

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

MUJEZINOVIĆ Indira^{1*}, SMAJLOVIĆ Ahmed¹, ČUPIĆ Vitomir²

¹University of Sarajevo, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Sarajevo, Bosnia and Herzegovina

²University of Belgrade, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Belgrade, Serbia

Received 23 October 2017; Accepted 14 March 2018

Available online: 16 April 2018

Copyright © 2018 Mujezinovic et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

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 α -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

*Corresponding author – e-mail: indira.mujezinovic@vfs.unsa.ba

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 γ -aminobutyric acid (GABA), into the synaptic cleft. The α -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 LD₅₀ 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.; mg/kg b.w.)	Survival time (h)		
Control	5	Tetanus toxin	0.2 $\mu\text{g}/\text{b.w.}$	89.20 ± 2.60		
			0.2 $\mu\text{g}/\text{kg}$, b.w. + 4 mg/kg , b.w.	113.55 ± 7.14		
			0.2 $\mu\text{g}/\text{kg}$, b.w. + 5 mg/kg , b.w.	106.95 ± 6.40		
			0.2 $\mu\text{g}/\text{kg}$, b.w. + 6 mg/kg , b.w.	102.00 ± 5.06		
			0.2 $\mu\text{g}/\text{kg}$, b.w. + 7 mg/kg , b.w.	107.85 ± 10.01		
	10	Tetanus toxin + haloperidol	0.2 $\mu\text{g}/\text{kg}$, b.w. + 8 mg/kg , b.w.	109.25 ± 4.01		
			0.2 $\mu\text{g}/\text{kg}$, b.w. + 4 mg/kg , b.w.	94.35 ± 3.97		
			5 mg/kg , b.w.			
			10	Tetanus toxin + nuciferine	0.2 $\mu\text{g}/\text{kg}$, b.w. + 4 mg/kg , b.w.	$116.94^* \pm 7.53$
					20 mg/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 mg/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 α -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 γ -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).

REFERENCES

- Atkinson W. 2012. Tetanus Epidemiology and Prevention of Vaccine-Preventable Diseases (12 ed.). Public Health Foundation, pp. 291-300.
- Baumert M., Maycox P.R, Navone F., De Camilli P., Jahn R. 1989. Synaptobrevin: an integral membrane protein of 18,000 daltons present in small synaptic vesicles of rat brain. *The EMBO Journal*, (8):379-84.
- Cato E.P., George W.L., Finegold S.M. 1986. Genus *Clostridium praemozski* 1880, 23AL. In: Sneath PHA, Mair NS, Sharpe ME, et al., eds: Bergey's Manual of Systemic Bacteriology. Vol II. Baltimore: Williams and Wilkins, pp. 1141-200.

- Collee J.G., van Heyningen S. 1990. Systemic toxogenic diseases (tetanus, botulism). In: Duerden BI, Draser BS, Eds. Anaerobes and human disease. London: Edward Arnold, pp. 372-94.
- Coyne C.P. 2006. Comparative Diagnostic Pharmacology: Clinical and Research Application in Living-System Models. (1 ed.). Blackwell Publishing. pp. 263-4.
- Doussau F, Humeau Y, Vitiello F, Popoff MR, Poulain B. 1999. Analysis of synaptic neurotransmitter release mechanisms using bacterial toxins. *Journal de la Société de Biologie*, 193 (6):457-67.
- Farkas S., Ono H. 1995. Participation of NMDA and non-NMDA excitatory amino acid receptors in the mediation of spinal reflex potentials in rats: an in vivo study. *British Journal of Pharmacology*, 114: 1193-205.
- Hadžović S., Brankov K., Hadžović J., Muminović M. 1976. Inhibitorni transmittori u terapiji tetanusa. *Veterinaria*, Sarajevo 25:1-2.
- Halpern J.L., Neale E.A. 1995. Neurospecific binding, internalization, and retrograde axonal transport. *Current Topics in Microbiology and Immunology*, 195:221-41.
- Hardman J.G., Limbird L.E. 1996. Goodman & Gilman's The Pharmacological Basis of Therapeutics. Ninth Edition. The McGraw-Hill Companies. New York, pp. 361-96.
- Lalli G., Herreros J., Osborne S.L., Montecucco C. 1999. Functional characterisation of tetanus and botulinum neurotoxins binding domains. *Journal of Cell Science*, 112:2715-24.
- Li Y., Foran P., Fairweather N.F. 1994. A single mutation in the recombinant light chain of tetanus toxin abolishes its proteolytic activity and removes the toxicity seen after reconstitution with native heavy chain. *Biochemistry*, 33:7014-20.
- Loscher W., Honack D., Gramer M. 1989. Use of inhibitors of gamma-aminobutyric acid (GABA) transaminase for the estimation of GABA turnover in various brain regions of rats: a reevaluation of aminooxyacetic acid. *Journal of Neurochemistry*, 53(6):1737-50.
- Maddison J.E., Page S.W., Church D.B. 2008. Small animal clinical pharmacology. (2 ed.). Elsevier Limited.
- Montecucco C., Schiavo G. 1994. Mechanism of action of tetanus and botulinum neurotoxins. *Molecular Microbiology*, 13:1-8.
- Mujezinović I., Muminović M., Smajlović A. 2011. The effect of caroverine and its combination with aminooxyacetic acid on survival time of mice with experimental tetanus. *Veterinaria*, 60 (1-2):1-8.
- Muminović M. 1983. Kombinovano funkcionalno i kompetitivno antagoniziranje djelovanja tetanus toksina kao prilog terapiji tetanusa. Magistarski rad. Sarajevo.
- Nakamura T., Uramura K., Nambu T., Yada T., et al. 2000. Orexin-induced hyperlocomotion and stereotypy are mediated by the dopaminergic system. *Brain Research*, 873:181-7.
- Rizwan A.M. 1999. Tetanus: A case report. *Can. J. Plast. Surg.* 7 (1):39-40.
- Schiavo G., Benfenati F., Poulain B. 1992. Tetanus and botulinum-B neurotoxins block neurotransmitter release by proteolytic cleavage of synaptobrevin. *Nature*, 359:832-5.
- Roberts P.J., Porter R.H.P. 1995. Characterization and modulation of binding glutamate receptors in: CNS Transmitters and Neuromodulators: Glutamate. T.W. Stone. (ed.) CRC Press, Boca Raton, FL. Chap. 5.
- Schiavo G., Matteoli M., Montecucco C. 2000. Neurotoxins affecting neuroexocytosis. *Physiological Reviews*, 80(2):717-66.
- Zamula E. 1996. Adults need tetanus shots, too. *FDA Consumer Magazine*.

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

MUJEZINOVIĆ Indira, SMAJLOVIĆ Ahmed, ČUPIĆ Vitomir

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