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EXPERIMENTAL VALIDATION OF APPROACHES TO PANCURONIUM BROMIDE USE FOR CORRECTION OF MYASTHENIC SYNDROME IN ANTICHOLINESTERASE POISONING

Mikhail A. Tyunin, Nikita S. Ilinskii✉, Sergey V. Chepur, Vladimir A. Matseychik, Elizaveta Yu. Izhorskaya

State Research and Testing Institute of Military Medicine, St. Petersburg, Russia

Introduction. The problem of intermediate syndrome following anticholinesterase poisoning remains poorly studied. This syndrome is clinically manifested as a myasthenic condition developing after the cholinergic crisis, affecting the muscles of the face, neck, proximal limbs, and respiratory muscles. The current literature describes limited attempts to use non-depolarizing muscle relaxants for the prevention and treatment of this condition. However, given the diversity of mechanisms underlying toxic myasthenia in anticholinesterase poisoning and the low safety profile of non-depolarizing muscle relaxants, research into the effective and toxic doses of these drugs is highly relevant for establishing the principles of their use in correcting neuromuscular transmission disorders.
Objective. To experimentally evaluate the efficacy of pancuronium bromide, a non-depolarizing muscle relaxant, in correcting neuromuscular transmission impairments in cases of anticholinesterase poisoning.
Materials and methods. Experimental, two-stage studies were conducted using male outbred white rats weighing 220–250 g (n = 78). Initially, pancuronium bromide was administered subcutaneously to intact animals to determine effective doses. The severity and duration of the muscle relaxant effect were assessed using clinical and functional tests (hanging on a horizontal bar, assessment of movement impairments according to the De Bleecker scale), and electromyography (single and rhythmic stimulation at 30 Hz). Subsequently, therapeutic doses of the drug were determined in a rat model of fenthion poisoning (12 h after its single subcutaneous administration at an LD_{50} dose of 479.4 mg/kg) based on data from the aforementioned methods and evaluation of changes in animal mortality rates. Statistical analysis of the results was performed using non-parametric statistical methods in the Prism GraphPad 9.0 software environment.
Results. Based on the assessment of neurological status and electromyography results, the median effective dose (ED_{50}) of pancuronium upon subcutaneous administration in intact rats was found to be 238.0 [95% CI: 219.8; 257.7] μg/kg. In contrast, against the background of severe fenthion poisoning, its therapeutic dose was statistically significantly lower ($p < 0.05$, Student's t-test), amounting to 90.1 [95% CI: 77.3; 105.1] μg/kg. Similar trends were observed for the median lethal doses (LD_{50}) of pancuronium bromide: 321.1 [95% CI: 305.8; 337.1] μg/kg and 152.3 [130.6; 177.6] μg/kg, respectively. Administration of pancuronium at the median therapeutic dose reduced the severity of the myasthenic syndrome induced by fenthion poisoning, manifesting as restored muscle strength and normalized electrophysiological characteristics of neuromuscular transmission.
Conclusions. The experiment demonstrated that pancuronium bromide can be used to correct neuromuscular transmission disorders underlying the intermediate syndrome emerging as a result of anticholinesterase poisoning. The effective dose for this correction is 90.1 μg/kg, which is 2.6-fold lower than the effective dose for healthy animals (238.0 μg/kg). The main electrophysiological criteria for the regression of the neuromuscular block should include a reduction in the number of repeated M-responses and the restoration of the decrement-increment pattern of the M-response series, which persist for 1 h after pancuronium bromide administration.

Keywords: pancuronium bromide; organophosphorus compound; intermediate syndrome; electroneuromyography; clinical-functional tests; toxic myasthenia

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✉ Nikita S. Ilinskii gniiivm_7@mil.ru

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ЭКСПЕРИМЕНТАЛЬНОЕ ОБОСНОВАНИЕ ПОДХОДОВ К ПРИМЕНЕНИЮ ПАНКУРОНИЯ БРОМИДА ДЛЯ КОРРЕКЦИИ МИАСТЕНИЧЕСКОГО СИНДРОМА ПРИ ОТРАВЛЕНИЯХ АНТИХОЛИНЭСТЕРАЗНЫМИ ЯДАМИ

М.А. Тюнин, Н.С. Ильинский✉, С.В. Чепур, В.А. Мацейчик, Е.Ю. Ижорская

Государственный научно-исследовательский испытательный институт военной медицины Министерства обороны Российской Федерации, Санкт-Петербург, Россия

Введение. До настоящего времени малоизученной остается проблема промежуточного синдрома при отравлениях антихолинэстеразными веществами, который клинически представляет собой развивающийся после холинэргического криза миастенический синдром, затрагивающий мышцы лица, шеи, проксимальных отделов конечностей и дыхательные мышцы. В литературе описаны немногочисленные попытки применения недеполяризующих миорелаксантов с целью профилактики и лечения промежуточного синдрома. Вместе с тем, учитывая многообразие механизмов токсической миастении при отравлениях антихолинэстеразными ядами и низкую безопасность недеполяризующих миорелаксантов, представляются актуальными исследования по определению эффективных и токсических доз рассматриваемых лекарственных средств в целях обоснования принципов их применения для коррекции нарушений нервно-мышечной передачи.

Цель. Экспериментально оценить эффективность недеполяризующего миорелаксанта панкурония бромида в отношении коррекции нарушений нервно-мышечной передачи при отравлениях антихолинэстеразными ядами.

Материалы и методы. Экспериментальные исследования проведены на самцах белых беспородных крыс массой 220–250 г (n = 78) в два этапа. Первоначально недеполяризующий миорелаксант панкурония бромид вводили подкожно интактным животным для определения его эффективных доз по развитию миорелаксации. Выраженность и длительность миорелаксантного эффекта оценивали при помощи клинико-функциональных тестов (подтягивание на горизонтальной перекладине, нарушения движений по шкале J. De Bleecker) и электромиографии (одиночная и ритмическая стимуляция с частотой 30 Гц). Затем определяли терапевтические дозы препарата на модели отравления крыс фентионом (через 12 ч после однократного подкожного введения в дозе $ЛД_{50}$ = 479,4 мг/кг) по данным указанных методов исследований и оценки изменения частоты гибели животных. Статистический анализ результатов проводили методами непараметрической статистики при помощи программного обеспечения Prism GraphPad 9.0.

Результаты. На основании данных оценки неврологического статуса и результатов электромиографии было установлено, что значение средней эффективной дозы панкурония при подкожном введении у интактных крыс составило 238,0 [95% ДИ: 219,8; 257,7] мкг/кг, в то время как на фоне тяжелого отравления фентионом значение его терапевтической дозы статистически значимо ниже (p < 0,05, t-критерий Стьюдента) и равно 90,1 [95% ДИ: 77,3; 105,1] мкг/кг. Аналогичные закономерности прослежены и для средних смертельных доз панкурония бромида: 321,1 [95% ДИ: 305,8; 337,1] и 152,3 [130,6; 177,6] мкг/кг соответственно. Введение панкурония в средней терапевтической дозе снижало выраженность миастенического синдрома, вызванного отравлением фентионом, в виде восстановления мышечной силы и нормализации электрофизиологических характеристик нервно-мышечной передачи.

Выводы. Экспериментально показано, что с целью коррекции расстройств нервно-мышечной передачи, лежащих в основе промежуточного синдрома при отравлениях антихолинэстеразными ядами, может быть использован панкурония бромид в сниженной в 2,6 раза (90,1 мкг/кг) относительно эффективной для здоровых животных (238,0 мкг/кг) дозе. К основным электрофизиологическим критериям регресса нервно-мышечного блока следует относить сокращение числа повторных М-ответов и восстановление декремент-инкремента серии М-ответов, сохраняющиеся в течение 1 ч после введения панкурония бромида.

Ключевые слова: панкурония бромид; фосфорорганическое соединение; промежуточный синдром; электронейромиография; клинико-функциональные тесты; токсическая миастения

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Финансирование: исследование выполнено без спонсорской поддержки.

Соответствие принципам этики: исследование выполнено с соблюдением правил биоэтики, утвержденных Европейской конвенцией о защите позвоночных животных, используемых для экспериментальных и других целей. Проведение исследований одобрено на заседании биоэтического комитета ФГБУ «ГНИИИ ВМ» МО РФ (протокол № 9 от 27.04.2020).

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✉ Ильинский Никита Сергеевич gniiivm_7@mil.ru

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INTRODUCTION

For decades, research conducted by the Russian school of toxicology has been focused on the development of approaches to preventing and treating cases of anticholinesterase poisoning. These efforts have resulted in the appearance of effective antidotes and agents for pathogenetic therapy [1, 2]. However, these antidotal drugs fail to counteract the entire spectrum of toxic effects produced by anticholinesterase compounds (AChEs), which are associated with severe delayed effects (intermediate syndrome, delayed polyneuropathies) [1–3].

To date, the intermediate syndrome (IS) emerging during poisoning with AChEs remains poorly studied [4]. Clinically, IS presents as a myasthenic syndrome developing after the cholinergic crisis, affecting the muscles of the face, neck, proximal limbs, and respiratory muscles [5]. According to clinical studies, IS develops 24–98 h after the poison enters the body. The duration of IS varies from 5 to 30 days. The most severe IS manifestation involves the paralysis of the respiratory muscles, leading to progressive respiratory failure. The incidence of IS ranges 10.0–68.0%, depending on the type of toxicant, with the mortality rate reaching 25.0–40.0% [7].

The Russian toxicology school regards IS as Phase II of toxic myopathy or as Type 2 paralysis [8]. Toxic myopathy is understood as a unified pathological process at the level of the neuromuscular synapse, characterized by the development of a neuromuscular block (NMB). Clinically, the course of toxic myopathy is conventionally divided into two phases. Phase I involves manifestations of a spastic neuromuscular block (Type 1), presenting as

widespread myofibrillations and muscle rigidity. Phase II corresponds to the onset of a paralytic NMB, manifesting as progressive weakness of the skeletal musculature [5, 8, 9].

Although the neuromuscular transmission impairments underlying IS have been studied [3, 5], a unified consensus on its pathogenesis and treatment has yet to be established. In particular, no effective methods for treating IS have been developed to date. It is believed that the main therapeutic measures are based on conventional approaches for etiotropic and detoxification therapy recommended for AChE poisonings. The cornerstone of IS management remains the timely detection of respiratory muscle paralysis; upon its onset, immediate initiation of artificial ventilation (AV) with prophylactic administration of antibacterial agents is indicated [6, 9].

Given the key role of NMB in the pathogenesis of IS [10], a limited number of studies have reported data on the successful use of non-depolarizing muscle relaxants (NDMRs) in the integrated therapy of AChEs poisoning [11]. Experimental and clinical studies have demonstrated the positive effects of rocuronium and atracurium [11, 12], leading the authors to recommend further investigation into the efficacy and safety of NDMRs. Therefore, the development of dosing strategies and optimal administration protocols for their use in AChE poisonings is a relevant research direction [3, 4].

In this work, we experimentally evaluate the efficacy of pancuronium bromide, a non-depolarizing muscle relaxant, in correcting neuromuscular transmission impairments in cases of anticholinesterase poisoning.

MATERIALS AND METHODS

The study was performed using male outbred white rats weighing 220–250 g (Rappolovo Breeding Nursery, Leningrad Oblast). The animals were housed in a vivarium under standard zoohygienic conditions and regulations. The experimental procedures were conducted in accordance with the requirements of the Order of the Ministry of Health of Russia.[1]

The study was conducted in two stages. During the first stage, the duration of muscle relaxation, effective doses, and toxic doses of the long-acting aminosteroid non-depolarizing muscle relaxant (NDMR) pancuronium bromide (Sigma-Aldrich, USA) were determined in intact rats following a single subcutaneous (s.c.) administration at doses ranging 200–350 μg/kg. Using a block randomization method, seven experimental groups were formed, each consisting of six rats: Group 1 — 200 μg/kg; Group 2 — 225 μg/kg; Group 3 — 250 μg/kg; Group 4 — 280 μg/kg; Group 5 — 300 μg/kg; Group 6 — 320 μg/kg; Group 7 — 350 μg/kg.

Electroneuromyography (ENMG) was performed using a Neuro-MVP-4 electromyograph (Neurosoft, Russia) and the Neuro-MVP-Omega software in accordance with a previously described methodology without the use of anesthetics [13]. To record the muscle relaxant effect of pancuronium bromide, ENMG was conducted in rhythmic stimulation (RS) mode at a frequency of 30 Hz, since high-frequency RS has been established as the most informative test for diagnosing neuromuscular transmission (NMT) impairments. The electrophysiological criteria for achieving the target effect of the muscle relaxant were defined as minimally evident signs of NMT block on ENMG, specifically a 10.0% decrease in the M-response amplitude (area) from baseline values upon single stimulation and/or the appearance of a decrement in amplitude (area) exceeding 10.0% during rhythmic stimulation. Neurological deficit and ENMG were monitored at two-minute intervals for 2 h after administration of the muscle relaxant.

During the second stage of the study, using the aforementioned diagnostic methods, the effect of pancuronium bromide administered subcutaneously across a dose range on the dynamics of NMT impairments in a toxic rat model was evaluated. This evaluation was conducted 12 h after the application of fenthion (IUPAC: dimethoxy-(3-methyl-4-methylsulfanylphenoxy)-sulfanylidene-λ5-phosphane, Adamanth, Russia) at the median lethal dose (LD_{50}/1 day = 479.4 mg/kg bw subcutaneously). The selection of fenthion as a model toxicant was based on the high frequency of IS manifestation in poisoning events [5, 7]. Using the block randomization method, six experimental groups were formed, each consisting of six rats: Group 1 — fenthion 479.4 mg/kg + pancuronium bromide 64.1 μg/kg; Group 2 — fenthion 479.4 mg/kg + pancuronium bromide 83.3 μg/kg; Group 3 — fenthion 479.4 mg/kg + pancuronium bromide 108.3 μg/kg; Group 4 — fenthion 479.4 mg/kg + pancuronium bromide 140.8 μg/kg; Group 5 — fenthion 479.4 mg/kg + pancuronium bromide 183.0 μg/kg; Group 6 — fenthion 479.4 mg/kg + pancuronium bromide 238.0 μg/kg.

Statistical analysis of the data was performed using the Prism GraphPad 9.0 software (GraphPad Software, USA) in the Windows 10 operating system. Multiple comparisons of unpaired samples were conducted using the Kruskal–Wallis test. Comparison of binary qualitative data was performed using Fisher's exact test. Effective and lethal doses were calculated using Finney's probit analysis in the Statistics 2005+ software (Russia). Doses are presented as the mean value and 95% confidence interval. Differences between groups were considered statistically significant at $p < 0.05$.

RESULTS

Determination of effective and toxic doses of pancuronium bromide in intact rats

During the first stage of the study, the effective and toxic doses of pancuronium bromide administered to

[1] Order of the Ministry of Health of the Russian Federation № 199n «On Approval of the Rules of Good Laboratory Practice» dated 01.04.2016.

Table 1. Results of the 30 Hz rhythmic stimulation test in rats after single subcutaneous administration of pancuronium bromide

Dose, μg/kg bw	Decrement incidence, %	Decrement value, %	Onset time of effect, min	Duration of effect, min	Mortality rate, %
200	0.0	4.5 [2.1; 6.3]	–	–	0.0
225	33.3	10.5 [8.4; 12.5]	8.0 [8.5; 9.5]	42.0 [41.0; 43.0]	0.0
250	66.7	26.0 [15.5; 36.4]	8.0 [7.5; 8.5]	41.0 [40.0; 42.5]	0.0
280	83.3	68.1 [48.6; 83.1]	6.0 [4.0; 6.0]	60.0 [58.0; 62.0]	0.0
300	100.0	79.7 [58.5; 87.9]	4.0 [2.5; 4.0]	70.0 [64.0; 74.0]	16.7
320	100.0	97.3 [96.0; 98.7]	2.0 [2.0; 3.5]	66.0 [62.0; 68.0]	50.0
350	100.0	98.2 [96.5; 99.5]	2.0 [2.0; 2.0]	8.5 [6.0; 9.5]	100.0

Table compiled by the authors based on original data

Note: quantitative values of the recorded parameters are presented as the median, first, and third quartiles — M_e [Q_1; Q_3].

intact animals were determined. Based on the assessment of the neurological status of rats using CFTs, a single subcutaneous administration of pancuronium bromide in the dose range of 200–225 μg/kg led to the development of myasthenic syndrome in 33.3–66.7% of cases, while administration of doses ranging 250–350 μg/kg resulted in its manifestation in 100% of cases. The speed of onset and severity of the myasthenic syndrome in intact rats exhibited a dose-dependent relationship. On average, the initial signs of the muscle relaxant effect were observed 5–7 min after administration, manifesting as reduced spontaneous motor activity, bilateral ptosis, and the inability to perform or maintain the position during the horizontal bar hanging test. The severity of paralysis then progressed: postural changes were detected, including loss of support on the distal limbs, inability to hold the head up, ataxia, and adynamia. Administration of pancuronium bromide at doses of 300–350 μg/kg bw resulted in animal death due to the neuromuscular form of acute respiratory failure within 5–10 min. The mortality rate in each group is presented in Table 1.

The dynamics of the NMB induced by the administration of various pancuronium doses were recorded using ENMG in rhythmic stimulation mode (Table 1). Figure 1 clearly illustrates characteristic changes in the M-response series, showing decrements in amplitude and area depending on the dose and time after muscle relaxant administration. The criteria for the NMB effect were defined as external signs of peripheral paresis combined with the illustrated electrophysiological changes.

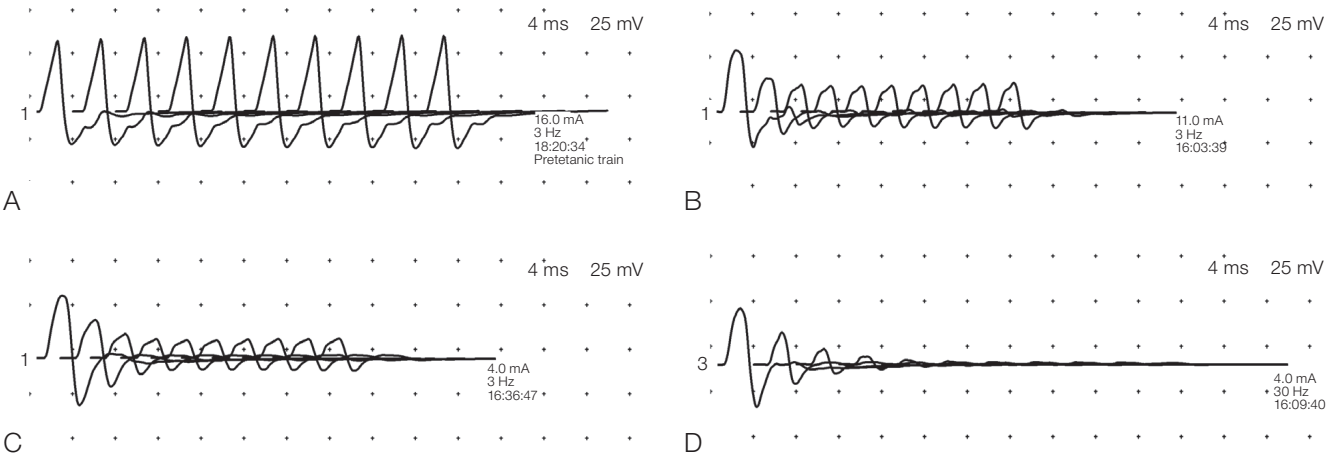

Figure prepared by the authors

Fig. 1. Typical M-response series of the gastrocnemius muscles in intact rats during the rhythmic stimulation test following a single subcutaneous administration of pancuronium bromide: A — no decrement observed during baseline assessment; B — 15 min after administration of a 280 μg/kg bw dose (decrement 50.6%); C — 20 min after administration of a 280 μg/kg bw dose (decrement 79.9%); D — 10 min after administration of a 300 μg/kg bw dose (decrement 96.7%); X-axis: M-response duration, ms; Y-axis: M-response amplitude, mV

The obtained data allowed us to establish that the median effective dose (ED_{50}) of pancuronium bromide following a single subcutaneous administration to intact rats was 238.0 [219.8; 257.7] μg/kg (ED_{16} = 211.4 μg/kg, ED_{84} = 274.4 μg/kg). The median duration of muscle relaxation was 60.0 [44.7; 64.0] min. Furthermore, the median lethal dose (LD_{50}) of pancuronium bromide following a single subcutaneous administration to intact animals was 321.1 [305.8; 337.1] μg/kg (LD_{16} = 298.1 μg/kg, LD_{84} = 339.1 μg/kg), and the median time to death was 26.0 [24.5; 27.5] min.

Thus, the therapeutic index, calculated as the ratio of LD_{50}/ED_{50}, for subcutaneously administered pancuronium bromide was 1.35. The ratio of LD_1 (255.0 μg/kg) to ED_{99} (292.0 μg/kg), referred to as the certain safety factor (CSF), equaled 0.87. These parameters indicate a narrow therapeutic range for pancuronium and dictate stringent requirements for dosing accuracy and the necessity for meticulous monitoring of its effects. The relationships between the ranges of effective and lethal doses of pancuronium are presented graphically in Figure 2.

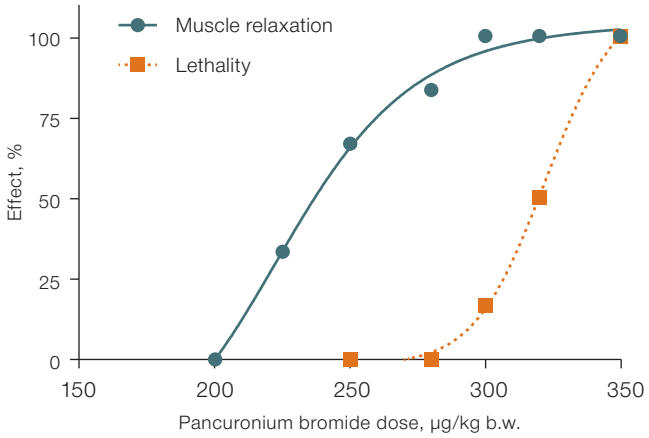

Figure prepared by the authors

Fig. 2. Dose-effect and dose-lethality relationships following single subcutaneous administration of pancuronium bromide to intact rats

Determination of effective and toxic doses of pancuronium bromide in a rat model of fenthion poisoning

According to preliminary toxicometric studies, the LD_{50} value of fenthion following a single subcutaneous administration in rats was 479.4 [465.3; 493.9] mg/kg. Animal deaths were recorded within the range of 6–24 h, with a median value of 11 h. The external signs of intoxication were represented by a set of cholinergic crisis symptoms (chromodacryorrhea, hypersalivation, incontinence, diarrhea, trismus, myofibrillations) and myasthenic syndrome, which appeared 15–30 min after administration and persisted for at least seven days. The residual activity of blood plasma acetylcholinesterase (AChE) in rats 12 h after pathology induction ranged 45.0–55.0% of the baseline level.

The neurophysiological profile of fenthion poisoning corresponded to the pattern previously described in a rat model of dimethoate poisoning [14]. In total, 12 h after fenthion administration, all animals exhibited numerous repeated M-responses to single stimulation, and in 80.0% of cases, a pronounced decrement-increment pattern was observed based on rhythmic stimulation data (Fig. 3A, B).

Administration of pancuronium bromide during the acute phase of poisoning at doses of 83.3 and 108.3 μg/kg bw contributed to the improvement of NMT, manifesting as restored muscle strength according to CFTs, a reduced number of repeated M-responses, and normalization of the response to rhythmic stimulation. From a neurophysiological perspective, the recorded changes

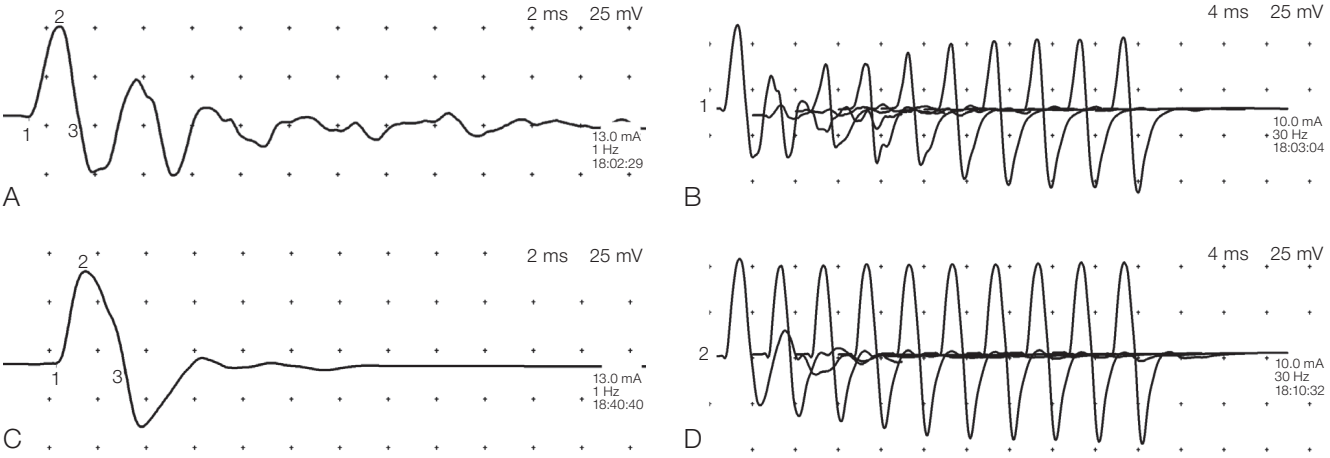

Figure prepared by the authors

Fig. 3. Changes in M-responses of the rat gastrocnemius muscle 12 h after fenthion administration: single stimulation (A) and rhythmic stimulation (B); reduction in the number and duration of repeated M-responses (C); restoration of the decrement-increment pattern to a normal M-response series (D): X-axis — M-response duration, ms; Y-axis — M-response amplitude, mV

Table 2. Results of assessing the effects of single subcutaneous administration of pancuronium bromide in rats during the acute phase of fenthion poisoning

Pancuronium dose, μg/kg bw	Reduction in repeated M-responses, %	Normalization of decrement-increment, %	Decrement detection frequency, %	Increase in muscle strength, %	Mortality rate, %
64.1	0.0	0.0	0.0	0.0	0.0
83.3	16.7	66.7	0.0	33.3	0.0
108.3	83.3	100.0	0.0	100.0	0.0
140.8	100.0	66.7	33.3	66.7	33.3
183.0	100.0	0.0	100.0	16.7	83.3
238.0	100.0	0.0	100.0	0.0	100.0

Table compiled by the authors based on original data

should be interpreted as a reduction in muscle hyperexcitability, restoration of nerve impulse discreteness, and, ultimately, an increase in the reliability of NMT. It was established that after administration of pancuronium bromide at a dose of 108.3 μg/kg bw, ENMG data indicated a decrease in the number of repeated M-responses, as well as restoration of the decrement-increment pattern to a normal M-response series (Fig. 3C, D).

Based on CFT data and neurophysiological monitoring, the range of effective doses of pancuronium for correcting NMT impairments in the acute phase of fenthion poisoning in rats was determined (Table 2). The efficacy criteria were the electrophysiological signs of NMT normalization described above and the restoration of muscle strength within 1 h after pancuronium bromide administration. Administration of pancuronium at doses exceeding 140.8 μg/kg bw resulted in an increased severity of NMB, manifesting as a decremental response instead of a decrement-increment pattern and

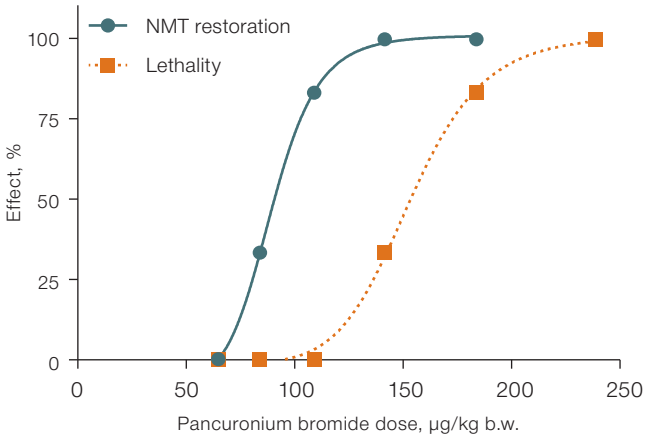

Figure prepared by the authors

Fig. 4. Dose-effect and dose-lethality curves obtained after subcutaneous administration of pancuronium bromide to rats 12 h after fenthion poisoning

progression of the myasthenic syndrome up to respiratory muscle paralysis.

The calculated ED_{50} value of pancuronium bromide, when administered during the acute phase of severe fenthion poisoning, was significantly lower than that in intact rats, amounting to 90.1 [77.3; 105.1] μg/kg (ED_{16} = 73.4 μg/kg, ED_{84} = 113.4 μg/kg). A leftward shift of the lethality curve was also observed against the background of fenthion poisoning: LD_{50} = 152.3 [130.6; 177.6] μg/kg (LD_{16} = 124.0 μg/kg, LD_{84} = 191.6 μg/kg). Graphs illustrating these relationships are presented in Figure 4. The therapeutic index of subcutaneously administered pancuronium bromide against the background of fenthion poisoning was 1.69, and the value of the certain safety factor was 0.59.

Thus, the necessity of reducing the effective dose by 2.64 times to correct the myasthenic syndrome, as well as maintaining a narrow therapeutic range of this medication, has been established. Moreover, the calculated ED_{50} value of pancuronium bromide for intact animals exceeds its LD_{99} value for rats under severe fenthion poisoning conditions.

DISCUSSION

Modern approaches to the treatment of anticholinesterase poisoning involve the use of atropine, oximes, and anticonvulsant drugs[2] [1, 3, 9]. Despite a long history of investigating the pharmacological potential of muscle relaxants in anticholinesterase poisoning, no validated approaches for their use as part of antidotal therapy have been established to date. This is due to a number of reasons, which, in our opinion, are related to specific features of the design of the conducted studies.

The scientific literature presents isolated clinical studies describing the experience of administering tubocurarine, pancuronium, and pipecuronium in organophosphorus (OP) poisoning [11, 12, 15, 17]. In particular,

[2] Kryukova EV, editor. Military Field Therapy: National Guide. 2nd ed., revised and expanded. Moscow: GEOTAR-Media; 2023.

the successful use of pancuronium bromide administered intravenously at doses of 0.5 mg [16] and 1.0 mg [15] was reported, which is 5–10 times lower than the recommended doses for achieving muscle relaxation. However, the data provided in these studies are not analytical in nature, preventing the determination of fundamental principles for dosing and assessing drug effects, considering their low safety margin. Our study is among those few performed using an *in vivo* model, which enabled the evaluation of complex interactions between OPs and muscle relaxants at the organismal level through clinical and instrumental monitoring of NMT impairments and provided a detailed rationale for the effective doses of the drug.

According to Besser et al. [15], the phenomena of decrement-increment and repeated M-responses reflect different degrees of AChE inhibition severity, which was also confirmed by our studies [14]. These phenomena are caused by two main electrophysiological mechanisms mediated by nicotinic acetylcholine receptors (nAChRs). In contrast, complete suppression of NMT, manifested as a decremental response, is caused by desensitization of postsynaptic nAChRs due to a depolarizing block [18]. Stimulus-induced antidromic backfiring (SIAB) is attributed to excessive stimulation of axonal presynaptic nAChRs, which mediate autofacilitation of neurotransmitter release, under conditions of incomplete AChE inhibition and the absence of a desensitization block in postsynaptic nAChRs.

Studies suggest that precisely this mechanism, which underlies the decrement-increment response to rhythmic stimulation and spontaneous myofibrillations, can be blocked by low concentrations of D-tubocurarine or pancuronium, insufficient to block postsynaptic nAChRs [18, 19]. Repeated M-responses (repetitive compound muscle action potentials) following a single supramaximal stimulus are caused by an increased duration of interactions between acetylcholine and postsynaptic nAChRs, as well as lateral diffusion of an excess amount of the neurotransmitter, leading to the activation of several adjacent receptors. Collectively, this results in prolonged end-plate potentials and impaired discreteness of the nerve impulse [14, 15, 20]. The differing sensitivity of pre- and postsynaptic nAChRs to the action of muscle relaxants, described in [15], was confirmed in our studies. For instance, restoration of the decrement-increment pattern to a normal M-response series was observed upon administration of lower pancuronium doses than those required to suppress repeated M-responses.

Pre- and postsynaptic nAChRs remain potential therapeutic targets at the neuromuscular junction (NMJ) for their competitive antagonists in OP intoxication. In this context, attempts to restore NMB through the application of muscle relaxants appear logical and justified. The concept behind their use in this situation is straightforward: it is necessary to administer a dose of a muscle relaxant that is sufficient to counteract the effects of excessive acetylcholine stimulation of nAChRs and leads to the normalization of NMJ function. Our studies have confirmed the hypothesis of Sheridan et al. that the dose of a muscle relaxant should not be sufficiently high to independently induce a non-depolarizing NMB in the “normalized” NMJ due to excessive antagonism [21].

According to Karalliedde, a marked increase in sensitivity to muscle relaxants should be anticipated in IS [22], analogous to cases of reduced vecuronium doses during the recovery phase from a depolarizing block induced by suxamethonium [23]. The results of our study support these hypotheses, demonstrating that the median effective dose of pancuronium in OP poisonings must be reduced by at least a factor of 2.6 compared to the median muscle-relaxing dose in intact animals. Furthermore, we observed a leftward shift in the dose-effect and dose-lethality curves while the narrow therapeutic range of the drug was maintained within the same limits.

Further research on extrapolating the obtained data on therapeutic doses of pancuronium bromide to humans in cases of OP poisoning is of significant interest. The recommended median effective doses for clinical use are 60.0–100.0 μg/kg upon intravenous administration [24], which is 2.4–4.0 times lower than the established analogous dose for rats (238.0 μg/kg). This fact indicates a comparable sensitivity to pancuronium between rats and humans, considering the traditionally accepted dose conversion based on body weight and surface area, thereby confirming the external validity of the selected experimental model. A more detailed analysis of preclinical data for their translation into clinical practice constitutes an independent task and represents a future direction of this scientific work.

It is widely acknowledged that administering muscle relaxants to patients who already require mechanical ventilation due to the acute respiratory failure typical of a cholinergic crisis (primarily of central origin) would help prevent subsequent excessive nAChR stimulation and NMJ dysfunction, without significantly altering the treatment strategy [11, 17, 21]. On the basis of this rationale, the authors conducted what they claimed to be the first pilot randomized clinical trial assessing the impact of muscle relaxants on the duration of the intubation period in patients with OP poisonings [12]. During prolonged intravenous infusion of high-dose rocuronium (1.5 mg/kg/h), the researchers observed a predictable increase in the intubation period compared to the control group. The study design and its results are debatable due to the insufficiently justified selection of rocuronium doses, the lack of dynamic neurological examination, and the failure to assess expected endpoints such as the development of IS, critical illness polyneuropathy/myopathy, or delayed organophosphate-induced polyneuropathy, since the anticipated effects of muscle relaxants are precisely the prevention of these pathological conditions.

It should be mentioned that priority in this direction belongs to Russian researchers who published a similar study over 20 years ago [11]. That study demonstrated

that the use of relaxant doses of pipecuronium (0.01–0.015 mg/kg intravenously, drip infusion) as part of a comprehensive therapy for karbofos (malathion) poisoning, which significantly reduced the severity of toxic neuromyopathy. This was evidenced by a faster recovery of muscle strength, a lower frequency and duration of peripheral respiratory paralysis, and a lower average number of hospital bed-days and mortality rates. The authors emphasized the importance of prolonged administration of muscle relaxants, selecting their optimal doses and administration regimens, considering the possibility of early administration at the prehospital stage in cases of OP poisoning.

The potential for prolonging the action of muscle relaxants was demonstrated by Iwasaki et al. in a small-size clinical study investigating the characteristics of the muscle relaxant effect of pancuronium following subcutaneous administration in patients [25]. It was established that subcutaneous administration in the upper limb region did not differ from intravenous administration in terms of the intensity and speed of the onset of the effect, as measured by acceleromyography in the "train-of-four" (TOF) mode. However, injection in the ankle region resulted in a significantly slower onset and prolonged duration of the muscle relaxant effect, the intensity of which was lower than with intravenous administration at an equal dose. On this basis, we specifically selected subcutaneous administration of pancuronium in rats to achieve an increased duration of effect without the need to induce deep NMB.

The obtained results underscore the necessity for precise calculation of muscle relaxant doses in cases of OP poisoning and the simultaneous monitoring of NMT status. The use of ENMG for this purpose is recognized as the most accurate and should be considered the "gold standard" [26, 27]. However, several publications have reported data indicating a good correlation between "train-of-four" (TOF) stimulation values determined by acceleromyography (AMG), mechanomyography (MMG), and ENMG. Although AMG is less accurate in assessing the depth of NMB compared to ENMG and MMG, it is the most widely used method in routine clinical practice due to its ease of use and applicability across various muscles [28]. The authors of these studies are unanimous in concluding that the choice of a specific monitoring method is less critical than the very fact of its presence and proper execution, since any of these methods allows for an objective assessment of NMT status. Furthermore, NMB monitoring is essential in cases of OP poisoning for the timely diagnosis of peripheral nervous system impairments.

CONCLUSION

In this study, we have determined experimentally electrophysiological criteria for correcting NMT impairments — underlying IS in AChE poisonings — using pancuronium bromide. These criteria specifically include a reduction in the number of repeated M-responses and a change in the pattern of the M-response series during high-frequency rhythmic stimulation from a decremental to a decrement-increment pattern. The regression of the NMB, as evidenced by this dynamic change in the ENMG profile, was further supported by an increase in muscle strength according to two CFTs and a reduction in the mortality rate among the experimental groups.

An experimental rationale for the range of effective (therapeutic) doses of pancuronium bromide in cases of OP poisoning has been provided, and the persistence of its narrow therapeutic range under conditions of pronounced AChE inhibition has been determined. Subcutaneous administration of pancuronium bromide at low doses (2.6 times lower than the median effective dose for intact animals) was found to alleviate severe NMT impairments in cases of severe OP poisoning through partial blockade of both pre- and postsynaptic nAChRs.

The presented data support the hypothesis that the use of muscle relaxants effectively mitigates the manifestations of OP-induced myasthenic syndrome. Given that virtually all patients with severe OP poisoning require mechanical ventilation as part of the standard treatment protocol, the early and prolonged administration of pancuronium bromide, a long-acting muscle relaxant, at low doses appears justified and promising for the prevention of intermediate syndrome. It is important to note that the dosing of muscle relaxants can be controlled in real-time using MMG or AMG modules to assess neuromuscular transmission and perform tests in the following sequence: single stimulation — train-of-four (TOF) — post-tetanic count (PTC). However, in order to develop robust clinical recommendations, further studies are needed to evaluate the efficacy of different muscle relaxants within antidotal therapy regimens, especially in combination with cholinesterase reactivators and atropine, under the condition of administering these drugs during different periods of intoxication.

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AUTHORS

Mikhail A. Tyunin, Cand. Sci. (Med.)
https://orcid.org/0000-0002-6974-5583
gniiivm_7@mil.ru

Nikita S. Ilinskii, Cand. Sci. (Med.)
https://orcid.org/0000-0001-7406-753X
gniiivm_7@mil.ru

Sergey V. Chepur, Dr. Sci. (Med.), Professor, Corr. Member of RAS
https://orcid.org/0000-0002-5324-512X
gniiivm_7@mil.ru

Vladimir A. Matseychik
https://orcid.org/0009-0007-2609-362X
gniiivm_7@mil.ru

Elizaveta Yu. Izhorskaya
https://orcid.org/0009-0005-5989-6985
gniiivm_7@mil.ru