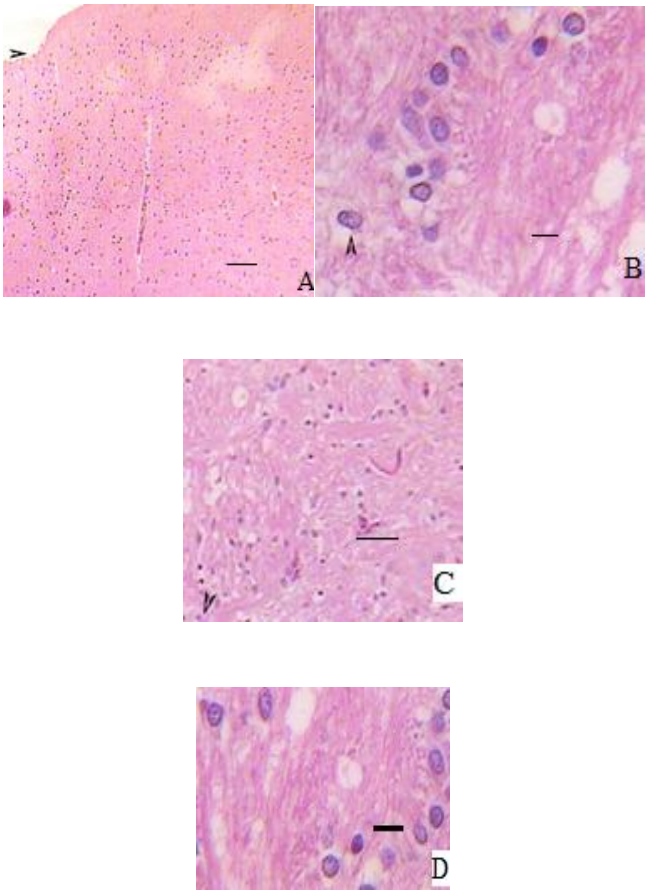

Figure 1: Tissue images of dorsal striatal regions both of control (A, B) and colchicine-treated rats (C, D). The neuronal degeneration in the target areas cannot be seen in none of groups. Lines denotes the magnifications (from 10 X in fig. A & C to 40 X in fig. B & D)

4. Discussion

This study was designed to povide a new animal model of epilepsy with the help of direct injections of a plant-derived alkaloid, colchicine, at the identified doses in the dorso-striatal complementary motor cortex of rat's brain. Present results, based on injections of identified doses of colchicine (IDC) into the dorsal striatum of rat's brain, show that a dose (9 µg/rat) of the colchicine is the most effective of all the dosages of the neurotoxin for induction of generalized epilepsy (GE).

It has previously and similarly been suggested an epileptogenic activity of thiocolchicoside, a natural glycoside and semi-synthetic derivative of the colchicine, which originates from the flower seeds of *Superba gloriosa*. It is a muscle relaxant with anti-inflammatory and analgesic effects (12, 13). Researcher has shown a definite activity of this material toward strychnine-binding sites in the rat brain regions (*e.g.*, amygdala) (14). Raynolds and Oakley (1984) (15) have also reported the stable epileptiform discharges due to colchicine while following intracellular studying the experimental epileptic focus.

So far, by reviewing the literatures, most animal models of epilepsy are kindled at the hippocampus and amygdala. However, Deransart et al. (16) have suggested that the dopaminergic neurotransmission within the striatum could be involved in the control of generalized epileptic seizures that accord with present results.

Biraben et al. (17) have recently reported the significant reduction of [18F]fluoro-L-DOPA uptake in both the caudate and the putamen of patients with ring chromosome 20 epilepsy as compared to healthy volunteers. There is also evidence supporting the preventive effect of either GABA antagonist

(bicuculline) or dopamine agonist (apomorphine) in a pilocarpine model in the rat striatum (18, 19). The importance of the caudate nucleus in the control of convulsive activity has previously been shown (20). Furthermore, the putamen cells have been involved significantly during both frontal and motor seizures (21). Thus, these findings support our hypothesis that the new epilepsy model can be induced in the dorsal region of striatum. Our findings (Tables 1-4) interestingly indicate that the movement area (dorsal striatum) is a suitable candidate to induce GE. In accordance, recently, Vuong and Devergnas (2018) (22) have indicated the role of basal ganglia in the control of motor cortical seizure development.

To discuss the disadvantages of some models, the administration of high doses of pentylenetetrazole (PTZ) has been shown to cause the acute tonic colonic attacks, while the administration of low and frequent doses of PTZ induces kindling (23). It works by opening the Ca channels and through changes in the activity of K and Na channels as well as obstruction of GABAa receptors in the nervous system (24), which is costly and long- lasting. In understanding the effects of penicillin, when penicillin has been injected into the neocortical regions, the areas come to be a focal point for epileptic seizures, which spreads over time and leads to an acute seizure (25, 26). The kainic acid as a glutamate-stimulating and powerful glutamate receptor agonist is also widely used to induce limbic seizures but, swelling of the neurons and surrounding vascular astrocytes is observed after injection of this substance. For reasons that are still unclear, this substance (the kainic acid) can cause high mortality of animals and severe damage to the hippocampus (27-29).

However, as an important finding, we should notify that according to our results, the best dose of colchicine, intra-dorsal striatal, for induction of GE is a low dose (9 µg/rat), which did not show the cellular destruction in the target area. Because of less toxicity of the low-doses colchicine, the model did create either without the mortality or damage to the brain. Furthermore, the animal recreates the seizure behaviors during the time intervals.

Conclusion

The substance colchicine is a disturbance alkaloid of neural processes that can be used (at the identified doses), intra-dorsal striatal, to definitely create the animal model of generalized epilepsy.

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