

PROKINETIC EFFECT OF CLARITHROMYCIN AND AZITHROMYCIN – IN VITRO STUDY ON RABBIT DUODENUM

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There is a continuous search for new prokinetic agents for use in gastro intestinal hypomotility. Erythromycin, a macrolide, is one of them. In this study we observed effect of other macrolides, i.e. clarithromycin and azithromycin on rabbit duodenum and compared with that of neostigmine. Both these drugs produced significant prokinetic effect with EC₅₀ 0.4 µg/ml and 0.29 µg/ml respectively. Effect of clarithromycin was well sustained as compared to that of azithromycin, so it seemed to be a better prokinetic agent.

Gastro intestinal hypomotility, acute or chronic, is a problem in many clinical conditions. Prokinetic agents, such as neostigmine, metoclopramide, cisapride are commonly used for treatment of such conditions but no agent has yet proved to be ideal. So, there is always a search for new agents. Neostigmine is used since times for post operative paralytic ileus but it acts on many systems producing a lot of muscarinic adverse effects. Metoclopramide produces extra pyramidal effects in some patients. Use of cisapride is limited because of its cardiac toxicity. For the past few years erythromycin, a macrolide antibiotic, has been used as prokinetic agent for post operative ileus¹, gastric atony² and intolerance to nasogastric feeding³ in critically ill patients. It has also been tried to treat children with cyclic vomiting⁴. Some chronic conditions in which erythromycin has been tried are idiopathic constipation⁵, chronic intestinal pseudoobstruction⁶⁻⁷, diabetic gastroparesis⁸ and functional dyspepsia⁹⁻¹⁰. Apart from erythromycin many macrolide antibiotics are available, out of these clarithromycin and azithromycin are widely used clinically. Very limited data is available on prokinetic effect of these two agents. Aim of the present study was to observe the effect of these agents on intestinal motility and determine whether this is class effect or the effect of individual molecules.

MATERIALS AND METHOD

Rabbits of either sex weighing 1-1.5 kg were taken. They were deprived of food but not water for 18 hours prior to experiment. Rabbits were sacrificed, abdomen was opened and duodenum was removed and put in Tyrode's solution. 1-3 cm long piece of duodenum was mounted in 50 ml organ bath containing Tyrode's solution of following composition; NaCl 8, KCl 0.2, CaCl₂ 0.2, MgCl₂ 0.1,

NaH₂PO₄ 0.05, NaHCO₃ 1, Dextrose 1 gm/liter, at 37°C aerated with oxygen. Thirty minutes were allowed for equilibration, during which period Tyrode's solution was changed every 10 minutes. Isotonic contractions were recorded with fronted writing lever on Harvard Kymograph. After recording normal contractions for 30 seconds, cumulatively increasing concentration of drugs were applied to tissues. Each concentration was applied for 1-2 minutes depending upon the response, till maximum response was obtained. Experiment with each drug was carried out on six animals and increase in response to each concentration was measured in mm and expressed as mean ± SEM. It was taken to construct dose response curve on semi log scale and EC₅₀ of each drug was determined from this curve. Drugs used were neostigmine, clarithromycin and azithromycin.

RESULTS

Neostigmine, a known prokinetic agent, produces dose dependent increase in contractions of intestine (Fig. 1). Both macrolides included in the study, i.e. clarithromycin and azithromycin also produced a dose dependent increase in contractions of intestine (Fig. 2, 3). It was compared with that of neostigmine. Neostigmine was more potent as well as more efficacious agent. Effect started appearing with 0.08 µg/ml and maximum effect was with 0.64 µg/ml. Average maximum was 52 ± 7.5 mm (Table 1). EC₅₀ was 0.17 µg/ml (Fig. 4). With clarithromycin effect appeared with 0.2 µg/ml and maximum was with 1.6 µg/ml. Average maximum was 41.5 ± 6.1 mm (Table 2). EC₅₀ was 0.4 µg/ml (Fig. 4). With azithromycin effect appeared with 0.2 µg/ml and maximum effect was with 1.6 µg/ml. Average maximum was 23.6 ± 2.7 mm (Table 3). EC₅₀ was 0.29 µg/ml (Fig. 4).

Although EC_{50} of azithromycin is slightly less as compared to clarithromycin its efficacy is considerably less, i.e. 23.6 mm increase in contractions as compared to 41.5 mm. Onset of action of azithromycin was quick as compared to clarithromycin. It appeared within one minute with each concentration as compared to clarithromycin and neostigmine which appeared after two minutes. But effect was less sustained, i.e. duration of action was short; it faded within five minutes while effects of clarithromycin and neostigmine were well sustained after five minutes.

DISCUSSION

In our study we observed prokinetic effect of both clarithromycin and azithromycin as well as that of neostigmine, which is a well known prokinetic agent. We observed this effect on isolated piece of rabbit duodenum. No invitro studies are available to compare our results. Bortolotti et al¹¹ studied effect of intravenous clarithromycin on gastroduodenal motility of patients with functional dyspepsia and *Helicobacter pylori* gastritis and concluded that clarithromycin is able to stimulate cyclic interdigestive gastroduodenal motility. Acalovschi

et al¹² studied effect of oral clarithromycin on gall bladder emptying of healthy subjects and concluded that clarithromycin has prokinetic effect on gall bladder which is of similar amplitude but of shorter duration than that of erythromycin.

In the present study concentration of drug producing prokinetic effect was also important. With both the drugs this effect was observed with concentration ranging from 0.2 to 1.6 $\mu\text{g/ml}$. With clarithromycin steady state peak concentrations in plasma are 2-3 $\mu\text{g/ml}$ after 2 hour from a regimen of 500 mg every 12 hour¹³. So this prokinetic effect is seen well below the antibacterial therapeutic concentration. This corresponds to prokinetic effect of erythromycin which is also seen with smaller doses¹⁴. With azithromycin peak plasma concentration after 500 mg loading dose is approximately 0.4 $\mu\text{g/ml}$. When this loading dose is followed by 250 mg once daily for 4 days, the steady state peak drug concentration is 0.24 $\mu\text{g/ml}$. In our study concentrations producing prokinetic effect corresponded to these plasma levels. But duration of action is also important to produce a useful therapeutic response. With

Table 1: Effect of neostigmine on rabbit duodenum.

Concentration ($\mu\text{g/ml}$)	Response in mm							Mean	SEM
	1	2	3	4	5	6			
0.08	7	5	10	8	12	9	8.5	0.9	
0.16	30	17	25	24	28	23	24.5	1.8	
0.32	55	34	60	27	41	36	42	5.2	
0.64	67	33	80	35	54	44	52	7.5	

Table 2: Effect of clarithromycin on rabbit duodenum.

Concentration ($\mu\text{g/ml}$)	Response in mm							Mean	SEM
	1	2	3	4	5	6			
0.2	4	6	6	4	12	7	6.5	1.2	
0.4	12	31	22	16	23	22	21	2.6	
0.8	38	58	38	27	24	35	36.6	4.8	
1.6	48	65	42	28	23	43	41.5	6.1	

Table 3: Effect of azithromycin on rabbit duodenum.

Concentration ($\mu\text{g/ml}$)	Response in mm							Mean	SEM
	1	2	3	4	5	6			
0.2	11	4	5	8	5	6	6.5	1.1	
0.4	22	9	10	15	14	20	16.6	5	
0.8	23	18	20	22	29	25	22.8	2.4	
1.6	21	19	22	23	30	27	23.6	2.7	

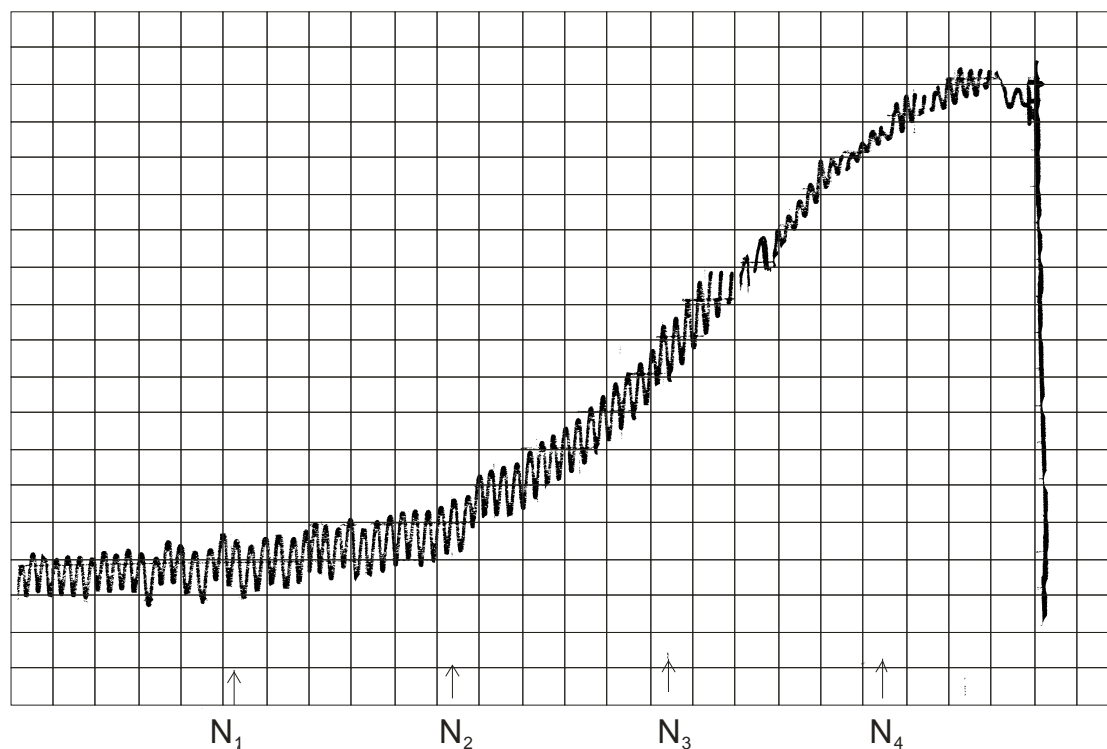


Fig. 1: Effect of neostigmine on rabbit duodenum.

N₁ = Neostigmine 0.08 µg/ml

N₂ = Neostigmine 0.16 µg/ml

N₃ = Neostigmine 0.32 µg/ml

N₄ = Neostigmine 0.64 µg/ml

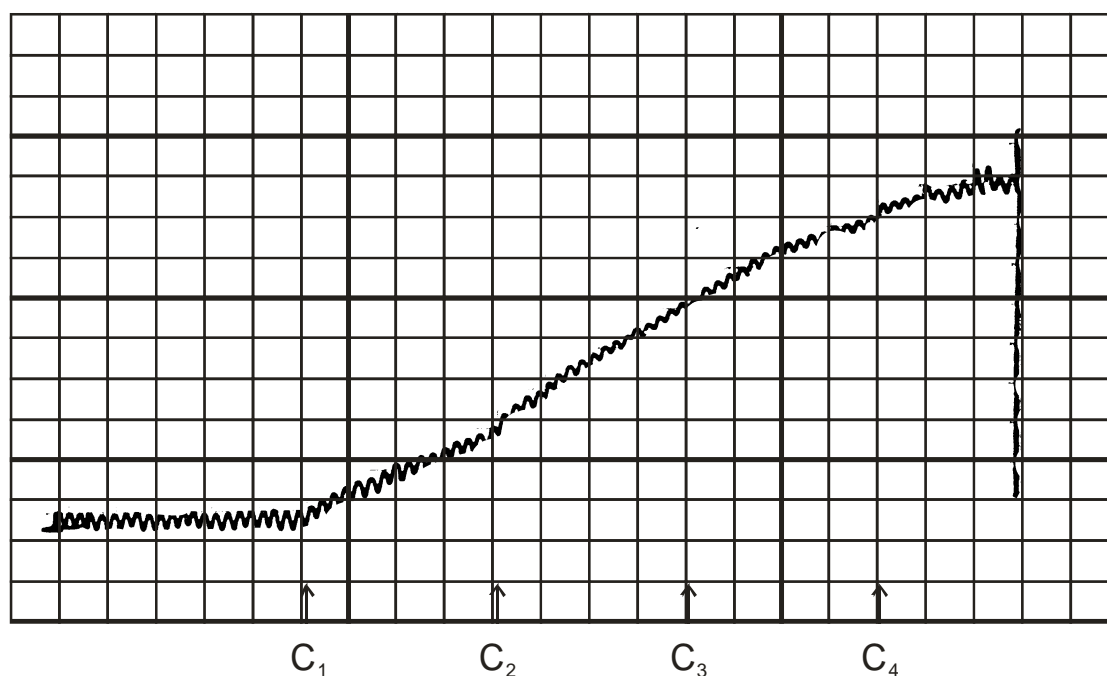


Fig. 2: Effect of clarithromycin on rabbit duodenum.

C₁ = Clarithromycin 0.2 µg/ml

C₂ = Clarithromycin 0.4 µg/ml

C₃ = Clarithromycin 0.8 µg/ml

C₄ = Clarithromycin 1.6 µg/ml

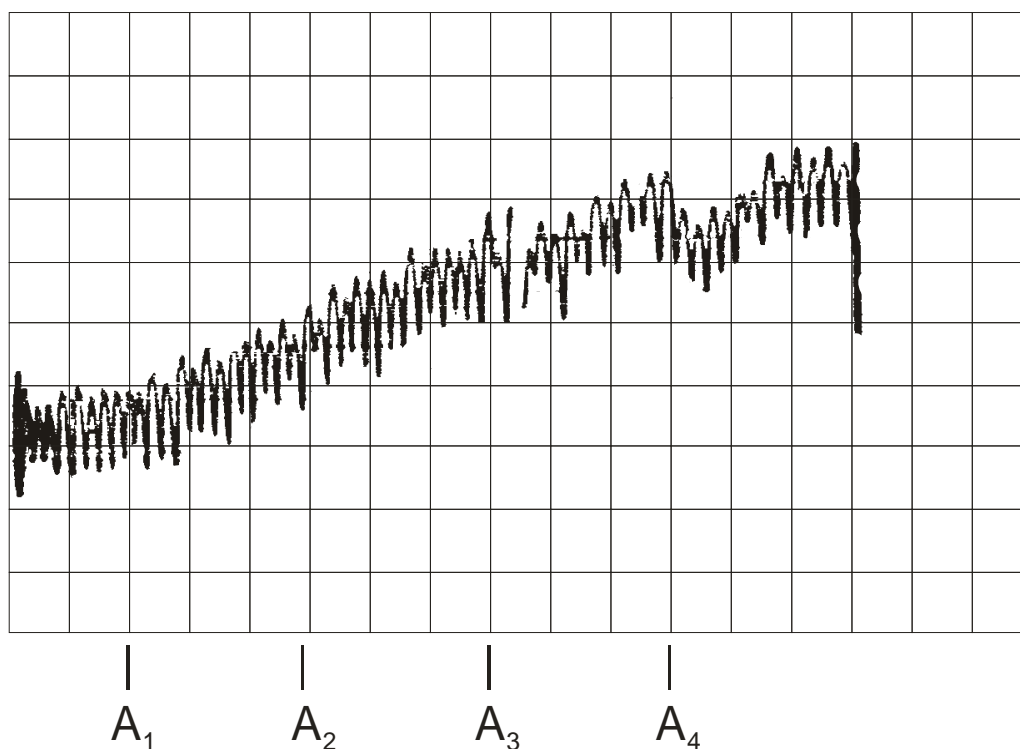


Fig. 3: Effect of azithromycin on rabbit duodenum.

A₁ = Azithromycin 0.2 µg/ml

A₂ = Azithromycin 0.4 µg/ml

A₃ = Azithromycin 0.8 µg/ml

A₄ = Azithromycin 1.6 µg/ml

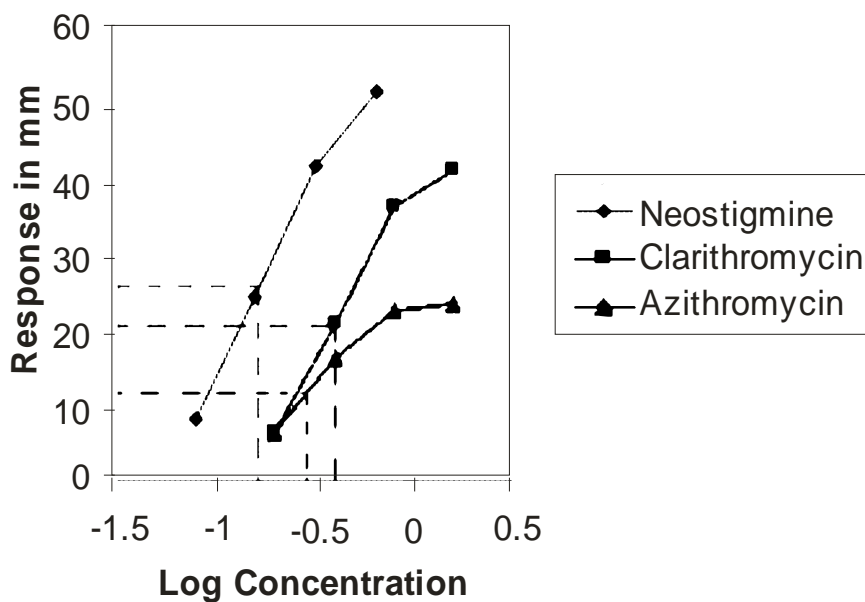


Fig. 4: Dose Response curves.

azithromycin this effect was not well sustained while with clarithromycin it was well sustained. So practically clarithromycin appears to be a useful

therapeutic prokinetic agent, but studies on intact animals and human beings are needed to confirm this usefulness.

We **conclude** from our study that clarithromycin is a useful prokinetic agent as compared to azithromycin. Beneficial effect of clarithromycin in *Helicobacter pylori* infection is mainly due to antibacterial action but improvement in motility will definitely enhance the effect.

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