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Investigation of In Vitro Antibacterial Activity of Suxamethonium Chloride and Rocuronium Bromide

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Abstract

Aim: Some non-antibiotic drugs may provide prevention against bacterial activity in their routine use. The antibacterial effects of two muscle relaxants (suxamethonium chloride and rocuronium bromide) were tested on bacteria using disk diffusion method. In addition, whether muscle relaxants altered the antibacterial activity of antibiotics was investigated with agar dilution method.
Methods: Dilutions of 6 bacteria (*S. aureus*, *S. epidermidis*, *E. faecalis*, *S. pyogenes*, *P. aeruginosa* and *E. Coli*) were prepared and inoculated onto the plates containing Mueller Hinton agar. Under sterile conditions disks of drugs (n=4) containing three different doses were prepared. Four disks of each doses of the drugs were placed onto each bacterium plate. The plates were incubated and the inhibition zones were measured. Mueller Hinton agar plates containing four different concentrations of muscle relaxants were prepared to investigate whether muscle relaxants made any differences in the efficiency of antibiotics. These plates were inoculated with the bacteria tested. Standard antibiogram disks were placed onto the plates. The measured inhibition zones were compared with the control (Mueller Hinton agar plates without drug).
Results: The investigated drugs did not exhibit any antibacterial activity on the bacteria tested and did not change the effects of the antibiotics.
Conclusion: Although no in vitro activity of suxamethonium chloride and rocuronium bromide on bacteria was not found, in vivo studies are needed to determine the interactions with antibiotics.

Key words: antibacterial activity, suxamethonium chloride, rocuronium bromide

Suksametonyum Klorür ve Rokuronyum Bromür'ün Antibakteriyel Aktivitesinin in vitro Araştırılması

Özet ;
Amaç: Antibiyotik olmayan bazı ilaçlar, rutin kullanımlarında bakteriyel aktiviteye karşı koruma sağlamaktadır. Bu çalışmada, iki kas gevşetici ajanın (suksametonyum klorür ve rokuronyum bromür) bakteriler üzerindeki antibakteriyel etkilerinin disk difüzyon yöntemi kullanılarak test edilmesi amaçlanmıştır. Ayrıca, kas gevşeticilerin, antibiyotiklerin antibakteriyel etkilerini değiştirip değiştirmedikleri agar dilüsyon metodu ile araştırılmıştır.
Yöntem: Altı bakteri suşunun (*S. aureus*, *S. epidermidis*, *E. faecalis*, *S. pyogenes*, *P. aeruginosa* ve *E. Coli*) dilüsyonu hazırlanmış ve Mueller Hinton agarı içeren plaklara uygulanmıştır. Steril şartlarda, üç farklı dozda kas gevşetici ilaç diskleri (n=4) hazırlanmıştır. Her bakteri plağına her bir ilaç dozundan dörder disk yerleştirilmiştir. Plaklar inkübe edilmiş ve inhibisyon zonları ölçülmüştür. Kas gevşeticilerin, antibiyotiklerin etkinliğinde farklılık yapıp yapmadığını araştırmak için ise kas gevşeticilerin dört farklı konsantrasyonlarını içeren Mueller Hinton agar plakları hazırlanmıştır. Bu plaklara test edilen bakteriler inokule edilmiştir. Standard antibiyogram diskleri plaklara yerleştirilmiştir. Ölçülen inhibisyon zonları kontrol (kas gevşetiçi içermeyen Müller Hinton agar plakları) ile karşılaştırılmıştır.
Sonuçlar: Çalışmada kullanılan kas gevşeticiler, test edilen bakterilerin hiçbirinde antibakteriyel etki yapmamış ve antibiyotiklerin etkilerinde değişikliğe neden olmamıştır.
Yorum: Suksametonyum klorür ve rokuronyumun bromür'ün altı bakteri üzerine in vitro hiçbir antibakteriyel etkisi saptanmasa da antibiyotiklerle etkileşimlerini göstermek için in vivo çalışmaları önermekteyiz.

Anahtar kelimeler: antibakteriyel aktivite, suksametonyum klorür, rokuronyum bromür

INTRODUCTION

Non-antibiotic drugs may show antibacterial activity in addition to their actual effects [1]. The activity of some drugs is limited to the clinically insignificant level, while certain drugs show antibacterial activity with marked clinical significance. Some of these drugs, in their routine use, may provide prevention against bacterial infections accompanying a disorder, and the prevention against bacterial contamination may be related to the contents of a preparation in some drugs. Furthermore, the combined use of various non-antibiotic drugs increases the efficiency of the antibiotic itself [2].

The antibacterial characteristics of some non-antibiotic drugs result from their protective

substances or excipiants. The active ingredient of the drug does not produce such an effect [3]. Thus, in determining whether daily use of a drug presents any antibacterial activity, the use of a prepared form of the drug rather than its pure active ingredient will yield more reliable results.

Antibacterial effects of anaesthetic agents, primarily lidocaine, have been known for a long time [4-6]. Muscle relaxants are widely used in the procedures conducted under general anaesthesia. In this study, two different muscle relaxant preparations containing suxamethonium chloride and rocuronium bromide were tested in vitro to evaluate if they had quantifiable antibacterial activities and whether their combined use with antibiotics caused any differences in the efficacy of antibiotics or not.

MATERIALS AND METHODS

The antibacterial effects of two different muscle relaxant preparations containing suxamethonium chloride (lysthenon® forte 2%) and rocuronium bromide (esmeron® 50 mg/5 mL) were evaluated using disk diffusion method [7]. Disk diffusion method is a practical current method and is also used for antibiogram tests. The effects of preparations against standard species of 6 bacteria [*S. aureus* (American Type Culture Collection, ATCC 29213), *S. epidermidis* (ATCC 25212), *E. faecalis* (ATCC 12228), *S. pyogenes* (ATCC 19615), *P.aeruginosa* (ATCC 27853), and *E. coli* (ATCC 25922)] were investigated. The dilutions of 0.5 Mc Farland units ($1.5X10^8$ CFU/mL) were prepared for each of the bacteria and inoculated onto the plates containing Mueller Hinton agar (for *S. pyogenes*, 5% sheep blood agar). Under sterile conditions, empty antibiogram disks were embedded in plates with bacteria, then disks of suxamethonium at doses of 200, 100, and 50 μg/disk and disks of rocuronium at doses of 100, 50 and 25 μg/disk were prepared, using sterile pipette. Four drug disks containing each of the doses were placed onto each bacterium plate (n=4). The plates were incubated at 35 ^{0}C for 18-24 hours and the inhibition zones of the disks were measured in millimetres.

In the second stage of the study, the effect of muscle relaxant preparations on the efficacy of of various antibiotics on the forementioned 6 bacteria was investigated. At the same time, antibacterial activity of neuromuscular blocking agents was tested using agar dilution method. To this end, Mueller Hinton agar plates containing 400, 200, 100, and 50 μg/mL concentrations of each muscle relaxants were prepared (for *S. pyogenes*, 5% sheep blood agar). The plates without drug were used as control. These plates were inoculated with 0.5 Mc Farland dilutions of the bacteria tested. Standard antibiogram disks of routine laboratory usage were placed onto the plates to test for disk diffusion sensitivity. The disks (oxoid) used were as follows: amoxicillin clavulanic acid (30 □ g) (AMC), ciprofloxacin (5 □ g) (CIP), gentamycine (10 □ g) (CN), meropenem (10 □ g) (MEM) for *E. coli*; AMC, CN, CIP and vancomycine (30 □ g)(VA) for *S. aureus* and *S. epidermidis*; VA, AMC, CIP, erythromycin (15 □ g) (E) for *E. feacalis*; AMC, CIP, CN, MEM for *P. aeruginosa* and VA, CIP, E, penicillin (10 Unit) (P) for *S. pyogenes.* The diameters of the inhibition zones that formed on the plates after 18-24 hours incubation at 35 ^{0}C were measured. The value obtained for each bacterium was compared with the diameter of the inhibition zone on the control plate.

Statistical analysis was performed by using Kruskal Wallis and Mann-Whitney U test. Statistical significance was considered at $P<0.05$.

RESULTS

In this study, it was found that muscle relaxants, at any concentrations, did not have an inhibitory effect on the bacteria.

In the second stage of this study, during which the interactions of muscle relaxants with antibiotics were tested using agar dilution method, we determined that muscle relaxants did not alter the bacterial growth and the efficiency of antibiotics, in spite of high concentrations like 400 μg/mL (Table 1).

DISCUSSION

Various non-antibiotic drugs including some anaesthetic agents have been shown to have antibacterial characteristics [4-6]. In addition, some drugs increase the efficiency of antibiotics when used in combination [8]. The awareness of these extraordinary effects and interactions will aid in selection and use of medications.

In a study by Memiş et al.concerning the antibacterial activity of muscle relaxants on *E. Coli*, it was shown that rocuronium had strong antimicrobial activity [9].

In this in vitro study investigating the antibacterial effects of the two commonly used drugs of general anesthesia, namely suxamethonium chloride and rocuronium bromide, no antibacterial effect could be documented. Although significantly higher doses of these drugs compared to their routine use were chosen, no antibacterial effects could be observed. In the light of these results, it can be said that suxamethonium chloride and rocuronium bromide do not show any antibacterial activity in their routine use. The reason of the difference in the results between our study and the study performed by Memis et al. may be related to the methods and the drug concentrations

Bacteria (n=4)	Muscle relaxant / Antibiotic	Suxamethonium				Rocuronium				Control
		400 □ g/mL	200 □ g/mL	100 □ g/mL	50 □ g/mL	400 □ g/mL	200 □ g/mL	100 □ g/mL	50 □ g/mL	
S. aureus	AMC (30 □ g)	30	30	32	31	30	30	30	30	29
	VA (30 □ g)	20	20	20	20	20	20	20	20	19
	CN (10 □ g)	23	24	25	24	24	24	24	23	23
	CIP (5 □ g)	30	30	30	30	30	30	30	30	30
S. epidermidis	AMC (30 □ g)	40	38	40	39	40	40	40	38	40
	VA (30 □ g)	21	23	24	23	23	23	23	24	22
	CN (10 □ g)	28	30	30	29	30	30	30	30	30
	CIP (5 □ g)	36	40	40	39	39	40	40	38	38
E. faecalis	AMC (30 □ g)	28	28	30	30	31	30	28	32	30
	VA (30 □ g)	20	20	20	20	20	20	20	20	20
	CIP (5 □ g)	25	26	25	26	25	25	28	26	24
	E (15 □ g)	26	27	27	27	26	27	27	25	25
E. coli	AMC (30 □ g)	21	20	24	24	21	21	22	21	20
	CIP (5 □ g)	38	38	40	40	40	38	40	42	38
	CN (10 □ g)	19	20	23	22	20	20	19	20	19
	MEM (10 □ g)	30	33	32	32	30	30	30	30	30
P.aeruginosa	AMC (30 □ g)	0	0	0	0	0	0	0	0	0
	CIP (5 □ g)	39	37	39	39	39	39	39	38	38
	CN (10 □ g)	23	24	24	24	22	22	21	22	26
	MEM (10 □ g)	31	31	32	31	31	32	34	33	31
S.pyogenes	VA (30 □ g)	25	24	23	26	25	23	27	28	22
	CIP (5 □ g)	25	26	27	27	28	27	32	30	26
	E (15 □ g)	34	34	36	36	35	36	36	34	32
	P (10 U)	28	31	31	30	28	29	30	30	27

Table 1: The diameters of inhibition zones (mm) performed at bacteria which were inoculated into the plates containing different concentrations of muscle relaxants (mean).

AMC: amoxicillin clavulanic acid, CIP: ciprofloxacin, CN: gentamycine, MEM: meropenem, VA: vancomycine, E: erythromycin, P: penicillin.

used. They inoculated the bacteria into the plates after keeping the bacteria in the dense mixture of drug. Thus, it may be claimed that muscle relaxants perform antibacterial activity, in case of bacterial contamination with drug preparation. The concentrations of the test drugs used in their study were very high for in vivo conditions. In clinical practise, the doses in plasma are lower than in commercial preparations due to dilution with body fluids. Therefore, antibacterial activity cannot be similar to that of plasma concentrations.

In addition, we found that when used in combination with antibiotics, suxamethonium chloride and rocuronium bromide did not create any difference in the effects of the antibiotics on 6 bacteria that were selected for the study.

Most drug interactions have been evaluated under in vivo conditions. Because of inactivation, penicillin must be given in a different solution when used with aminoglycoside. The absorption of tetracycline has been prevented by Fe, Mg and Ca [10] while Mg and Al containing antiacids have inhibited the absorption of quinolone [11]. Macrolide antibiotics have increased the serum levels of digoxin [12,13] and benzodiazepines [14] but isepamicin (aminoglycoside) therapy has reduced the activity and duration of mivacurium and rocuronium [15]. The effects of oral contraceptive, anticoagulant and barbiturate have been decreased in patients used rifampine [16].

Although antibacterial activity of non-antibiotic drugs are positive, it might be expected that some drugs combined with antibiotics will decrease the activity of antibiotic and there are examples of this condition. The muscle relaxants tested in our study neither showed any antibacterial activity nor altered the effectiveness of antibiotics under in vitro conditions in case of combined use. Further studies are needed to show the interactions in living subjects.

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