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Klinik Çalışma - Araştırma / *Original Article*

Growth of Escherichia Coli in Atracurium, Rocuronium, Mivacurium, Cisatracurium, Pancuronium, and Vecuronium

Atrakuryum, Rokuronyum, Mivakuryum, Sisatrakuryum, Pankuronyum ve Vekuronyumda Escherichia Coli Üremesi

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Objectives: We studied in vivo growth of *Escherichia coli* in atracurium, rocuronium, mivacurium, cisatracurium, pancuronium, and vecuronium.

Patients and Methods: The pathogen was exposed to atracurium, rocuronium, mivacurium, cisatracurium, pancuronium and vecuronium for 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h at room temperature, respectively.

Results: The mean colony counts of *Escherichia coli* after exposure to rocuronium was significantly lower than the counts after exposure to atracurium, mivacurium, cisatracurium, pancuronium and vecuronium (p=0.002, 0.000, 0.000, 0.001, and 0.002, respectively). No significant difference was found with respect to the mean colony counts with atracurium, mivacurium, cisatracurium, pancuronium and vecuronium (p<0.05).

Conclusion: Rocuronium had more powerful antimicrobial effects than the other neuromuscular agents.

Key words: Atracurium, rocuronium, mivacurium, cisatracurium, pancuronium, vecuronium, bacterial contamination, Escherichia coli.

Amaç: Biz çalışmamızda atrakuryum, rokuronyum, mivakuryum, sisatrakuryum, pankuronyum ve vekuronyumun *Escherichia coli* üremesi üzerine olan etkinliğini araştırdık.

Hastalar ve Yöntemler: Patojen bakteri, atrakuryum, rokuronyum, mivakuryum, sisatrakuryum, pankuronyum ve vekuronyum ile 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, ve 24 saat oda sıcaklığında temas ettirildi.

Bulgular: *Escherichia coli* ortalama koloni sayısı rokuronyumda istatiksel anlamlı olarak atrakuryum, mivakuryum, sisatrakuryum, pankuronyum ve vekuronyumdan daha düşük saptandı (sırasıyla p=0.002, 0.000, 0.000, 0.001, ve 0.002). Atrakuryum, mivakuryum, sisatrakuryum, pankuronyum ve vekuronyumda ortalama koloni sayısı açısından anlamlı fark saptanmadı (p<0.05).

Sonuç: Rokuronyum, diğer nöromusküler kas gevşetici ajanlardan daha kuvvetli antimikrobiyal etki gösterdi.

Anahtar sözcükler: Atrakuryum, mivakuryum, sisatrakuryum, pankuronyum, vekuronyum, bakterial kontaminasyon, Escherichia coli.

Drugs used in anesthesia may influence bacterial growth.[1] Used ampules and syringes may be contaminated in a busy environment.[2] Neuromuscular-blocking drugs block neuromuscular transmission at the neuromuscular junction, causing paralysis of the affected skeletal muscles. This is accomplished either by acting presynaptically via the inhibition of acetylcholine synthesis or release, or by acting postsynaptically at the acetylcholine receptor. While there are drugs that act presynaptically, the clinically-relevant drugs work postsynaptically. Clinically,

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neuromuscular block is used as an adjunct to anesthesia to induce paralysis.[3]

There is little evidence to suggest that neuromuscular blocker agents would increase or facilitate bacterial growth; however, such data are important to ensure that their use does not increase the risk of bacterial contamination or sepsis.[4] Therefore, we conducted an in vitro study to test the hypothesis that atracurium, rocuronium, mivacurium, cisatracurium, pancuronium and vecuronium may exhibit an antibacterial activity against common etiologic agents encountered during infectious complications after anesthesia. The purpose of this study was to determine the potential for growth of a microorganism (*Escheria coli*) in atracurium, rocuronium, mivacurium, cisatracurium, pancuronium, and vecuronium.

PATIENTS AND METHODS

Bacterial strains were isolates of *Escherichia coli* (ATCC 25922). The tested pharmaceutical preparations were atracurium (Tracrium® 50 mg/5 mL, GlaxoSmithKline, Italy), rocuronium (Esmeron® 50 mg/5 mL, Organon, Holland), mivacurium (Mivacron® 10 mg/5 mL, GlaxoSmithKline, Italy), cisatracurium (Nimbex® 5 mg/2.5 mL GlaxoSmithKline, Italy), pancuronium (Pavulon® 4 mg/2 mL, Organon, Holland), and vecuronium (Norcuron® 4 mg, Organon, Holland). Presumptive identification of the pathogen was achieved by screening non-sorbitol-fermenting colonies on sorbitol McConkey agar and by use of an identification kit. The identified strain was inoculated into 5 mL of brain-heart infusion broth and incubated overnight at 37 °C. The suspension containing approximately $5x10^8$ to 10^9 colony forming units (CFU/mL) was used as a test inoculum.

Ten microuters of the test inoculum was added to 5 mL of atracurium, rocuronium, mivacurium, cisatracurium, pancuronium and vecuronium. The solutions were vortexed for 15s, obtaining approximate bacterial density of $2x10^6$ CFU/mL. The pathogen was exposed to test drug for 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h at room temperature (26 °C). All procedures were performed by the same anesthetists (D.M and M.B).

In order to assess bactericidal activity of the test drug, the drugs present in the suspensions were inactivated by diluting 10 μL of each suspension with 10 mL of distilled water (1:1000 dilution) after exposure at room temperature.[5] Control suspensions were prepared by adding the test inoculum to distilled water. Antimicrobial activity was considered to be bactericidal when a 10^3-fold reduction (99.9% kill) in colony count from control was achieved.[6]

To test the validity of inactivation of antimicrobial activity, a pair of suspensions, one containing 10 μL of the test inoculum in 10 mL of distilled water (control) and another containing 10 μL of the test inoculum and 10 μL of atracurium, rocuronium, mivacurium, cisatracurium, pancuronium and vecuronium in 10 mL of distilled water respectively, were prepared at room temperature. Antimicrobial activity was considered to be inactive when there was no significant difference in the colony count from the control.

Due to large variability in the data, all analyses for the difference in colony count between test solutions was performed on the natural logarithm of the colony counts using a one-way analysis of variance. Individual comparisons between group means were made using the Bonferroni post-hoc test. $P<0.05$ was regarded as significant.

RESULTS

Table 1 outlines the composition of the drugs in this study. Table 2 summarizes colony counts of *E. coli* after exposure of the microorganism to the test solutions for various periods of time and distilled water. Some organisms grew after exposure to rocuronium. The mean colony counts of *E. coli* after exposure to rocuronium was significantly lower than the counts after exposure to atracurium, mivacurium, cisatracurium, pancuronium and vecuronium ($p=0.002$, 0.000, 0.000, 0.001, and 0.002, respectively). No significant difference was found with respect to the mean colony counts with atracurium, mivacurium, cisatracurium, pancuronium and vecuronium ($p<0.05$). The colony count with pancuronium was significantly higher than with other neuromuscular blockers in first 2 h. ($p<0.001$).

Table 1. The composition of the study drugs

Medication/Manufacturer	Type of ampoule	Preservatives	pH
Atracurium (Tracrium® 50 mg/5 mL, GlaxoSmithKline, Italy)	Glass	Benzenesulfonic acid	3.2-3.7
Rocuronium (Esmeron® 50 mg/5 mL, Organon, Holland)	Glass	Sodium acetate, sodium chloride, asetic acid	4
Mivacurium (Mivacron® 10 mg/5 mL, GlaxoSmithKline, Italy)	Glass	Benzenesulfonic acid	3.5
Cisatracurium (Nimbex® 5 mg/2.5 mL GlaxoSmithKline, Italy)	Glass	Benzenesulfonic acid	3.3-3.8
Pancuronium (Pavulon® 4 mg/2 mL, Organon, Holland)	Glass	Sodium acetate, sodium chloride, asetic acid	4
Vecuronium (Norcuron® 4 mg, Organon, Holland)	Glass	Citric acid, disodium phosphate, mannitol	4

DISCUSSION

Although the impact of drugs used in anesthesia on bacteria has been extensively studied,[1,7-12] this study was devoted to the bactericidal properties of common neuromuscular blockers used in anesthesia. We found that rocuronium had more powerful antimicrobial effects than the other neuromuscular agents.

Neuromuscular-blocking drugs block neuromuscular transmission at the neuromuscular junction, causing paralysis of the affected skeletal muscles. The ammonio steroids have structures containing the same steroid nucleus as steroid hormones like pancuronium, vecuronium and rocuronium.[13] Benzylisoquinolines have complex ring structures and are included in atracurium and mivacurium.[3] Cisatracurium is the 1R-cis 1'R-cis isomer of atracurium.[14] Roy and Varin[15] demonstrated that the basic characteristics of neuromuscular blocking drugs, namely, molecular weight, lipid solubility and protein binding, were strongly associated with the kinetics of the drug response. For a series of aminosteroidal agents, Wierda et al.[16,17] reported a relation between lipid solubility or protein binding and various pharmacokinetic and pharmacodynamic descriptors. We thought that although pancuronium, vecuronium and rocuronium have the same chemical structure, small changes of the chains might influence the antibacterial activity, but this should be confirmed by further clinical studies.

Many factors may affect the sterility of drugs after they have been drawn up. These include

Table 2. Colony counts of *Escheria coli* after exposure to atracurium, rocuronium, mivacurium, cisatracurium, pancuronium and vecuronium

	2h	4h	6h	8h	10h	12h	14h	16h	18h	20h	22h	24h	Mean count (ln)
Atracurium	1	1	2	2	5	5	6	8	8	15	16	31	1.4998*
Rocuronium	0	1	0	0	0	0	0	0	0	0	0	2	0.1033
Mivacurium	1	1	2	3	6	8	15	22	37	41	45	81	2.0798*
Cisatracurium	1	4	4	5	6	8	9	13	13	14	24	32	1.9531*
Pancuronium	0	0	0	1	0	5	4	5	8	19	61	475	1.6225*
Vecuronium	0	0	1	1	2	2	4	5	16	27	47	72	1.4633*
Distilled water	0	0	0	0	0	0	0	0	0	0	0	0	0

Control colony count at 0 h-exposure was 487.
*The colony counts of *E. coli* after exposure to rocuronium was significantly lower than the counts after exposure to atracurium, mivacurium, cisatracurium, pancuronium and vecuronium (p=0.002, 0.000, 0.000, 0.001, and 0.002, respectively).

the type of ampoule, syringe preparation technique, presence of drug preservatives, intrinsic bactericidal property of the drug in question, pH of the pharmaceutical preparation, duration of storage of the drawn-up drug and environmental temperature.[18]

Zacher et al.[19] showed bacterial contamination of the contents of glass ampoules upon opening to be a frequent occurrence. They investigated this subject and concluded that swabbing the neck of glass ampoules with alcohol swabs before opening reduced the incidence of bacterial contamination. Many factors determine the appropriate final pH of a pharmaceutical preparation. Lowering of the pH enhances the antimicrobial activity of preservatives.[20] In our study, all drugs present in glass ampoules, syringe preparation technique, pH levels (changes 3.3-4) and environmental temperature were the same.

Concerning infection control, results obtained at room temperature should be considered. Bacteria can be introduced into ampules during manufacture (intrinsic contamination) or during preparation and administration in the hospital (extrinsic contamination). Contaminated glass particles, rubber diaphragm, or needles may introduce bacteria into the fluids. The likelihood of fluid becoming contaminated during use is directly related to the duration of uninterrupted infusion through the same administration set and the frequency with which the set is manipulated.

Graystone et al.[4] determined that the potential for commonly infused drug solutions to support or inhibit microbial growth was explored in their study. Drugs examined were midazolam HCl, morphine sulphate, fentanyl citrate, pethidine HCl, bupivacaine HCl, atracurium besylate, vecuronium bromide, adrenaline, dopamine, dobutamine, noradrenaline, isoprenaline, glyceryl trinitrate, sodium nitroprusside and propofol. They found that there was no significant difference between atracurium besylate and vecuronium bromide groups. Our results showed that *E. coli* grows in atracurium, mivacurium, cisatracurium, pancuronium and vecuronium at room temperature so they may cause nosocomial infection if contaminated. Indeed, the antibacterial effect of a drug against bacteria depends on many factors, such as the size of the inoculum, the concentration of the antibacterial drug in vivo, or the host-defense mechanisms. The antibacterial activity of these drugs should be investigated in further studies. However, less bacterial growth observed for each concentration of rocuronium might ensure that the use of these drugs does not increase the risk of bacterial contamination or sepsis.

The antibactericidal activity of rocuronium in vitro on the microorganisms most frequently decreased in infectious complications after anesthesia might be useful to ensure that the use of this drug does not increase the risk of bacterial contamination or sepsis, although this should be confirmed by further clinical studies. Moreover, the mechanisms of the bactericidal activity of rocuronium are not known and should be elucidated by further studies.

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