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Original Article

Antibacterial activity of ondansetron and granisetron in vitro

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Abstract: To study the antibacterial activity of antiemetic drugs ondansetron and granisetron in vitro. Escherichia coli (E. coli), Staphylococcus aureus (S. aureus) and Pseudomonas aeruginosa (P. aeruginosa) were grown in medium containing 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL and 0.0625 mg/mL ondansetron or granisetron. The number of colonies was counted after 24 hours. Normal saline was use as control. Each experiment was repeated ten times. At ≥0.25 mg/ml, ondansetron inhibits the growth of E. coli and P. aeruginosa. S. aureus growth was inhibited at concentration of ≥0.5 mg/ml. Granisetron inhibits E. coli growth beginning at 0.0625 mg/ml and growth of both P. aeruginosa and S. aureus at ≥0.25 mg/ml. Almost in all strains, there was a significant difference in the antibacterial activity between 6 and 24 h incubation times when the concentration of agents inhibited bacterial growth. We found that ondansetron and granisetron have inhibitory activity on growth of E. coli, S. aureus and P. aeruginosa in vitro.

Keywords: Ondansetron, granisetron, inhibitory activity, vitro

Introduction

In recent years, the proportion of epidural anesthesia was very high in China, especially these patients after obstetrics and gynecology surgery under this anesthesia often selected postoperative epidural analgesia [1, 2]. Postoperative analgesia medication generally lasts more than 40 hours, leading to potential epidural infection, and the infection rate has been on the rise [3, 4].

Anti-nausea and vomiting drugs are commonly used in combination with local analgesics for postoperative epidural analgesia [5]. Previous studies have showed that commonly used local anesthetics such as lidocaine and bupivacain have a certain antibacterial effect [6-9]. Antiemetics such as ondansetron [10] and granisetron [11] are used widely as additive agents for analgesia, and because of the antibacterial activity of 5-HT uptake inhibitor, sertraline [1, 12], we hypothesis ondansetron and granisetron which are the 5-HT3 serotonin antagonist may have antibacterial activity.

In the present study, we research the inhibitory effects of ondansetron and granisetron on different bacteria growth with variedagent concentrations in vitro.

Materials and methods

The ethics committee of the Suzhou BenQ medical center approved the protocol:

Drugs

Preservative-free ondansetron hydrochloride (2 mg/mL, Shandong Qilu Pharmaceutical, China) and granisetron hydrochloride (1 mg/mL, Suzhou Changzhen-Xinkai Pharmaceutical, China) were diluted with sterile saline to final concentrations of 1, 0.5, 0.25 and 0.125 mg/mL. Sterile saline alone was used as control.

Bacteria strains

Standard clinical laboratory strains of Escherichia coli (E. coli) (ATCC25922), Staphylococcus aureus (S. aureus) (ATCC25923) and Pseudo-

Table 1. Bacterial colony numbers at different concentrations of ondansetron

Ondansetron (mg/mL)	Incubation time (hours)	E. coli (CFU/mL)	S. aureus (CFU/mL)	P. aeruginosa (CFU/mL)
1	6	$(6.3\pm0.6)\times10^{5*}$	$(8.6\pm1.3)\times10^{5*}$	$(1.3\pm0.3)\times10^{4*}$
	24	$(3.5\pm1.2)\times10^{4*,\#}$	$(1.7\pm0.6)\times10^{4*,\#}$	$(9.0\pm3.4)\times10^{2*,\#}$
0.5	6	$(1.0\pm0.2)\times10^{6*}$	$(3.2\pm1.2)\times10^{6}$	$(1.3\pm0.1)\times10^{5*}$
	24	$(4.6\pm1.9)\times10^{5*,\#}$	$(3.9\pm0.7)\times10^{5*,\#}$	$(1.7\pm0.6)\times10^{4*,\#}$
0.25	6	$(6.8\pm1.7)\times10^{6}$	$(7.7\pm1.6)\times10^{6}$	$(3.3\pm0.6)\times10^{6}$
	24	$(9.8\pm0.9)\times10^{5*,\#}$	$(8.9\pm2.8)\times10^{6}$	$(3.4\pm1.5)\times10^{6}$
0.125	6	$(7.9\pm1.6)\times10^{6}$	$(8.5\pm1.5)\times10^{6}$	$(3.5\pm1.9)\times10^{6}$
	24	$(8.5\pm2.3)\times10^{6}$	$(9.0\pm3.6)\times10^{6}$	$(7.3\pm1.3)\times10^{6}$
0	0		5×10^{6}	
	6	$(1.1\pm0.3)\times10^{7}$	$(1.0\pm0.1)\times10^{7}$	$(4.1\pm1.1)\times10^{6}$
	24	$(1.3\pm0.4)\times10^{7}$	$(3.9\pm0.7)\times10^{7}$	$(6.5\pm0.6)\times10^{6}$

*P<0.01, versus bacterial cultured with 0 mg/ml for 6 hours or 24 hours correspondingly; #P<0.01, versus bacterial cultured with different drug concentrations for 6 hours correspondingly.

Table 2. Bacterial colony numbers at different concentrations of granisetron

Granisetron (mg/mL)	Incubation time (hours)	E. coli (CFU/mL)	S. aureus (CFU/mL)	P. aeruginosa (CFU/mL)
0.5	6	$(3.1\pm1.1)\times10^{4*}$	$(2.8\pm0.5)\times10^{4*}$	$(3.3\pm0.2)\times10^{5*}$
	24	$(6.7\pm2.6)\times10^{2*,\#}$	$(7.6\pm3.5)\times10^{3*,\#}$	$(5.7\pm2.4)\times10^{4*,\#}$
0.25	6	$(9.6\pm1.8)\times10^{4*}$	$(5.5\pm1.3)\times10^{5*}$	$(1.1\pm0.7)\times10^{6}$
	24	$(2.4\pm0.9)\times10^{3*,\#}$	$(1.3\pm0.5)\times10^{4*,\#}$	$(8.2\pm0.9)\times10^{5}$
0.125	6	$(6\pm2.1)\times10^{5}$	$(8.3\pm2.1)\times10^{6}$	$(1.3\pm0.6)\times10^{6}$
	24	$(1.3\pm0.6)\times10^{5*,\#}$	$(9.5\pm2.3)\times10^{6}$	$(4.8\pm2.1)\times10^{6}$
0.0625	6	$(5.1\pm1.3)\times10^{6}$	$(5.3\pm1.8)\times10^{6}$	$(2.7\pm0.3)\times10^{6}$
	24	$(1.7\pm0.7)\times10^{6*}$	$(1.7\pm0.7)\times10^{7}$	$(2.3\pm0.9)\times10^{6}$
0	0		5×10^{6}	
	6	$(1.1\pm0.3)\times10^{7}$	$(1.0\pm0.1)\times10^{7}$	$(4.1\pm1.1)\times10^{6}$
	24	$(1.3\pm0.4)\times10^{7}$	$(3.9\pm0.7)\times10^{7}$	$(6.5\pm0.6)\times10^{6}$

*P<0.01, versus bacterial cultured with 0 mg/ml for 6 hours or 24 hours correspondingly; #P<0.01, versus bacterial cultured with different drug concentrations for 6 hours correspondingly.

monas aeruginosa (P. aeruginosa) (ATCC27853) were obtained from Jiangsu Province Center for Clinical Standards.

In vitro bacteria culture

Bacteria were grown in soybean casein digest medium for 18 hours to reach the exponential growth phase and diluted with sterile saline to reach a McFarland unit. Each McFarland unit corresponds to an initial concentration of about 3×10^{8} colony forming units (CFU)/mL. Each standard inoculum was diluted using sterile saline and inoculated onto a single blood agar and cultured for 6 hours or 24 hours at 37°C. 20 μL of bacteria containing approximately 5×10^{6} CFU were added to 980 μL of ondansetron and granisetron solutions at different concentrations, followed by incubation at 37°C. An equal volume of sterile saline was used as control. After incubation for 24 hours, 100 μL of 1:10,000 bacteria dilution was then inoculated on blood agar medium and cultured at 37°C. Colony numbers were counted after 24 hours. Each experiment was repeated ten times.

Statistics analysis

All data are presented as mean ± standard deviation (SD). Statistical analyses were performed with the Prism software package (GraphPad v5, San Diego, CA, USA). Data were analyzed using one-way ANOVA. A *P*-value less than 0.01 was accepted as statistically significant.

Results

Both ondansetron and granisetron showed significant antimicrobial effect with a time- and dose-dependent manner on E. coli, S. aureus,

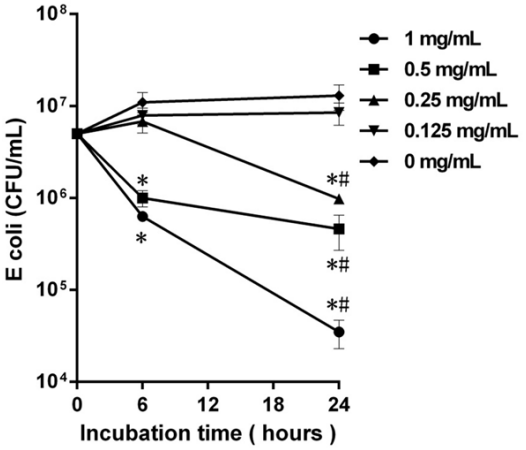

Figure 1. Colony counts of E. coli after 6 and 24 h incubation with various concentrations of ondansetron. *Colony count of each group is significantly lower (P<0.01) than control. #For each concentration of ondansetron, colony count is significantly different (P<0.01) between 24 and 6 h incubation.

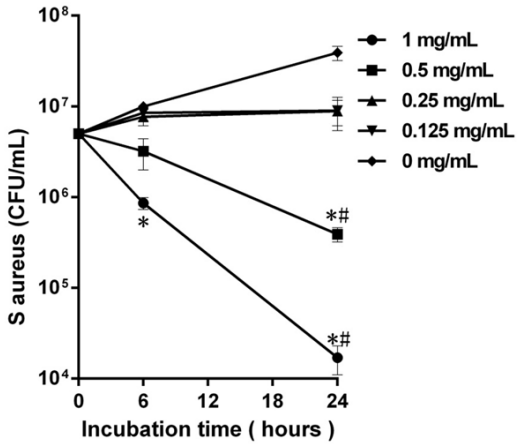

Figure 2. Colony counts of S aureus after 6 and 24 h incubation with various concentrations of ondansetron. *Colony count of each group is significantly lower (P<0.01) than control. #For each concentration of ondansetron, colony count is significantly different (P<0.01) between 24 and 6 h incubation.

and P. aeruginosa (**Tables 1**, **2** and **Figures 1**-**6**).

The concentrations of ondansetron inhibited growth of E. coli are higher than 0.25 mg/ml (P<0.01) (**Table 1**; **Figure 1**). The concentrations of S. aureus and P. aeruginosa are both higher than 0.5 mg/ml (P<0.01) (**Table 1**; **Figures 2**, **3**), and with The higher concentration, the inhibitive effect on bacterial growth is greater. The concentrations of granisetron inhibited bacterial growth are lower than ondansetron. The inhibitory effect started at 0.25 mg/ml for S. aureus, and at 0.5 mg/ml for P. aeruginosa (P<0.01) (**Table 2**; **Figures 5**, **6**). Granisetron exhibited growth inhibitory effect on E. coli even at a concentration of 0.0625 mg/ml after 24 h incubation times (P<0.01) (**Table 2**; **Figure 4**). Almost inall strains, there was a significant difference in the antibacterial activity between 6 and 24 h incubation times when the concentration of agents inhibited bacterial growth.

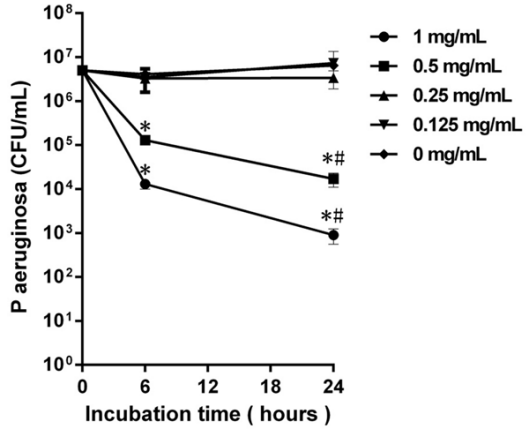

Figure 3. Colony counts of P. aeruginosa after 6 and 24 h incubation with various concentrations of ondansetron. *Colony count of each group is significantly lower (P<0.01) than control. #For each concentration of ondansetron, colony count is significantly different (P<0.01) between 24 and 6 h incubation.

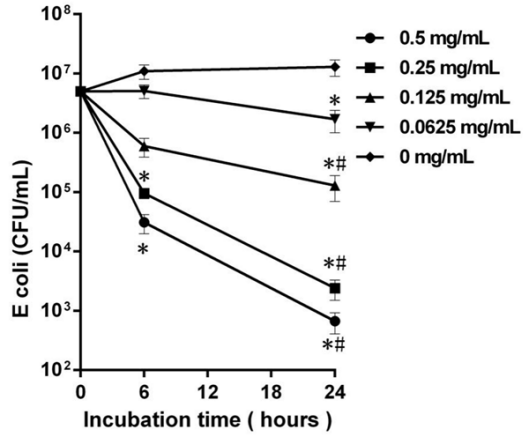

Figure 4. Colony counts of E. coli after 6 and 24 h incubation with various concentrations of granisetron. *Colony count of each group is significantly lower (P<0.01) than control. #For each concentration of ondansetron, colony count is significantly different (P<0.01) between 24 and 6 h incubation.

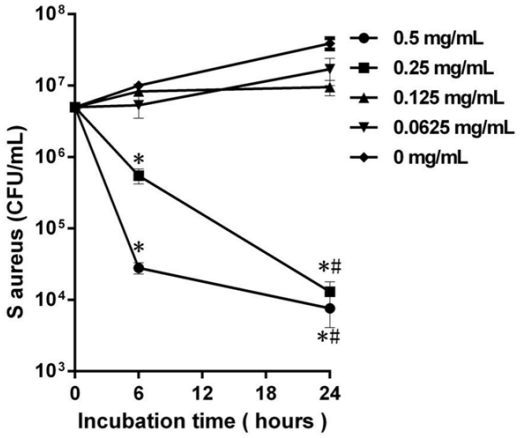

Figure 5. Colony counts of S aureus after 6 and 24 h incubation with various concentrations of granisetron. *Colony count of each group is significantly lower (P<0.01) than control. #For each concentration of ondansetron, colony count is significantly different (P<0.01) between 24 and 6 h incubation.

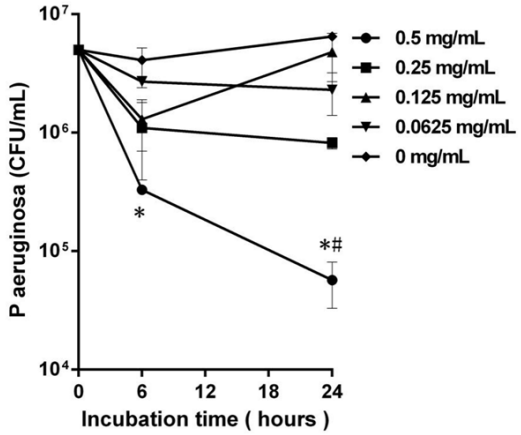

Figure 6. Colony counts of P. aeruginosa after 6 and 24 h incubation with various concentrations of granisetron. *Colony count of each group is significantly lower (P<0.01) than control. #For each concentration of ondansetron, colony count is significantly different (P<0.01) between 24 and 6 h incubation.

Discussion

Although the overall incidence of epidural infection is still relatively low, the infection rate has been on the rise since the application of patient-controlled epidural analgesia (PCEA) [4]. PCEA after surgery commonly used local anesthetic and combines with anti-nausea, antivomiting drugs [5]. So the research on whether the analgesic and antiemetic drugs have any antibacterial effect has important clinical significance. Previous studies showed that the local anesthetics such as lidocaine and ropivacaine have antibacterial effects, but the antibacterial activities of antiemetic drugs have not been reported. So this study focused on the most common clinical antiemetic drugs, ondansetron and granisetron, to show that they can both inhibit the growth of such bacteria as E. coli, S. aureus, and P. aeruginosa in vitro. E. coli, S. aureus and P. aeruginosa were chosen for the study because they are widely present on the human skin surface, and are commonly used as surveillance targets for hospital infection [7].

PCEA was widely used, especially in obstetrics and gynecology. The PCEA pumps were often filled with ropivacaine (1.5 mg/ml) and ondansetron (160 μg/ml) or granisetron (90 μg/ml) in saline solution and set with a bolus of 2 ml, a lockout interval of 10 min. The ondansetron have not IV administration, so the concentration of ondansetron was higher in epidural space (160 μg/ml) than that in the blood (5 μg/ml) after intravenous injection [13].

The potential mechanism by which ondansetron and granisetron inhibit bacteria growth is unclear. Previous studies have shown that the bacterial inhibitory effect of local anesthetics is due to interference with the function of the prokaryotic cell membranes. Local anesthetics interfered with E. coli respiration lead to the outflow of the cell contents [14]. Lidocaine has also been reported to inhibit neutrophil adhesion and chemotaxis. Research has also shown that by changing the permeability of the outer membrane of the bacterial cell, and lidocaine can cause bacterial cell depolarization [15]. Tanji, et al. found that local anesthetics inhibit the intracellular ATP production, meanwhile upregulate heat shock proteins [16]. Whether antiemetics such asondansetron and granisetron employ similar mechanisms to inhibit bacteria growth requires further study. Bacteria often exist in an extracellular polymeric matrix composed of proteins, polysaccharides and nucleic acids, which confer a physical barrier to diffusion of molecules such as antibiotics. One of the mechanisms of antibiotic resistance is the formation of a large matrix [17, 18]. The higher mRNA level of polysaccharide biosynthesis genespsi A, algD, pelAin P. Aeruginosa may contribute to the creation of the matrix [19, 20].

Interestingly, in this study P. aeruginosa is also more resistant to ondansetron and granisetron compared to E. coli.

In summary, ondansetron and granisetron inhibited the growth of E. coli, S. aureus, and P. aeruginosa growth in the time- and dose-dependent manner in vitro. Moreover, at the concentrations commonly used in practice, ondansetron and granisetron significantly inhibited the growth of E. coli, which showing clinical potential for the prevention of anesthesia-related infections.

Disclosure of conflict of interest

None.

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