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RESEARCH ARTICLE

EXPERIMENTAL MODEL TO INVESTIGATE DRUGS FOR EPILEPSY

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Abstract

Introduction. Epilepsy is one of the most common neurological disorders characterized by seizures. Epileptic seizures result from excessive, abnormal, or hyper synchronous neuronal activity in the brain. It is a public health problem that is not yet curable but which can be controlled. A high percentage of patients who suffer from epilepsy do not have seizures control even when using the existing treatments. Therefore, an experimental study was conducted with the **objective** of determining the effect of the Bufo-toxin in rats belonging to the BALB/c and the Wistar strainsin order to diagnose the group of symptoms that confer lethality to this toxin and which can work as a treatment to such disease.**Methodology** the bufo-toxin was obtained from toads, then it was placedin alcoholic solution and it was applied to the rats in doses of 5 by 5 for up to 20 Units with an insulin syringe though the intramuscular via. Observations were registered and when the rats died a post-mortem examination was conducted in order to describe the effect of the toxin in the internal organs. **Results.** The rats that were inoculated within 20 units of the toxin showed epileptic seizures and finally cerebral spill or heart attack. **Discussion and conclusion.** This model can be used to investigate on useful drugs against epilepsy and even heart diseases, such as, hypertension andheart attacks.

Introduction

Epilepsy represents a public health problem whose prevalence occurs in 18 out of 1,000 inhabitants.It can manifest itself at any age or during childhood. Its main cause is interrelated with hereditary factors and even with the administration of medical treatments, as well as it can occur after traumatic actions or accidents.[1]

This type of problem mainly affects children and young people (0-20 years), but it is most frequent to happen in children who are younger than 9 years old, which truly represents a public health problem.[2] This research aims to provide an analysis that could lead to further research on new natural treatments which could help people who need to mitigate or cure epilepsy, especially because nowadays there are no drugs that could solve this health condition.

An epileptic *seizure*(word that comes from the Latin "sacire" which means“takes possession”) is a paroxysmal phenomenon that occurs due to abnormal, excessive and hyper synchronous discharges of a group of neurons in the central nervous system (CNS). Depending on the distribution of the seizure, this abnormal activity of the CNS can manifest itself in different ways, ranging from a striking seizure activity to phenomena of subjective experience difficult to warn by an observer. Although a variety of factors influence the incidence and prevalence of epileptic seizures, a 5 to 10 per cent of the world's population will have at least one seizure

during their lifetime, the incidence is higher at early ages, during childhood and adulthood. Epilepsy is described as a disorder in which a person has recurring seizures due to an underlying chronic process. This definition implies that a person who has suffered a single seizure or a secondary one (due to avoidable or correctable factors) does not necessarily have epilepsy. [3]

The main feature that distinguishes the different categories of seizures is ifthe epileptic activity is partial or generalized. Partial seizures are those in which the epileptic activity is limited to small areas of the cerebral cortex. Generalized epileptic seizures simultaneously affect large brain regions, of bilateral and symmetrical shape. As a general rule the partial seizures are typically associated with structural brain lesions. By contrast, generalized seizures may occur by cellular, biochemical or structural abnormalities of more widespread distribution. There are two types of seizure disorders: an isolatednon-recurring episode which may be accompanied of a febrile illness or after a traumatic brain injury and, epilepsy: paroxysmal disorder and recurrent cerebral function characterized by short and sudden crisis of disturbance of consciousness accompanied by motor activity, sensory phenomena or misconduct caused by an excessive neuronal discharge.[4]

The first concepts of epilepsy were developed in ancient India, between 4500-1500 BC. In the literature the Charakas Samhita (400 b.c.) Ayurvedic, epilepsy was described as APASMARA, which meant “the loss of consciousness”. *The Charakas Samhita* contains abundant references to all aspects of epilepsy, including its symptoms, etiology, diagnosis, and treatment.

The tabula of ancient Babylon dates 2000 BC and provides records of different types of attacks associated with the name of an evil spirit, describes a treatment mainly of aspiritual kind. The Babylonian approach was the predecessor of the Greek concept of the sacred disease. Etymologically the word epilepsy is derived from the Greek "*Epilambanein"* which means to be shuddered suddenly. In 400 BC Hippocrates was the first to describe the disease as a brain abnormality: "disease consider sacred," he described the recommended physical treatments and stated that if the disease was chronic it was incurable.

In the 18th century, Neurology soon emerged, a new discipline different from psychiatry, the concept of epilepsy as a brain disorder became widely accepted, especially in Europe and the United States of America.[5] In 1873, in London, the neurologist John Hunglings Jackson suggested that the attacks were the result of an abnormal electric "shock occasionally suddenintense, rapid and repeated grey matter".

In 1929, Hans Berger, Professor of Psychiatry at the University of Jena, in Germany, published for the first time that brain electrical activity was likely to be recorded from the surface of the skull. Later, in 1931, Berger published electrographic changes associated with epilepsy. The EEG (Electro electroencephalogram) helped to locate the site of the seizures or attacks and expanded the possibilities of neuro-surgical treatments, which were made available to people from the 1950s onwards in London, Montreal and Paris. Finallyin 1973, the World Health Organization published a dictionary that defined epilepsy as a chronic condition of different etiology, characterized by recurrent seizures, due to an excessive discharge of brain neurons (seizures), associated eventually with different clinical and paraclinical manifestations".[5]

Seizures tend to be varied because partial seizures affect only a part of the brain, partial simple (focal), complex partial [6] must be supported on the characteristics of an electroencephalogram. Other studies such as computed tomography or magnetic resonance imaging are not initially required. Clinical and Electroencephalographic tests are very precise in this case to get the diagnosis. [7]

Regarding the treatment of partial seizures in epilepsy, some commonly used medications are: Felbamate (Felbatol) ®, Gabapentin (Neurontin) ®, levetiracetam (Keppra) ®, lamotrigine (Lamictal) ®, Oxcarbazepine (Trileptal) ®, pregabalin (Lyrica) ®, topiramate (Topamax) ® Tiagabine (Sabril) ®. For generalized seizures: levetiracetam (Keppra) ®, lamotrigine (Lamictal) ®, topiramate (Topamax) ®. Anti-seizure medications require regular blood checks in order to evaluate the appropriate doses and prevent side effects or toxic consumption.[8] There is no cure to epilepsy so far; this provides an added value to this experimental model.Epilepsy is one of the most common neurological disorders characterized by seizures. In India, the epileptic seizures resulted from excessive, abnormal, or hyper synchronous neuronal activity in the brain. It is clearly a worldwide public health that can be found in 5 out of every 10 people per 1000 inhabitants. Onset of new cases occur most frequently in infants and the elderly. Epilepsy is generally controlled, although not cured. In other words, over 30% of people with epilepsy do not have seizure control even with the best available medications.[9,17]

Pharmacotherapy eliminates completely the crises in a 1/3 of the patients and it reduces in a meaningful way their frequency in another third of them. About 2/3 of patients with well controlled seizures can discontinue medication without causing relapse.

The majority of people with epilepsy are neurologically normal except for their crisis, although

excessive use of antiepileptic drugs can decrease their level of alertness. Progressive mental decline is usually related to the neurological disorder that causes seizures. When having epilepsy the left temporal lobe can be associated with alterations of the verbal memory and the right temporal lobe can cause disorders on the viso-spatial memory. The prognosis is better when there is a demonstrable brain injury.[10] The presence of recurrent seizures of etiology are unknown[11] Epilepsy is defined as a disorder of the central nervous system characterized by the repetition of two or more seizures not caused by an immediately identifiable cause. The occurrence of a single crisis does not allow diagnosing epilepsy.[12]

The bufo-toxin is a toxin derived from the skin of the toad. [13] in its biochemical composition. It is a component that is formed as a result of the union of the bufofagin with one molecule of arginine. [14] its action is toxic and is observed at the level of enzymes by inhibiting the ATPase of the pump of Na + - k + cardiac muscle fiber, by blocking the activity of Na channels, elevates intracellular Ca ++ concentration, causing an increment in the contraction of the heart and a reduction ofcardiac frequency.[15]

Two fundamental features on which this study focused were: determining the dose of the diluted bufo-toxinin order to induce epilepsy in rats of the Balb-c strain and Winstar strain and describing the epileptic symptoms induced after the application of the bufo-toxin. The effect of bufo-toxin in rats has apparently not been studied. Therefore, the implementation of this project will allow to answer the following questions:

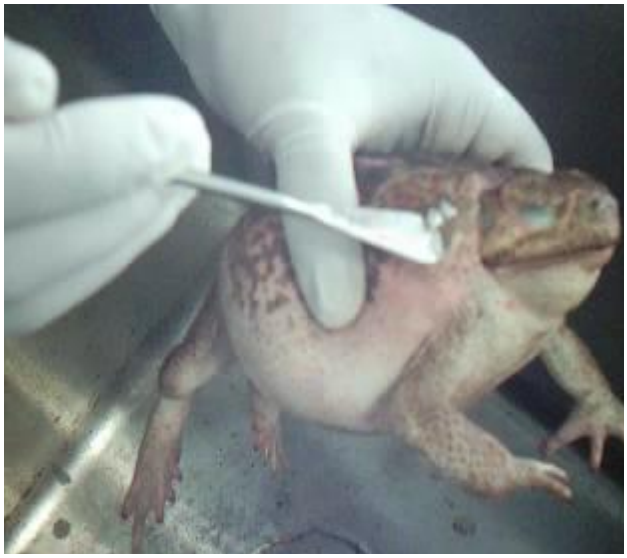

Figure1. Obtaining of bufo-toxin; considered as an oily poisonous secretion that is locatedin the dorsal cutaneousglands of the frog, commonly known as toad.

Source: Direct source, samples taken at the Physiochemical and biological Laboratory,UMG. Marista University of Guadalajara. (Ruvalcaba, 2013)

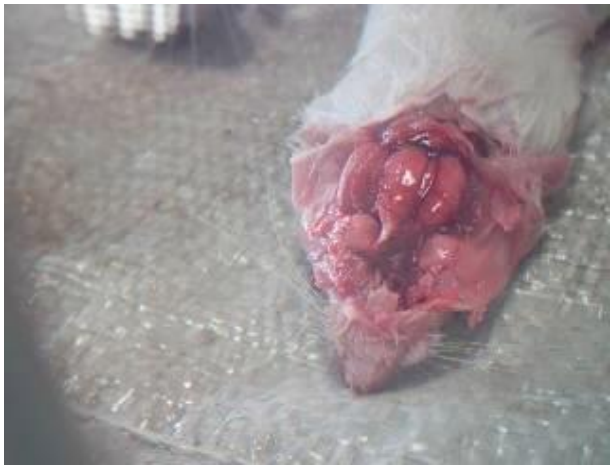

Figure 2. Exhibition of the brain of a rat which is under the effects of the bufo-toxin.

Source; Direct source, samples taken at the Physiochemical and biological Laboratory, at the University Marista of Guadalajara.

Research Questions

What is the effect of the bufo-toxin in two strains of rat?

Will it be possible to implement an experimental animal model to encourage further research on new epilepsy treatments?

Will it be possible to implement an experimental animal model in order to do research on new medical treatments that could treat heart diseases, such as hypertension and heart attacks?

HYPOTHESIS

Hi = the administering of the bufo-toxin in rats triggers induced epileptic seizures in both rat strains.
Ho = the administering of the bufo-toxin in rats does not trigger induced epileptic seizures in both rat strains.

VARIABLES

Table 1.-interelationsamong variables:

Independent Variable	Intervening Variable	Dependent Variable
Bufo-toxin intoxication.	Acquisition of a disease during their stay in the laboratory.	Epilepsy induced by the action of bufo-toxin.

Methodology

Type of study: anexperimental research design was employed; the researcher intervened by modifying the conditions under which the research was conducted.

Universe: Rats of the BALB/c and Wistar strain (8 days old, at a reproductive age)

Sample size: 50 rats of the BALB/c strain and 50 rats of the Wistar strain.

Method of sampling: probabilistic, randomized in blocks of 10 rodents.

Unit of analysis: rats of the BALB/c strain and Wistar strain (with epilepsy) which weregiven the bufo-toxin. Symptomatology and mortality of rats from the Balb/c and Wistar strains and whose epilepsy was induced through thebufo-toxin.

Methodology: Rats were kept in rooms of polycarbonate with food and water, they were fed by croquettes and the health conditions of the rats were constantly monitored. Since they remained in healthy spaces the inoculation of the toxin took place, by placing each rat in a separate space from the rest of the rats.

2 frogs from the *Bufonidae* family were bought for the extraction of the bufo-toxin, they were settled in a tank with a few water and they were fed by adding pieces of apples in order to attract insects for them so that they could eat. The toxin was removed from the glands of toadsunder controlled conditions,such glands are located on the sides of their neck, in the cephalic region; the toxin was stored in a previously sterilized autoclave test tube and diluted in alcohol and was conserved, such toxin was kept at minus 4 Celsius degrees. Each rat was weighted and measured in order to keep track of their appearance and see if their weight was correlated with the amount of toxin that were given. In a sterile lab area, the rat was placed and was administered 20 units of medication through aninsulin syringe because that was the amount the pilot study hinted and in which most of the symptomatology occurred without damaging the life of the rat in a short time.

During the piloting to standardize samples, there was difficulty to establish the pathogenesis in the balb-c strain, although the pathogenesis is similar, since seizures were less perceptible in the Wistar strain than in the BALB/c strain. The optimal standardized time to observe the symptomatology is 10 minutes after the inoculation of the toxin took place. Symptoms expressed were recorded in a log for further use (database). The rats that did not survive were subjects of a complete necropsy in order to record the findings in the log.

At the end of the experiment, the analysis of the results in SPSS-15 was conducted. The Marist University of Guadalajara provided itssupport for this research in terms of providing the physical laboratory and other facilities and the costs for conducting the research were subrogated by the researcher.To carry out this research project various resources wereincluded, both financial and materials.This research project was carried out at the biology of the Francisco Febres Cordero, La Salle College in its first phaseand the second phase at the physiochemical laboratory at the high school known as Cervantes, whose facilities belong to the Marist University of Guadalajara.

Inclusion criteria: rats of the BALB/c strain and the Wistar strain which were 4 to 45 weeks oldwere handled and controlled at the laboratory and did not show symptoms suggestive of a disease.

Criteria of non-Inclusion: rats less than 4 weeks and older than 45 weeks with symptoms suggestive of a disease.

Exclusion criteria:those rats that were not inoculated with the toxin and died of unknown causes.

Considerations of animals:they were kept under controlled conditions, in a healthy environment and were handled with respect and dignity.

Results

The results of this research make reference to the laboratory work performed with 50 rodents of the Balb/c strain and 50 of the Wistar strain, from which 40 (80%) of the Balb/c strain were female and 10 (20%) males, about the Wistar strain; 39 (78%) were female and only 11(22%) males. (Table 1).The gathering of thebufo-toxin; considered as an oily poisonous secretion was located in the dorsal cutaneous glands of the frog commonly known as Toad (Figure 1) The exhibition of brain under the effects of the bufo-toxin (Figure 2).

Regarding the age of rodents used in this research, it can be stated that Balb/c strain group was between 14 and 16 weeks old. The results obtained with the Balb/c strain denote that with among 10-15 units of toxin symptoms take 30 minutes to appear; this is somewhat invisible in some cases, so these rats were administered with 20 units and symptoms increased

its manifestation. Approximately in a period from 1 to 10 minutes the of effect can be clearly seen and the symptoms are initially presentedin a quiet period with bristly hair and tremors in their limbs. Within a few more minutes, tremor in their muscles was widespread, it was observed that their eyes have them somewhat bulging and that seizure-like movements started in their extremities, i.e., the movement performed was in clockwise direction beginning in the left upper extremity, then the right, follow the left lower extremity and thus continuing the right lower extremity showing the same pattern in the mobility of the tail.

During the epileptic seizure one position of dorsal decubitus was commonly seen. Onaverage, 30 minutes after convulsions dies, except in some cases, the death of rodent was not verified but apparently could have happened between the first 2 and 15 hours. The Necropsy revealed that stroke occurred as well as myocardial infarction as the main cause of death, apparently the effect is neurotoxic but it ends heart attacks. In reference to the ECA Wistar the same symptomswere observed and in this case thesymptoms were quantified (Table 1, 2)

The symptoms that the Wistar strain group manifested during the experimental phase were observed, there was broad similarity regarding the BALB/c strain, where 2 rats (4%) did not present any symptom and a rat presented 23 symptoms, 17 presented from 3 to 6 symptoms which represents 34% of the sample, regarding the necropsy performed to rodents their death was related to the administration of the toxin; 31 (62%) were not assigned to necropsy;it indicated a neurotoxic effect, mainly with hepatic and cardiovascular impact (Table 2,3) in the winstar strain seizures occurred very quickly compared to the Balb/c strain and were not as characteristic as in the BALB/c strain. Wistar strainsare apparently more sensitive to the toxin, but there is some concordance between both strains regarding the effect observed from the administration of the toxin. Finally, the highest percentage was registeredas a heart attack effect. (Table 4).

Table 1 Gender distribution in both rat strains.

Género	% Balb/c strain	% strain Wistar
Male	**80**	**22**
Female	**20**	**78**

Direct Source: physiochemical-biological laboratory, UMG. Marist University of Guadalajara. Biology laboratory, La Salle.

Table2. Percentage of symptoms with greater presence in rodents which were administeredbufo-toxin

Syntoms	percentage	Síntoms	percentage
Pilo-erection	85.4	Tearing	20.8
Hyperventilation	54.2	Defecates toxin effect	20.8
Hypersensibity to noice	45.8	Urine toxin effect	20.8
He Stratches his head	41.7	hyperactivity	18.8
Trembling jaw drooling	37.5	sialorrhea	14.6
Stupor	35.4	Clonic movements of head	12.5
Whiskers extended forward.	29.2	Dificulty opening eyes	10.4

Direct Source:physiochemical-biological laboratory, UMG. Marist University of Guadalajara. Biology laboratory, La Salle

Table 3. Findings at the necropsies.

Findings	percentage	Findings	percentage
Heart attack	**100**	Splenomegaly	32
Hepatomegaly	84	Testicular hypertrofy	11
Distended colon	68	Collapsed lung	5
Distended Stroke	53		

Direct Source: physiochemical-biological laboratory, UMG. Marist University of Guadalajara. Biology laboratory, La Salle

Table 4. Most commonly seen findings at the necropsies (Wistar strain group under the effects of bufo-toxin).

Main effect of the toxin	Percentage

Strok	**57**
Distended colon	**100**
Hepatomegaly	**71**
Heart attack	**100**

Source;Direct samples taken at the Physiochemical and biological Laboratory at the Universidad Marista of Guadalajara.

Discussion

The findings obtained at the laboratory and the knowledge generated, both, from the effect of bufo-toxin in rodents of the Balb-c strainsand in the Winstar strainswere of great importance, since they represent an option to do further research on this field and therefore implementnew drugs against epilepsy. Studies have been conducted on the intoxication with bufo-toxin located on the Toad Parotid gland and which has been implemented to dogs who experience for a 40 minute period epileptic symptoms, included those related to the heart rate increase, a negative deviation of the QRS complex, fibrillation, and death which occurs if the animal is not treated. Symptoms can be classified into three; slightly moderate and which lead to seizures, without providing any explanation on how they are and how they occur. Apparently there has not been publications where the effect of bufo-toxin is described; this constitutes an area of opportunity to conduct further research on epilepsy and on cardiology as well because this toxin also has effects on cholesterol, ergosterol and gamasistosterol, in reference to the nervous system, catecholamines, and adrenaline because it may trigger symptoms that are compatible with the same seizures or those of cardiac type; as case studies have been conducted with dogs which wereadministeredwith bufo-toxin.[15]

The induced symptomatology and the bufo toxin effects are compatible with real epilepsy episodes and among both rat strains this adds value to the use of new drugs on the eve of finding an effective cure against this disease. Induced seizures began on limbs and rat tails which marked the final phase of their lives, a situation in which the Winstar strains seizures occurred in a very fast way and were not as characteristic as in the Balb-c strain. Therefore, it is important to note that there is some relationship among both strains in reference to the effects of the toxin that they experienced.

The design of research models in order to conduct further research on this fieldis necessary, and should be randomized, multi-centered, and controlled in the future in order to assess the role that drugs play for the treatment of epilepsy. The bufo-toxin causes epilepsy in rats with lethal effect, which is a reversible situation by employing the same toxic substance but in a homeopathic preparationat 7 CH.[16]

Conclusions

1. The effect of the toxin is lethal.

2.-Rats which were 16 weeks old responded more clearly to the effect of the toxin and epilepsy was more clearly induced in rats that were 40 weeks old.

3. The Administering of the bufo-toxin allows obtaining an animal model that can be used to study epilepsy and myocardial infarction and that even can help find animal, vegetable or mineral treatments to reduce the effect of epilepsy.

4. The epilepsyis a public health problem;it not is curable and requires constant research; this is why experimenting models are important in assessing epilepsy treatments, the model of bufo-toxin aims to test acute lethal effects and it represents an option to test drugs against epilepsy or even against heart attacks and other cardiac diseases.

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