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RESEARCH ARTICLE

COMPARISION OF POTENCY OF PANCURONIUM, VECURONIUM AND ATRACURIUM AGAINST THE ACETYLCHOLINE INDUCED CONTRACTION ON ISOLATED FROG RECTUS ABDOMINIS MUSCLE

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ABSTRACT

Objective: To compare potency of pancuronium , vecuronium and atracurium against the acetylcholine induced contraction on isolated rectus abdominis muscle of frog.
Material and Methods : In the present study the neuromuscular blocking effect of pancuronium, vecuronium and atracurium was compared by using acetylcholine as standard on the height of frog rectus abdominus muscle contraction.
Results : Pancuronium , vecuronium and atracurium in the doses of 0.06 mg/lit, 0.12 mg/lit, 0.25 mg/lit produced dose dependent reduction in the amplitude of contraction of acetylcholine (32µg, 64 µg , 128 µg, 256µg, 512µg).
Conclusion : Among the three neuromuscular blocking agents pancuronium appears equally potent to vecuronium as there is no statistically significant difference in half maximal inhibitory (IC50) values as compared to half maximal inhibitory (IC50) concentration value of atracurium.
Keywords: Pancuronium, vecuronium, atracurium , non depolarizing blocking drugs

INTRODUCTION

Drugs that affect skeletal muscle function include two different therapeutic groups : those used during surgical procedures and in the intensive care unit (ICU) to produce muscle paralysis (neuromuscular blockers) and those used to reduce spasticity in a variety of painful conditions (spasmolytics) . Neuromuscular blocking agents act peripherally at neuromuscular junction to reduce muscle tone and cause paralysis of skeletal muscles. They are used mainly as a part of balanced anesthesia to provide muscle relaxation during surgery. The most important use of neuromuscular blockers is as adjuvant to general anaesthesia where adequate muscle relaxation can be achieved at lighter plane, particularly of abdominal wall and lower limbs so that operative manipulations become easier. This situation minimizes the risk of respiratory and cardiovascular depression besides shortening the post-anaesthetic recovery period because a much lighter level of anaesthesia is sufficient. These drugs block the post-synaptic actions of acetylcholine at motor end plate and are generally referred to as skeletal muscle relaxants or at times simply as muscle relaxants because smooth muscle relaxants are specifically designated as either antispasmodics or vasodilators.[1]

Neuromuscular blockers drugs which are acting peripherally are called non-depolarizing blockers (competitive blocker) and persistant depolarizing blockers based on the mechanism of action. Directly acting muscle relaxants (dantrolene) and centrally acting muscle relaxants (mephenesin and its congeners) are the other types of skeletal muscle relaxants.[2] Competitive blockers (curare like drugs) are bulky quaternary compounds with two positively charged nitrogen. They shadow over the N_M receptor, and prevent its binding to acetylcholine. As a result the conformational change in the N_M receptor, which is needed for the opening of the Na^+ channel is prevented. Therefore in the absence of end plate potential, the motor nerve cannot elicit the contractions and hence skeletal muscle relaxation ensues. They do not cause depolarization by themselves but prevent endplate depolarization by acetylcholine. The antagonism is surmountable and reversal can be achieved with anticholinesterase drugs like neostigmine. At high concentrations these directly block the Na^+ channels which further causes weakening of neuromuscular

transmission but at the same time reduces the ability of drug like neostigmine to reverse the actions of non-depolarizing muscle relaxants.

In the multiple innervated contracture muscle like rectus abdominis muscle of frog, stimulation is prolonged resulting in sustained contraction.

Non-depolarizing neuromuscular blocking agents produce flaccid paralysis and depolarizing agents produce spastic paralysis. The main purpose of this study is to find out the potency and of three commonly used neuromuscular non-depolarizing muscle relaxants like pancuronium, vecuronium and atracurium in vitro experimental models of isolated frog rectus abdominis muscle .[3]

MATERIAL AND METHODS

The study was conducted in amphibian laboratory in the department of pharmacology , kamineni institute of medical sciences, narketpally , Andhra Pradesh during the period 1/8/2011 to 12/7/2012 . Frogs (Rana tigrina) 30 in number , weighing 150-250 grams, reared in central animal house of the kamineni institute of medical sciences were used. The present study was approved by institutional animal ethics committee.

Dissecting and Mounting of the tissue

The standard procedure for dissecting and mounting of the tissue is followed. A pithed frog (150-250 grams) is laid on its back in a tray. The skin on the abdominal wall is removed. The two recti muscles in the midline extending from xiphisternum to the symphysis pubis is identified. The borders of muscle is dissected out from the adjacent wall muscles. The two recti muscles are separated with a scissors. With the help of a needle and thread , a tight ligature round the muscle is applied just above the symphysis pubis and just below the xiphisternum with a long thread attached. The muscle strip is cut and removed from the symphysis pubis and xiphisternum.

Mounting

The lower end of the isolated rectus muscle is tied closely to the bent portion of the (lower end) tissue holder at its centre . The upper end of the tissue with the long thread is tied to the simple lever placed above , so that when the muscle contacts , the writing point goes up. During the dissection and attaching muscle to the tissue holder, the muscle must be kept moist with the frog ringer solution. Allow the tissue to relax for 30minutes and wash the tissue for 2 minutes by allowing the nutrient solution (ringer) in and out of inner organ bath. The normal contraction of the isolated rectus muscle is recorded for three minutes by using frog ringer solution and tissue is washed with frog ringer solution in between each contraction and relaxed for 2 minutes.[4] Acetylcholine 32µg was added and height of contraction of rectus muscle was recorded to test the sensitivity of the tissue. Then pancuronium 0.06mg/litre was added to inner organ bath and height of contraction was recorded. The above proceedure was repeated with acetylcholine different concentrations 64 µg,128 µg, 256µg, 512µg by adding Pancuronium dose concentrations of 0.12mg/litre and 0.25mg/litre after each acetylcholine dose. Sensitivity of vecuronium dose concentrations 0.06 mg/lit, 0.12 mg/lit , 0.25 mg/lit and atracurium dose concentrations 0.06 mg/lit, 0.12 mg/lit and 0.25 mg/lit was also tested by using the above procedure.

Table 1: Grouping of animals for comparision of potency of drugs

Animals were divided in to 5 groups each containing 6 animals (n=6 in each group , a total of 5 groups , 30 frogs required)

Groups (n=30)	Drugs	Concentration of drugs
1	Frog ringer	-
2	Acetylcholine	32µg, 64 µg , 128 µg, 256µg, 512µg
3	Pancuronium	0.06 mg/lit, 0.12 mg/lit, 0.25 mg/lit
4	Vecuronium	0.06 mg/lit, 0.12 mg/lit, 0.25 mg/lit
5	Atracurium	0.06 mg/lit, 0.12 mg/lit , 0.25 mg/lit

RESULTS

As shown in the (table-2) pancuronium different dose concentrations treatment has decreased acetylcholine induced contraction of frog rectus abdominus muscle from 0 minute to 90 minute. Further (table-3 and table -4) vecuronium and atracurium treated animals group also decreased acetylcholine contractions compared to 0 minute. The above observations indicate stastically significant ($P<0.001$).

Table 2 : Comparision of antagonist effect of pancuronium in minutes (mean ± sd) with different concentrations (0.06mg/lit , 0.12mg/lit and 0.25mg/lit) against acetylcholine height of contractions

Frog ringer, acetylcholine, pancuronium height of contractions response in mm

Treatment/ Minutes	Frog ringer	ACH	ACH + Pancuronium 0.06mg/lit	ACH + Pancuronium 0.12mg/lit	ACH + Pancuronium 0.25mg/lit	P value
0 min	5.91±0.41	11.17±4.99	8.10±6.07	4.09±5.06	2.00±3.04	0.04
15min	5.08±0.35	22.33±11.11	17.83±7.83	14.83±1.17	10.00±4.03	0.05
30min	5.25±0.25	29.33±9.69	10.17±9.45	2.33±2.42	0.40±0.55	0.001*
60min	5.15±0.22	36.67±8.24	21.67±3.26	5.50±2.56	1.40±0.55	0.001*
90min	5.30±0.20	43.33±9.44	26.33±4.76	10.00±2.09	4.80±1.30	0.001*

ACH= Acetylcholine, P<0.001 is highly significant

Table 3 : Comparision of antagonist effect of vecuronium in minutes (mean ±sd) with different concentrations (0.06mg/lit , 0.12mg/lit and 0.25mg/lit) against acetylcholine height of contractions

Frog ringer , acetylcholine, pancuronium height of contractions response in mm

Treatment/ Minutes	Frog ringer	ACH	Ach + Vecuronium 0.06mg/lit	Ach + Vecuronium 0.12mg/lit	Ach + Vecuronium 0.25mg/lit	P value
0 min	5.00±0.38	14.00±7.09	11.33±1.03	8.00±3.09	5.00±2.01	0.04
15 min	5.00±0.30	24.33±1.03	17.33±5.89	11.17±0.17	8.00±3.06	0.05
30min	5.20±0.24	35.67±10.84	11.17±6.85	3.33±3.08	1.00±1.55	0.001*
60min	5.10±0.20	43.50±9.61	24.83±7.33	10.33±6.69	4.20±3.54	0.001*
90min	5.25±0.15	51.83±7.83	25.83±2.32	15.33±8.41	5.00±5.87	0.001*

Table 4 : Comparision of antagonist effect of atracurium in minutes (mean ±sd) with different concentrations (0.06mg/lit , 0.12mg/lit and 0.25mg/lit) against acetylcholine height of contractions

Frog ringer , acetylcholine, pancuronium height of contractions response in mm

Treatment/ Minutes	Frog ringer	Ach	Ach + Atracurium 0.06mg/lit	Ach + Atracurium 0.12mg/lit	Ach + Atracurium 0.25mg/lit	P value
0min	5.45±0.40	16.83±3.32	13.00±3.58	9.33±2.82	3.00±1.03	0.04
15min	5.12±0.28	19.83±4.45	13.00±5.67	10.67±1.03	6.00±2.04	0.05
30min	5.30±0.22	25.17±6.85	13.00±8.29	5.00±2.53	2.33±1.97	0.001*
60min	5.15±0.15	35.83±10.65	22.33±8.21	9.83±4.9	4.83±2.93	0.001*
90min	5.28±0.10	41.17±12.70	28.17±9.58	16.67±7.03	5.33±4.27	0.001*

Table 5 : IC_{50} (Half maximal inhibitory concentration **)** value of pancuronium different concentrations 0.06mg/lit, 0.12mg/lit and 0.25mg/lit against acetylcholine (submaximal dose) dose 256µg/30ml frog ringer solution.

Number of frogs	Mean± sd
6	0.066 ± 0.0046

Sd – Standard deviation

Table 6 : IC_{50} (Half maximal inhibitory concentration **)** value of vecuronium different concentrations 0.06mg/lit, 0.12mg/lit and 0.25mg/lit against acetylcholine (submaximal dose) dose 256µg/30ml frog ringer solution.

Number of frogs	Mean± sd
6	0.067 ± 0.0035

Table 7 : IC_{50} (Half maximal inhibitory concentration **)** value of atracurium different concentrations 0.06mg/lit, 0.12mg/lit and 0.25mg/lit against acetylcholine (submaximal dose) dose 256µg/30ml frog ringer solution.

Number of frogs	Mean± sd
6	1.18 ± 0.1204

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DISCUSSION

Skeletal muscle relaxants are drugs that are able to reduce unwanted spasm or spasticity with out interfering with consciousness and normal voluntary movements finds an important application in various neurological or painful musculo – skeletal disorders. [5]

The first competitive blocking agent which was developed was d-tubocurarine . Presently it is not used as it produces hypotension due to histamine release and ganglion blocking property. The newer competitive blockers pancuronium , vecuronium and atracurium which were developed used therapeutically have less or no histamine releasing property.

The therapeutically used doses of pancuronium, vecuronium and atracurium for initiation of skeletal muscle relaxation in human beings are 0.08 - 0.1mg/kg i.v, 0.1mg/kg i.v and 0.5mg/kg i.v respectively.[6,7]

Isolated frog rectus abdominis muscle motor end plate contain nicotinic N-type of acetylcholine receptors. Acetylcholine produces contraction of frog rectus abdominis muscle by stimulating the nicotinic receptors present at the motor end plate of isolated frog rectus abdominis muscle.

The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function.This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme ,cell, cell receptor or microorganism) by half. It is commonly used as a measure of antagonist drug potency in pharmacological research.

Height of acetylcholine induced contraction on frog rectus muscle is most common method for testing the antagonism of neuromuscular blocking agent in biological animal. Among the three competitive blockers pancuronium appears euipotent to vecuronium as maximal reduction in IC_{50} (Half maximal inhibitory concentration **)** value of acetylcholine with dose concentration of 256µg (table-5 and table-6). Atracurium (table-7) is less potent than pancuronium and vecuronium as evident by increase in IC_{50} (Half maximal inhibitory concentration **).**

CONCLUSION

Pancuronium, vecuronium and atracurium reduced the height of acetylcholine induced contractions on isolated frog rectus abdominis muscle in a dose dependent manner. Pancuronium and vecuronium showed more significant promising neuromuscular blocking property. How ever further invitro/invivo studies in other experimental models may be required to conform the potency of all the three neuromuscular blocking drugs.

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