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REPORT NO T2-91

RESPIRATORY AND SKELETAL MUSCLE FUNCTION AFTER ACUTE PYRIDOSTIGMINE BROMIDE ADMINISTRATION

U S ARMY RESEARCH INSTITUTE
OF
ENVIRONMENTAL MEDICINE
Natick, Massachusetts

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RESPIRATORY AND SKELETAL MUSCLE FUNCTION AFTER ACUTE PYRIDOSTIGMINE BROMIDE ADMINISTRATION

by

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13. ABSTRACT *(Maximum 200 words)*

This study examined respiratory and skeletal muscle function in response to an acute oral administration of pyridostigmine bromide (PYR). Since PYR is currently issued to U.S. Army personnel to use as a prophylactic drug for protection against chemical (organophosphate) warfare nerve-agents, should they be a threat, our purpose was to document changes that might occur in respiratory and skeletal muscle function. We found that one single, orally administered dose (30mg) of PYR did not significantly alter any measurement of respiratory function made in this study: forced vital capacity, forced expiratory volume in one second, maximal voluntary ventilation in 15 seconds, carbon dioxide sensitivity, or maximal inspiratory and expiratory flow rates. Neither did we note significant changes in the measurements we made of skeletal muscle strength, endurance and damage: peak hand-grip strength and 60% peak hand-grip endurance time, peak torque for leg extension at 30 and 180°·sec^{-1}, and serum enzymes representative of muscle tissue damage. Since soldiers are instructed to take PYR in 30mg doses, three times daily, for seven or more days during threat of chemical nerve-agent exposure, these data may not be directly applicable to multiple dose or chronic administration of pyridostigmine bromide.

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EXECUTIVE SUMMARY

This study examined respiratory and skeletal muscle function in response to a single oral administration of pyridostigmine bromide (PB). Since PB is currently available for U.S. Army personnel as a pretreatment drug against chemical (organophosphate) warfare nerve-agents, our purpose was to document changes that might occur in respiratory and skeletal muscle function. We found that one single, orally administered dose (30mg) of PB did not significantly alter any measurement of respiratory function made in this study: forced vital capacity, forced expiratory volume in one second, maximal voluntary ventilation in 15 seconds, carbon dioxide sensitivity, or maximal inspiratory and expiratory flow rates. Neither did we note significant changes in the measurements we made of skeletal muscle strength, endurance and damage: peak hand-grip strength and 60% peak hand-grip endurance time, peak torque for leg extension at 30 and 180°•sec^{-1}, and serum enzymes representative of muscle tissue damage. Since we studied only one 30-mg administration of PB, these data may not be directly applicable to multiple dose or chronic administration of pyridostigmine bromide.

INTRODUCTION

Pyridostigmine bromide is a reversible anticholinesterase with a half-life of approximately 2.5 hours (1,4). Anticholinesterase drugs exert their actions at cholinergic synapses by inactivating acetylcholinesterase. As the neurotransmitter acetylcholine is released upon neural stimulation, instead of being hydrolyzed by acetylcholinesterase, it continues to bind with available receptors in the synaptic area, thus repeatedly stimulating cholinergic fibers (24,25).

Pyridostigmine bromide is used therapeutically (oral dose 200-1400mg/day) for individuals with myasthenia gravis, a disease characterized by muscular weakness. At these doses PB stimulates gastro-intestinal motility and secretory activities, stimulates salivary and sweat gland secretions, induces miosis, causes bradycardia and in the myasthenic individual, increases skeletal muscle contractions (24). Individuals taking this or similar cholinesterase inhibitors may also experience other physiologic symptoms consistent with cholinergic stimulation, such as increased contraction of smooth airway muscle, as well as increased bronchiole and upper airway secretions (23,25).

Upper airway secretions and/or constriction could increase airway resistance. These physiologic events might contribute to decreased pulmonary function and potentially to decrements in exercise performance (5). Even the perception of respiratory discomfort has been associated with compromised performance (15). Cholinergic stimulation of muscle tissue is associated with increased muscle blood flow even in inactive muscle tissue (12,23,25), and microscopic changes in muscle ultrastructure have been reported in rodents receiving pyridostigmine (8,9,10,18,19). Increased serum concentrations of muscle enzymes (including creatine phosphokinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH)) are associated with muscle damage which can occur during exercise, acute heart attack, hypothermia or heatstroke. Elevated serum CK is also associated with irritability in fatigued muscle (without frank damage), due perhaps to altered membrane permeability (17).

The purpose of the present study was to characterize changes which may occur in respiratory and skeletal muscle function, and in markers of muscle damage, in healthy young men following a single 30 mg oral dose of pyridostigmine bromide.

MILITARY RELEVANCE

Under current U.S. Army doctrine, soldiers will be issued a blister pack containing 21 tablets of pyridostigmine bromide, one 30 mg tablet to be administered three times daily (t.i.d.), for 7 days, if their deployment subjects them to the risk of chemical warfare (CW). Because it is a spontaneously reversible cholinesterase inhibitor, pyridostigmine can be used as a pretreatment in situations where the irreversible anticholinesterase agents (organophosphate CW nerve-agents) are a threat (6). PB inhibited cholinesterase activity is restored within minutes, whereas with irreversible inhibitors, acetylcholinesterase activity may take weeks to recover in the survivor (6,22). PB offers protection with few side effects, against irreversible antichclinesterase drugs. When PB is used as a pretreatment, in combination with atropine (used during or immediately post-organophosphate exposure), the greatest protection is attained (6).

MINIMIZING RISKS TO SUBJECTS

With the exception of the ingestion of pyridostigmine bromide, all of the procedures in this study fell within the framework, restrictions and safety limitations of the USARIEM Type Protocol for Human Research Studies in the areas of Thermal, Hypoxic and Operational Stress, Exercise, Nutrition and Military Performance.[1] To minimize risks associated with pyridostigmine, volunteers were given medical examinations prior to acceptance as subjects. No one with a history of asthma or hepatic, renal or cardiovascular disturbances or hypersensitivity to pyridostigmine or related drugs was included as a test subject. (A trial administration of one 30mg dose was given at least three days prior to any testing. Inhibition of red cell cholinesterase activity was not allowed to exceed 60%).

[1]Approved 1 June 1990, The type protocol provides information and explanations about conditions, standards and safeguards, in order to serve as an encompassing framework for specific in-house studies in its general subject area. It is to be used as a reference to facilitate the understanding and review of specific study protocols which conform to its provisions, and thus do not exceed the degree of risk, and safety limits herein stipulated (USAMRDC Reg 70-25, 30 September 1988).

METHODS

Ten healthy male soldiers volunteered to participate in this study (approved by the USARIEM Human Use Review Committee and the US Army Surgeon General's Human Use Review Office) after signing a statement of informed consent. Their characteristics are presented in Table 1. Subjects were tested (one at a time) for respiratory function on four separate test days. For each subject, all four tests were conducted at the same time of day. On two of the test days the subject ingested 30 mg pyridostigmine bromide (Roche UK, Lot BK94626) and on two control days (CON), they received no drug. The order of drug and control days was counterbalanced, and at least 48h separated each test. Immediately preceding and 150 min after receiving the medication, red blood cell cholinesterase (AChE) activity was determined (7). Cholinesterase activity was also measured on both control days. Beginning 100 min after PB administration, each subject participated in a series of pulmonary function tests which took about 45 min. Forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) were determined using a wet seal bell spirometer (Collins 9L respirometer). Maximal voluntary ventilation for 15 seconds (MVV_{15}) was determined using an open circuit valve connected to a dry gas meter (Harvard and Parkinson Cowan) for measuring expiratory volumes. Maximal inspiratory and expiratory flow rates (MIF, MEF) were measured from photographs taken of flow volume curves obtained with a wedge spirometer (Med-Science Electronics), recorded on and photographed from an oscilloscope. All of the above respiratory measurements were made (2-3 trials/test) while the subjects were standing at rest. Carbon dioxide (CO_2) sensitivity (one trial/test) was measured during a closed system CO_2 rebreathing test (21). This rebreathing test began with a volume of air ~1L greater than FVC, comprised of 7% CO_2 and 93% O_2, following 10 min of resting breathing. During this test, the subject was comfortably seated in a semi-supine position, in a quiet room. The rebreathing test was terminated when the end-tidal CO_2 concentration reached 9.5%, which was ~4 min. Minute ventilation ($\dot{V}E$), measured with an HP pneumotachometer, and $\%CO_2$, measured with a Beckman LB2 gas analyzer, were continuously recorded on a direct writing polygraph (Western Graphtec). For each 30 second segment of the rebreathing test, the partial pressure of alveolar carbon dioxide (P_ACO_2) was calculated from end tidal CO_2. Carbon dioxide sensitivity was determined as the slope of $\dot{V}E$ plotted as a function of P_ACO_2.

Seven of the ten volunteers performed four tests of gross muscular strength and endurance on one control day and on one test day, 210-240 minutes after the 30 mg oral PB administration. The volunteers performed peak hand-grip strength and 60% peak hand-grip endurance trials using a hand-grip dynamometer (20). Peak hand grip strength was determined as the mean of two trials. Endurance time was determined as the subjects maintained a constant pressure at 60% (±2%) of peak hand-grip strength until either volitional release or an inability to remain within the range for three seconds. Two leg extension tests at 30 and 180°·sec^{-1} were done on a CYBEX apparatus. Subjects completed 2-3 trials on the CYBEX at each speed, with changes of <10% peak torque between trials as the criterion for stopping the test. Aliquots of blood samples taken for the AChE determination 150 min after PB administration or at the same time of day for CON, were used for serum enzyme analyses. Serum enzymes analyzed were CK, LDH and AST, as representative markers of muscle damage (SIGMA kits, Procedure Nos. 47-UV, 228-UV, 58-UV for CK, LDH, and AST, respectively).

An analysis of variance with repeated measures was performed for all respiratory data collected. Least squares regression procedures were used for CO_2 sensitivity determinations. Dependent t-tests were performed on muscle strength and enzyme data.

Table 1. SUBJECT CHARACTERISTICS

	AGE (yr)	HEIGHT (cm)	WEIGHT (kg)	SURFACE AREA (m^2)	FVC (%)*	FEV_1 (%)*
1	20	188.5	81.0	2.06	111	106
2	22	177.8	73.9	1.92	101	113
3	19	165.1	78.4	1.82	85	91
4	28	170.0	68.7	1.80	99	105
5	26	7 3	73.3	1.91	103	114
6	31	165.5	74.0	1.82	105	107
7	26	172.7	66.4	1.79	96	98
8	24	176.5	70.0	1.86	111	93
9	19	172.7	58.5	1.69	90	99
10	19	188.5	88.5	2.15	107	108
MEAN ±SD (n=10)	24 4	175.5 8.2	73.3 8.3	1.88 0.14	101 9	103 8
For the seven subjects who participated in the muscle testing portion of this study (omit #s 6,7,8), means are as follows:						
MEAN ±SD (n=7)	22 4	177.2 8.9	74.6 9.5	1.91 0.16	99 9	105 8
* percent of age/height/weight predicted norm (2)						

RESULTS

In the ten subjects performing the pulmonary function tests, PB decreased the activity of red blood cell cholinesterase by 37 (±10)% compared to CON. FVC and FEV_1 were not different between CON and PB test days, and were not different between the two CON tests or the two PB tests (Table 2). MIF and MEF were also not different on any of the four test days (Table 2). MVV_{15} and CO_2 sensitivity are shown in Table 3. There were no differences in these parameters between CON and PB, or among any of the four test days. CO_2 sensitivity on consecutive test days (counterbalanced for each subject) also did not change as testing progressed. Mean (±SD) CO_2 sensitivities ($\Delta \dot{V}E \cdot \Delta P_ACO_2^{-1}$) for days 1, 2, 3 and 4 were 3.34 (1.42), 3.34 (1.26), 3.22 (1.24), and 3.60 (1.08), respectively.

Table 2. FVC, FEV_1, MIF, MEF (at BTPS), mean (±SD), n=10

	FVC (L)	FEV_1 (L)	MIF ($L \cdot sec^{-1}$)	MEF ($L \cdot sec^{-1}$)
CONTROL 1	5.21 (0.84)	4.39 (0.58)	8.89 (1.36)	10.99 (1.85)
CONTROL 2	5.19 (0.90)	4.29 (0.68)	9.01 (1.47)	11.37 (1.58)
PB 1	5.17 (0.87)	4.32 (0.64)	8.62 (1.08)	11.06 (2.22)
PB 2	5.11 (0.93)	4.26 (0.60)	8.88 (1.43)	10.78 (1.62)

Table 3. MVV_{15} (BTPS) AND CO_2 SENSITIVITY, mean (±SD), n=10

	MVV_{15} ($L \cdot min^{-1}$)	CO_2 SENSITIVITY ($\Delta \dot{V}E \cdot \Delta P_ACO_2^{-1}$)
CONTROL 1	180.2 (14.9)	3.27 (1.37)
CONTROL 2	179.6 (24.6)	3.30 (1.15)
PYRIDOSTIGMINE 1	174.5 (11.8)	3.41 (1.31)
PYRIDOSTIGMINE 2	179.5 (18.4)	3.51 (1.20)

In the seven subjects who performed the skeletal muscle testing, cholinesterase activity was decreased 34(±10)% . There were no differences in the serum enzyme activities of CK, LDH, or AST between CON and PB. These data are presented in Table 4.

Table 4. SERUM ENZYME ACTIVITY, mean (±SD), n=7

	CK ($U \cdot L^{-1}$)	LDH ($U \cdot L^{-1}$)	AST ($U \cdot L^{-1}$)
CONTROL	98.0 (58.1)	61.6 (17.4)	11.9 (4.3)
PYRIDOSTIGMINE	76.9 (28.1)	57.9 (13.6)	13.9 (4.4)

Table 5. PEAK TORQUE DURING LEG EXTENSION, mean (±SD), n=7

	$30° \cdot sec^{-1}$ (Nm)	$180° \cdot sec^{-1}$ (Nm)
CONTROL	243.4 (52.7)	151.5 (35.1)
FYRIDOSTIGMINE	243.1 (38.8)	148.9 (30.9)

Peak torque at both speeds measured (30 and $180° \cdot sec^{-1}$) in CON and PB is shown in Table 5. There were no differences between the treatments. Peak hand-grip strength and endurance time for 60% peak hand-grip strength are presented in Table 6. These measurements were also not affected by PB.

Table 6. PEAK HAND-GRIP STRENGTH AND 60% PEAK HAND-GRIP ENDURANCE TIME, mean, (±SD), n=7

	PEAK GRIP (kg)	60% GRIP TIME (sec)
CONTROL	57.4 (7.8)	55.4 (19.1)
PYRIDOSTIGMINE	57.2 (8.4)	60.7 (15.2)

DISCUSSION

In this study, a single oral administration of the anticholinesterase drug pyridostigmine bromide (30mg) did not adversely affect normal respiratory or skeletal muscle function. Since the synaptic function in both the respiratory airways and the neuromuscular junction is cholinergic we expected that it would be subjected to overstimulation as acetylcholine accumulated in the synaptic area. The cholinesterase inhibition for the group of 10 and 7 subjects, 27 and 34%, is consistent with values measured in other studies, up to 2 h post drug ingestion, and somewhat lower than levels at 24 h during chronic (30 mg t.i.d.) administration (6, 13). However, after one single 30 mg dose, we found no adverse effect on any measured parameter which may have resulted from increased airway resistance due to an accumulation of airway secretions or contraction of airway smooth muscle, or from muscle damage or changes in muscle cell permeability due to increased muscular stimulation.

In this study, PB did not have significant effects on respiratory function, but it is possible that even small effects of PB (possibly added airway resistance) could have significant consequences in situations where soldiers must wear chemical protective masks which increase resistance to breathing and increase external dead space, which increases P_ACO_2 (15). Inhalation of increased concentration of CO_2 is not well tolerated when combined with increased resistance to breathing (3). Individuals most sensitive to hypercapnia respond by increasing $\dot{V}E$ and inspiratory flow rates to minimize P_ACO_2, and consequently compromise exercise intensity and/or duration (16). Perception of respiratory discomfort could itself compromise performance as seen in U.S. Army field studies (15). However, data reported from two studies indicate that even chronic (30mg, t.i.d. for 14 days) PB adminstration has little or no effect on respiratory function in men either wearing (14) or not wearing (11) chemical protective masks.

The administration of pyridostigmine (either acute large dose or chronic lower dose) in rodents has been associated with ultrastructural changes in the area of the neuromuscular junction (8). An acute, subclinical dose as was given to the men in this study had no measurable effect on skeletal muscle function, although we did not evaluate fine motor function such as dexterity or steadiness. The leg extension strength and hand-grip strength and endurance tests were chosen as representative of skeletal muscle function used in common tasks, and are standard tests frequently used to assess muscular strength. Serum enzyme

markers of muscle damage also did not change with PB administration. Our blood samples, drawn 150 min after PB administration, may have been too early to show changes in serum enzymes, although data from several studies show cholinesterase inhibition is relatively stable even during chronic PB administration (6, 13). When muscle enzymes enter the bloodstream following prolonged exercise, CK and AST peak between 24 and 48 h post exercise, compared to LDH, which peaks immediately or within 8 h. Chronic PB administration or PB combined with prolonged or intense exercise, might also have an effect on muscle ultrastructure or cell membrane permeability, where one single dose did not. In this study, results from all the pulmonary and muscle function tests show that there are no significant changes with one 30 mg dose of pyridostigmine bromide.

CONCLUSION

Results from the present study demonstrate that a single 30 mg dose of pyridostigmine bromide, which significantly decreases red blood cell cholinesterase activity, does not appear to exert a significant effect on the performance of standard pulmonary function tests or standard tests of skeletal muscle function in young healthy adult males.

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