

## THE EFFECT OF HALOPERIDOL, AMINOXYACETIC ACID AND (-)-NUCIFERINE ON PROLONGING SURVIVAL TIME OF MICE WITH TETANUS

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Received 23 October 2017; Accepted 14 March 2018

Available online: 16 April 2018

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### Abstract

*Introduction.* Tetanus, also known as lockjaw, is a very dangerous, infectious, acute, usually afebrile disease characterised by muscle spasms. The causative agent of the disease is the bacterium *Clostridium tetani*. This pathogen produces a specific neurotoxin, termed tetanus toxin, with two components: tetanospasmin and tetanolysin. Light chains of tetanospasmin cleavage synaptobrevin, which in turn prevent release of the inhibitory neurotransmitter GABA into the synaptic cleft. The  $\alpha$ -motor neurons are, therefore, under no inhibitory control, as a result of which they undergo sustained excitatory discharge causing the characteristic motor spasms of tetanus.

*Materials and Methods.* In this research, we attempted to normalise disorders caused by tetanus toxin by using haloperidol (at doses of 4, 5, 6, 7 and 8 mg/kg b.w.), alone and in combination with (-)-nuciferine (at a dose of 5 mg/kg b.w.) or aminooxyacetic acid (at a dose of 20 mg/kg b.w.). Experiments were conducted on albino mice. Experimental tetanus was induced by application of tetanus toxin.

*Results and Conclusions.* Application of haloperidol (alone and in combination with (-)-nuciferine and aminooxyacetic acid) was carried out 24 h following the application of tetanus toxin. It was found that haloperidol, given alone in a dose of 4 mg/kg, prolonged the average survival time of mice with experimental tetanus by 24.35 h compared to the control animals. Additionally, the combination of haloperidol with (-)-nuciferine slightly, but non-significantly, extended survival time, while the combination of haloperidol with aminooxyacetic acid produced the best effect on extension of survival time (mice survived on average 27.74 h longer than control mice).

Key Words: tetanus, tetanus toxin, haloperidol, (-)-nuciferine, aminooxyacetic acid

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## INTRODUCTION

Tetanus is a very dangerous infectious disease occurring in humans and various animal species which characterised by muscle spasms. The causative agent of the disease is *Clostridium tetani*, a gram positive, anaerobic bacillus. Older or stressed cells of *Cl. tetani* lose their flagella after the development of spores (Cato et al., 1986). The bacteria usually enter the human or animal body through damaged skin (Collee & van Heyningen, 1990).

*Cl. tetani* produces a specific neurotoxin – tetanus toxin (Atkinson, 2012), which consists of two components: tetanospasmin and tetanolysin (Schiavo et al., 1992). Tetanospasmin is responsible for the appearance of clinical signs of disease, while the function of tetanolysin is still unknown (Rizwan, 1999). Tetanospasmin consists of heavy (H) and light (L) chains (Doussau et al., 1999). The carboxyl terminal portion of the H chain, termed HC, mediates attachment to gangliosides (GD1b and GT1b) on peripheral nerves (Li et al., 1994; Collee & van Heyningen, 1990). It is then moved from the peripheral to the central nervous system by retrograde axonal transport and trans-synaptic spread. The entire toxin molecule is internalised into presynaptic cells and, in a process requiring the HN fragment, the L chain is released from the endosome. The L chain cleaves synaptobrevin (Schiavo et al., 2000). Synaptobrevin is an integral membrane component of synaptic vesicles and it is essential for the fusion of synaptic vesicles with the presynaptic membrane (Hardman & Limbird, 1996). Outside the nervous system, synaptobrevin is found in endocrine cells (Baumert et al., 1989). Cleavage made by tetanus toxin L chain prevents the release of their contents, i.e. the inhibitory neurotransmitter  $\gamma$ -aminobutyric acid (GABA), into the synaptic cleft. The  $\alpha$ -motor neurons are, therefore, under no inhibitory control, and thus, they undergo sustained excitatory discharge causing the characteristic motor spasms of tetanus. The toxin exerts its effects on the spinal cord, the brain stem, peripheral nerves, neuromuscular junctions, and directly onto muscles. The extent to which cortical and subcortical structures are involved remains unknown. However, it is well known that the toxin is a potent convulsant when injected into the cortex of experimental animals (Montecucco & Schiavo, 1994).

Therefore, in this research, we examined the effect of haloperidol (a butyrophenone neuroleptic), a substance that acts as a tranquillizer, sedative and muscle relaxant and its combination with compounds that antagonise the central effects of tetanus toxin on prolonging survival time in mice with experimental tetanus.

## MATERIALS AND METHODS

### Materials

The study was conducted on albino mice of both sexes weighing approximately 20-25 grams (strain Pasteur Institute Novi Sad; bred at the Department of Pharmacology

and Toxicology of the Veterinary Faculty in Sarajevo). For the purposes of this study, we used a control group (5 animals per group) and experimental groups (10 animals per group). The Ethics Committee of the Veterinary Faculty, Sarajevo, approved the research and experimental procedures (Approval No. 1/17, date 20.05.2017). The animals were kept in conventional conditions and were treated according to the Animal Welfare Regulations. The animals were maintained on standard diet with free access to water and housed in groups of 10 mice per cage for seven days prior to the experiment.

### **Substances used in the study**

- tetanus toxin (Institute of Immunology, Zagreb, Croatia);
- haloperidol (Sigma-Aldrich, USA);
- (-)-nuciferine (Pharmaceutical Research Department, F. Hoffmann-La Roche CO., Ltd., Ch-4002 Basel, Switzerland);
- aminooxyacetic acid (Sigma-Aldrich, USA);
- water for injection, used to dilute all the above substances.

### **Methods**

Prior to its administration, tetanus toxin was diluted in water for injection and was then administered intramuscularly (i.m.) in the *m. gastrocnemius* of the mouse's right leg, at a dose of 0.2 µg per animal. We used this dose of tetanus toxin on the basis of previously conducted studies (Hadžović et al., 1975; Muminović, 1983), when it was used to determine the period of time for LD<sub>50</sub> of tetanus toxin for each group. At that time, it was established that a dose of 0.2 µg per animal administered i.m. kills 50% of experimental animals within 48 hours, while a dose of 0.1 µg per animal kills 50% of experimental mice within 7 days.

Specific doses of haloperidol, (-)-nuciferine and aminooxyacetic acid, intended for use in this study, were established in a preliminary study due to lack of bibliographic data. Consequently, the substances were administered at the following doses: haloperidol was administered subcutaneously (s.c.) (at doses of 4, 5, 6, 7 and 8 mg/kg b.w.), whilst (-)-nuciferine (at a dose of 5 mg/kg b.w.) and aminooxyacetic acid (at a dose of 20 mg/kg b.w.) were administered intraperitoneally. These above mentioned substances were administered to mice in experimental groups after the occurrence of local tetanus in the right leg, approximately 24 h after administration of tetanus toxin. The substances were administered once per day, which was continued until the animal died. Each experimental group had its own control group, with tetanus toxin and the solvent (water for injection) administered in an equivalent way as the tetanus toxin/water for injection in the experimental mouse groups.

Basic statistical data diagnostics was conducted by using Microsoft Excel® (Microsoft Office package, Microsoft, USA).

## RESULTS AND DISCUSSION

### Induction of experimental tetanus:

In animals with no application of life-extending substances, the first signs of local tetanus in each animal's right leg were registered 24 h after application of tetanus toxin. The leg was stiff and extended, and during this period, no death of experimental animals was recorded. In the next 48 h, general tetanus started to develop, followed by the animal's death.

### Application of substances:

Effects of haloperidol (central dopamine receptor antagonist) and its combination with (-)-nuciferine (competitive antagonist of glutamate) and aminoxyacetic acid (inhibits 4-aminobutyrate aminotransferase or GABA-T activity) on prolonged survival time in mice with experimental tetanus are presented in Table 1.

**Table 1.** Effects of haloperidol (in different doses), and combination of haloperidol with (-)-nuciferine, as well as combination of haloperidol with aminoxyacetic acid on the survival time of tetanus toxin-affected mice

Groups	N	Substances	Doses ( $\mu\text{g}/\text{kg}$ , b.w.; $\text{mg}/\text{kg}$ b.w.)	Survival time (h)	
Control	5	Tetanus toxin	0.2 $\mu\text{g}/\text{b.w.}$	$89.20 \pm 2.60$	
			0.2 $\mu\text{g}/\text{kg}$ , b.w. + 4 $\text{mg}/\text{kg}$ , b.w.	$113.55 \pm 7.14$	
	10	Tetanus toxin +	0.2 $\mu\text{g}/\text{kg}$ , b.w. + 5 $\text{mg}/\text{kg}$ , b.w.	$106.95 \pm 6.40$	
			0.2 $\mu\text{g}/\text{kg}$ , b.w. + 6 $\text{mg}/\text{kg}$ , b.w.	$102.00 \pm 5.06$	
			0.2 $\mu\text{g}/\text{kg}$ , b.w. + 7 $\text{mg}/\text{kg}$ , b.w.	$107.85 \pm 10.01$	
			0.2 $\mu\text{g}/\text{kg}$ , b.w. + 8 $\text{mg}/\text{kg}$ , b.w.	$109.25 \pm 4.01$	
	10	Tetanus toxin +	0.2 $\mu\text{g}/\text{kg}$ , b.w. +	$94.35 \pm 3.97$	
			haloperidol +		
	Experimental	10	Tetanus toxin +	5 $\text{mg}/\text{kg}$ , b.w.	$116.94^* \pm 7.53$
				haloperidol +	
10		Tetanus toxin +	4 $\text{mg}/\text{kg}$ , b.w. +	$116.94^* \pm 7.53$	
			aminoxyacetic acid 20 $\text{mg}/\text{kg}$ , b.w.		

N = number of animals; b.w. = body weight; \*significant

The first part of the experiment was carried out to determine the effective dose of central dopamine receptor antagonist haloperidol (Table 1). Haloperidol at s.c. doses of 5, 6, 7 and 8  $\text{mg}/\text{kg}$  b.w. did not effectively prolong mouse survival times

compared to the control animals. However, a dose of 4 mg/kg b.w. achieved the best results, prolonging the average survival time of the mice to almost 114 h, while the average survival time of control mice was around 89 h (Table 1). Haloperidol leads to relaxation of skeletal muscles from the spasm due to its direct interaction with inhibitory transmitter receptors on the body (soma) of  $\alpha$ -motor neurons in the spinal cord (Coyne, 2006; Muminović, 1983; Nakamura et al. 2000). Haloperidol also acts as a tranquillizer (Maddison et al., 2008), which is very important, especially in tetanus, where reactions to external stimuli are extremely strong.

In order to expand the research, we combined haloperidol with (-)-nuciferine, a competitive antagonist of glutamate (Farkas & Ono, 1995; Roberts & Porter, 1995). Co-administration of haloperidol and (-)-nuciferine caused a slight extension of survival time in mice with experimental tetanus (Table 1). However, the applied (-)-nuciferine dose of 5 mg/kg b.w. did not give the expected results, so this substance was not further used in this study. We had assumed that concomitant use of haloperidol and (-)-nuciferine could lead to summation of their effects, which was confirmed in our research (data not shown).

We also combined haloperidol with aminooxyacetic acid. This combination produced the longest extension of mice survival time, to around 117 h (Table 1). Aminooxyacetic acid inhibits (GABA-T) activity *in vitro* and *in vivo*, leading to less  $\gamma$ -aminobutyric acid (GABA) being broken down. Subsequently, the level of GABA is increased in tissues (Loshner et al., 1989; Mujezinović et al., 2011).

## CONCLUSION

In conclusion, haloperidol at a dose of 4 mg/kg b.w. prolonged the average survival time of tetanus toxin-affected mice by 24.35 h compared to the control group of mice with experimental tetanus. However, the application of higher doses (5, 6, 7 and 8 mg/kg b.w.) did not produce further increases of survival time. Co-administration of haloperidol with (-)-nuciferine led to a slight extension of survival time in mice with experimental tetanus, whereas the combination of haloperidol with aminooxyacetic acid produced the best effect on the extension of survival time (mice survived 27.74 h longer than control mice).

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## DELOVANJE HALOPERIDOLA, AMINOOKSISIRČETNE KISELINE I (-)-NUCIFERINA NA PRODUŽENJE PREŽIVLJAVANJA MIŠEVA S TETANUSOM

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### Kratak sadržaj

*Uvod.* Tetanus je veoma opasna, akutna, obično afebrilna bolest koju karakteriše mišićni spazam. Uzročnik obolenja je bakterija *Clostridium tetani*, koja proizvodi specifičan neurotoksin ili tetanus toksin. Toksin se sastoji od dve komponente: tetanospazmina i tetanolizina. Laki lanac tetanospazmina cepa sinaptobrevin, što za posledicu ima sprečavanje oslobađanje inhibitorynog neurotransmitera GABA u sinaptički procep. Funkcija ekscitatornih neurotransmitera ostaje očuvana, te pod njihovim uticajem dolazi do grča skeletne muskulature.

*Materijali i metode.* U ovom istraživanju pokušali smo normalizovati poremećaje uzrokovane tetanus toksinom koristeći haloperidol (u dozama od 4, 5, 6, 7 i 8 mg/kg t.m.), samostalno i u kombinaciji sa (-)-nuciferinom (u dozi od 5 mg/kg t.m.) i aminooksisirčetnom kiselinom (u dozi od 20 mg/kg t.m.). Kompletno istraživanje je sprovedeno na albino miševima. Eksperimentalni tetanus izazvan je aplikacijom tetanus toksina.

*Rezultati i zaključak.* Primena haloperidola (samo i u kombinaciji sa (-)-nuciferinom i aminooksisirčetnom kiselinom) vršena je 24 sata nakon aplikacije tetanus toksina. Haloperidol, aplikovan u dozi od 4 mg/kg t.m., produžio je vreme preživljavanja miševa s eksperimentalnim tetanusom za oko 24.35 sati u odnosu na kontrolnu grupu životinja, te smo ovu dozu smatrali jedino opravdanom za dalja istraživanja. Kombinacija haloperidola s (-)-nuciferinom neznatno produžava vreme preživljavanja, dok je kombinacija haloperidola sa aminooksisirčetnom kiselinom imala nabolji efekat na produženje ovog perioda. Period preživljavanja je produžen oko 27.74 sata u odnosu na kontrolnu grupu životinja.

**Ključne reči:** tetanus, tetanus toksin, haloperidol, (-)-nuciferin, aminooksisirčetna kiselina