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SURGICAL RESEARCH

DO ANTIBIOTICS CONTRIBUTE TO POSTOPERATIVE ILEUS? CONTRACTILE RESPONSES OF ILEUM SMOOTH MUSCLE IN GUINEA PIGS TO LONG-TERM PARENTERAL CEFTRIAXONE AND AMPICILLIN

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Background: Antibiotics may impair small bowel smooth muscle contractility and contribute to postoperative ileus. The aim of this study was to compare the contractile responses of ileum smooth muscle to different agonists in guinea pigs treated with ceftriaxone (Rocephin; F. Hoffman–La Roche, Kaiseraugst, Switzerland) or ampicillin (Ampisina; Mustafa Nevzat İlaç Sanayii AŞ, Istanbul, Turkey).

Methods: Twenty-four adult guinea pigs were randomly divided into three groups. Whereas eight of these received ceftriaxone sodium (100 mg/kg per day, i.m.) for 10 days, another eight guinea pigs received ampicillin (50 mg/kg per day, i.m.) for 10 days and the remaining eight served as the control group receiving 1 mL distilled water during 10 days as placebo. By the end of 10 days, the animals were killed and their ilea were excised. Ileum segments were placed in an organ bath; concentration–response relationship for carbachol and histamine were obtained by adding the reagent cumulatively to the bath.

Results: pD_2 values being the same, maximum contractile responses (E_{max}) to carbachol and histamine were significantly reduced in the ceftriaxone sodium group compared with the control group. No significant differences in E_{max} and pD_2 values to carbachol and histamine were observed between the ampicillin group and the control group.

Conclusion: These data indicate that whereas ceftriaxone may impair small bowel smooth muscle contractility, ampicillin does not. There are implications for the long-term use of parenteral antibiotics in the postoperative period.

Key words: ampicillin, ceftriaxone, contractile response, ileum.

Abbreviations: AMP, ampicillin; CTX, ceftriaxone.

INTRODUCTION

Besides their primary antimicrobial activity, antibiotics also have significant additional effects on many tissues, including smooth muscles. It is well known that aminoglycoside antibiotics have a neuromuscular blocking effect of clinical importance during general anaesthesia.[1] Erythromycin, a macrolide antibiotic, has a contractile effect similar to motillin on the gastrointestinal system smooth muscle.[2] There is a very limited number of studies on effects of β-lactam antibiotics on smooth muscles. Recently, we studied the effect of ceftriaxone (CTX) on gall bladder smooth muscle in guinea pigs and found that CTX decreases the contractile response to carbachol and histamine.[3] In this study, at laparotomy we noted that small intestine of the guinea pigs was dilated and intestinal content was thicker. From this observation, we suggested that CTX may affect also ileum and could interfere with intestinal motility. If antibiotics have such properties, their prolonged administration may decrease intestinal contractility and may contribute to postoperative ileus. In this study we aimed to evaluate the effect of β-lactam antibiotics; CTX and ampicillin (AMP) treatment on ileum smooth muscle in guinea pigs.

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METHODS

Twenty-four male guinea pigs each weighing approximately 500 g were maintained in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals. The study was carried out by the approval of the Ethical Committee of Animal Research of Cumhuriyet University. Guinea pigs were divided randomly into three groups. All animals were fed with same standard chow in three groups: (i) *control group* (C) received 1 mL distilled water i.m. as placebo for 10 days ($n = 8$); (ii) the *CTX group* received ceftriaxone sodium (100 mg/kg per day) i.m. for 10 days ($n = 8$); and (iii) the *AMP group* received ampicillin (50 mg/kg per day) intramuscularly for 10 days.

Tissue preparation

On the 10th day of antibiotic treatment, the guinea pigs were killed by cervical dislocation. The abdomen was opened with a midline incision. Ileus was constituted by laparotomy and manipulation of the intestine.[4] Ileum was removed and placed in previously aerated (95% O_2 and 5% CO_2) Krebs' solution (the compositions were NaCl, 115.48 mmol; KCl, 4.61 mmol; $CaCl_2$, 2.5 mmol; $MgSO_4$, 1.16 mmol; $NaHCO_3$, 21.9 mmol;

NaH_2PO_4, 1.14 mmol and glucose 10.09 mmol). Whole full-thickness segments of ileum in Krebs' solution were allowed to equilibrate for 4 h at 4°C in refrigerator. This procedure decreases spontaneous ileal contractions and stabilizes subsequent contractile responses to carbachol and histamine. After this procedure, whole full-thickness segments of the ileum were placed in longitudinal direction in a 10-mL muscle bath, filled with pre-aerated Krebs' solution at 37°C. The upper end of the preparation was tied to an isometric transducer (Grass FT 03; Quincy, MA, USA) and preloaded with 1–1.5 g. Tissue was allowed to equilibrate for 30 min until a stable baseline was attained.

Isometric measurements

At the beginning of each experiment, 80 mmol KCl was added to the tissue bath and the contraction was considered as the reference response. Following the KCl response, tissues were washed twice and after a period of 30 min the concentration–response curves to carbachol (10^{-8}–10^{-4} mol/L) and histamine (10^{-8}–10^{-4} mol/L) were obtained cumulatively. Isometric tension was recorded on a Grass model 79 E polygraph.

Three sets of experiments were carried out on ileal smooth muscle obtained from guinea pigs. In the first set of experiments, we evaluated the concentration-dependent effects of carbachol and histamine on ileal smooth muscle isolated from control group. In the second set of experiments, we evaluated the concentration-dependent effects of carbachol and histamine on ileal smooth muscle isolated from CTX group. In the third and final set of experiments, we evaluated the concentration-dependent effects of carbachol and histamine on ileal smooth muscle isolated from AMP group (n = 8).

The contractile responses to carbachol and histamine were calculated as a percentage of the contraction caused by KCl (80 mmol). To evaluate the effects of agonists, the maximum contractile responses (E_{max}) and pD_2 values (i.e. the negative logarithm of the concentration for the half-maximal response, ED_{50}) were calculated. The concentration response data obtained in each experiment were plotted as the response/concentration against the response, producing a sigmoid curve in each experiment, as predicted from the Scatchard equation for drug–receptor interaction. Agonist pD_2 values (apparent agonist affinity constants) were calculated from each agonist concentration–response curve by linear regression of the linear median part of the sigmoid curve and taken as a measure of the sensitivity of the tissues to each agonist.

Drugs

Chemicals used in the experiments were carbachol and histamine from Sigma (St Louis, MO, USA). Drugs were dissolved in distilled water. The volume that was added to the muscle bath never exceeded 5% of its total volume. All solutions were prepared just before use.

Statistical analysis

All data were expressed as mean ± standard error of the mean. Data were analysed by repeated measured ANOVA and groups were compared statistically using general linear models of ANOVA followed by Newman Keul's test. Differences were considered to be significant when $P < 0.05$.

RESULTS

At the beginning of each experiment, 80 mmol KCl was added to the tissue bath. Contractions elicited by 80 mmol KCl were not significantly different in ileal smooth muscles isolated from guinea pigs in experiment groups from those in control group (3.04 ± 0.21 g; 2.96 ± 0.18 g; 3.02 ± 0.12 g, n = 8 in all groups) (Fig. 1).

The cumulative addition of carbachol (10^{-8}–10^{-4} mol) and histamine (10^{-8}–10^{-4} mol) produced concentration-dependent contractions in ileal smooth muscles obtained from guinea pigs in control and experimental groups (n = 8 in three groups) (Fig. 2 a,b). The concentration–response curves for carbachol and histamine were shifted to the right, with significantly lower E_{max} values in CTX group in comparison to the control group ($P < 0.05$), but there was no significance among the corresponding pD_2 values (Fig. 2a,b; Table 1). E_{max} and pD_2 values for carbachol and histamine were not significantly different in the AMP group in comparison to the control group ($P > 0.05$), (Fig. 2a,b; Table 1).

DISCUSSION

Paralytic postoperative ileus continues to be a significant problem after abdominal and other types of surgery. Three main mechanisms are involved in its causation, namely neurogenic, inflammatory and pharmacological mechanisms.[5–7] Anaesthetic agents, opioids, laparatomy and intestinal manipulation can cause impaired intestinal motility. Despite frequent use of antibiotics in the perioperative period, their actions on the intestinal smooth muscles have not been thoroughly studied yet. In a recent study we found that CTX treatment decreases the contractile responses of the gall bladder smooth muscle in guinea pigs.[3] Current study was conducted to evaluate the ileum smooth muscle contractile responses during treatment with two β-lactam antibiotics; CTX and ampicillin.

β-lactam antibiotics have been scarcely tried experimentally on excitable tissues including isolated smooth muscles, although their epileptogenic effect is well known since the early days of intrathecal administration of penicillin for treatment of bacterial meningitis.[8] There are limited and conflicting data in the published work about the effects of β-lactam antibiotics on smooth muscles.

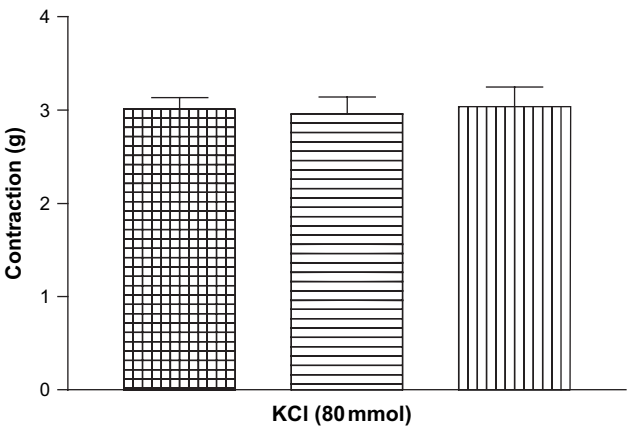

Fig. 1. Mean values obtained when 80 mmol KCl was added on ileum smooth muscles isolated from control, ceftriaxone (CTX) and ampicillin (AMP) groups. Data are expressed as the means ± standard error of mean (SEM) of eight experiments. ⊞, AMP group; ⊟, CFX group; ⊞, control group.

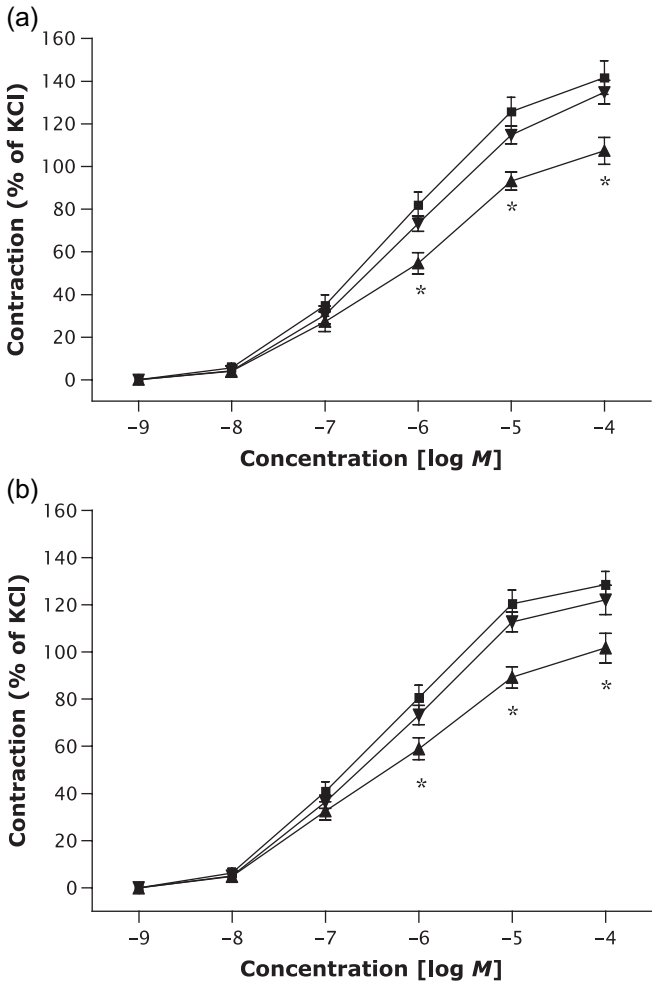

Fig. 2. Concentration–response curves of (a) carbachol and (b) histamine in ileum smooth muscles isolated from guinea pigs in control and experiment groups. Each point is expressed as a percentage of the contraction induced by 80 mmol KCl. Data are expressed as the means ± standard error of mean of eight experiments. *$P < 0.05$ denotes significant difference from control. ▼, Ampicillin group; ▲, Ceftriaxone group; ■, Control group.

Percy and Christensen reported that AMP depresses tone and spontaneous contraction of the muscularis mucosa of the opossum distal colon.[9] Said *et al.* showed that AMP had no blocking effect on the transmurally elicited twitches of the guinea pig ileum.[10]

Table 1. E_{max} (% of KCl) and pD_2 values of carbachol, histamine in ileum smooth muscles from guinea pigs in control, CTX sodium and AMP groups

	Control group	CTX group	AMP group
Carbachol			
E_{max}	147.7 ± 7.9	107.3 ± 6.3*	134.8 ± 5.6
pD_2	6.18 ± 0.08	6.16 ± 0.08	6.12 ± 0.10
Histamine			
E_{max}	128.6 ± 5.6	101.7 ± 6.3*	122.1 ± 6.2
pD_2	6.38 ± 0.09	6.28 ± 0.07	6.34 ± 0.08

*$P < 0.05$ denotes significant different from E_{max} values in the control group. Data are expressed as the means ± standard error of means of eight experiments. AMP, ampicillin; CTX, ceftriaxone.

Jankovic *et al.* reported that cefuroxime, cefamandole and AMP have relaxant effects on isolated rat uterus.[11] Graham *et al.* treated pregnant women for urinary tract infection with cefazoline or AMP and noted increased uterine activity 1–4 h after antibiotic administration.[12] Contrarily, Millar *et al.* reported that uterine contractility decreased steadily and significantly after CTX administration in pregnant women treated for urinary tract infection.[13] It was reported that some penicillin derivatives have reducing effect on the intramural electrical stimulation-induced contractile response to exogenous acetylcholine of guinea pig urinary bladder.[14]

In this study, we evaluated the contractile effects of carbachol and histamine on ileal smooth muscles isolated from guinea pigs treated with parenteral CTX or AMP for 10 days. E_{max} values for carbachol and histamine were significantly decreased in the CTX group in comparison to the control group, with no significant difference of pD_2 values ($P < 0.05$). The lower E_{max} values obtained with carbachol and histamine in CTX group may be related to an alteration in the regulation of receptor/post-receptor excitation–contraction coupling. Comparison of the pD_2 ($-\log10$ EC50) values of the control and experimental groups obtained in response to carbachol and histamine did not show any significant difference. This indicates that the potency of carbachol and histamine are not significantly different in the CTX or AMP groups compared with controls.

It is well known that the KCl-induced contraction in smooth muscle is due to an increase in Ca^{2+} influx through voltage operated Ca^{2+} channels.[15] We used the KCl responses to classify observed changes due to non-receptor-mediated mechanisms in control and experiment groups. The contractile responses for KCl in ileum smooth muscle were not different in all groups. This result rules out the possibility of functional and morphological damage in ileum smooth muscle after CTX or AMP treatment.

Acetylcholine and histamine are the classic excitatory neurotransmitters in guinea pig ileum. Carbachol is a muscarinic agonist that mimics the neurotransmitter acetylcholine in smooth muscles.[16,17] In our study, we used carbachol and histamine responses to classify observed differences as due to receptor-mediated mechanism. Our results show that E_{max} values for carbachol and histamine were significantly decreased, with no difference in the pD_2 values, in CTX group compared with control group. The pD_2 values are indicators of the affinity of the agonists to their receptor. In this study, unchanged pD_2 values with decreased E_{max} showed us that carbachol and histamine coupling with their receptors were normal and the receptors affinity to this agonists were normal. Probably postreceptor activation events or intracellular signal transduction were responsible decreased contractile responses. A decrease in maximal response to carbachol and histamine in CTX group found in this study indicates a post-receptor defect rather than a decrease in the affinity of receptors.

Jankovic *et al.* reported that effects of β-lactam antibiotics on smooth muscles are tissue and species dependent, indicating selectivity of their action.[11] In a study the effect of antibiotics on cholinergic autonomic nervous transmission were evaluated in isolated guinea pig ileum and AMP were found had no blocking effect.[10] Percy and Christensen reported that AMP is depressed tone and spontaneous contractions of the muscularis mucosa of the opossum distal colon.[9] The present study has shown that contractile responses to carbachol and histamine in guinea pigs treated with AMP were not different from those in controls.

The results of the present study clearly show that the contractile response of guinea pigs ileum to carbachol and histamine

significantly decreases after CTX treatment, whereas it does not change after ampicillin treatment. We suggested that decreased contractile responses to carbachol and histamine after prolonged use of parenteral CTX in the postoperative period may contribute to the ileus, but more studies are required to clarify the mechanism of the decrease in the contractile responses after CTX treatment. The effect of β-lactam antibiotics in different smooth muscles in humans should be further investigated with *in vivo* and *in vitro* studies.

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