Evaluation of Prokinetic Action of Clarithromycin using Orocecal Transit Time in Healthy Human Subjects

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Citation

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Abstract

Clarithromycin is a synthetic analogue of Erythromycin and shares its prokinetic action to various extents. The objective of this study is to investigate the prokinetic action of clarithromycin by a non-invasive and reproducible technique compared against placebo and loperamide. The randomized, open, placebo-controlled, crossover study in healthy human subjects approved by Institutional Ethics Committee. Eleven healthy male volunteers were enrolled in the study. The subjects were given placebo or 2mg loperamide or 500mg clarithromycin and crossover was done after a seven day washout period. Orocecal transit time was evaluated by measuring saliva sulfapyridine appearance time after the administration of sulfasalazine. Mean salivary sulfapyridine lag time with placebo was 313.64 ± 49.05 minutes, with loperamide >480 minutes and with clarithromycin 245.45 ± 56.63 minutes. As compared to placebo and loperamide, sulfapyridine lag time with clarithromycin was significantly reduced ($p<0.001$). The results indicate the prokinetic effect of clarithromycin similar to erythromycin.

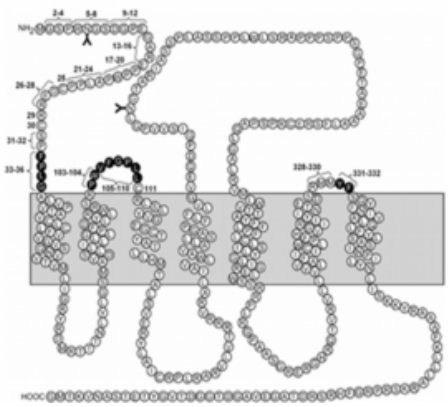
INTRODUCTION

Macrolide antibiotics like erythromycin and clarithromycin are widely used in the clinical practice for various infections including Helicobacter pylori eradication. [1] Clarithromycin is more acid-stable than erythromycin and is rapidly absorbed after oral administration with a bioavailability of about 55%. Clarithromycin is a well tolerated antibiotic; the frequency of gastrointestinal side effects is much lower than with erythromycin. Oral erythromycin and its analogues have been shown to significantly increase gastrointestinal motility by acting on motilin receptors. [2] Endogenous agonist of these receptors is motilin. Motilin is 22-aminoacid peptide found in gastrointestinal M cells, some enterochromaffin cells and proximal small intestine. [3,4] Motilin, erythromycin and other motilides show prokinetic action mainly by acting on motilin receptor and causing prolonged depolarization in a subset of neurons [4,5] and to certain extent through the release of acetylcholine from intrinsic cholinergic neurons and by direct muscular action. [6]

Motilin receptor (Fig.1) is a G protein-coupled receptor whose loop and the tail regions are quite varied and provide the diversity of themes allowing the binding of structurally diverse ligands. [7] Motilin binds with the residues at the membrane interface at each end of the long loop [Val ^{179}, Leu ^{245}, Arg ^{246}] and the residues at the amino-terminal tail and extracellular loop domains [Gly ^{36}, Pro ^{103}, Leu ^{109} and Phe ^{332}]. [7] Erythromycin and other non-peptidyl motilin receptor agonists bind to intramembranous regions of the receptor. The intradomain disulfide bond between two cysteine residues [Cys ^{25} and Cys ^{30}] within the amino-terminal tail domain is shown to have functional significance for both motilin and erythromycin action. [8]

Figure 1

Fig 1. Human motilin receptor: schematic diagram of the primary sequence and possible membrane topology (Matsuura B. et al, 2006)



Clarithromycin is a macrolide derivative, structurally related to erythromycin (Fig.2,3) and shares the prokinetic action of the later. [9,10,11] Its prokinetic action is studied by measuring the orocecal transit time (OTT). Orocecal transit time is

measured by non-invasive methods like sulfapyridine appearance in saliva after sulfasalazine intake, [12] isotope scintigraphy [13] and lactulose-hydrogen breath test. [14] Isotopic methods involve exposure to radiation and the use of expensive equipment and the lactulose used in the breath test accelerates OTT and gives a non-physiologic measurement of OTT, [12] hence we have chosen sulfapyridine appearance time in saliva.

Figure 2

Fig 2. Erythromycin [$C_{37}H_{67}NO_{13}$]

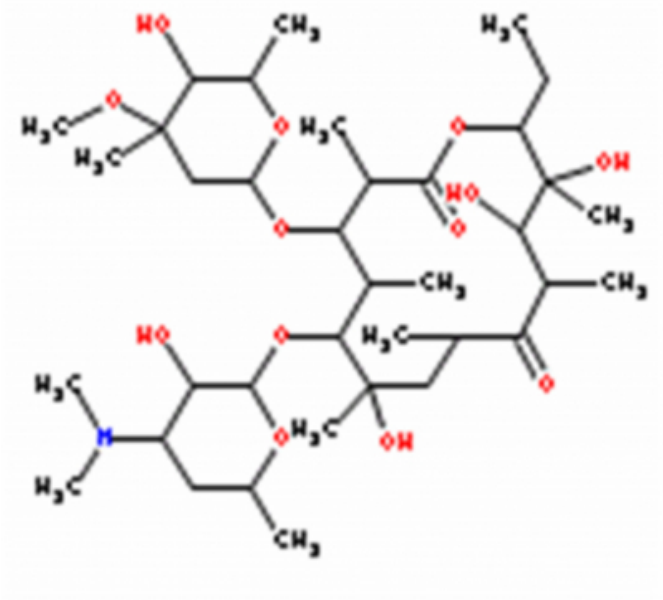
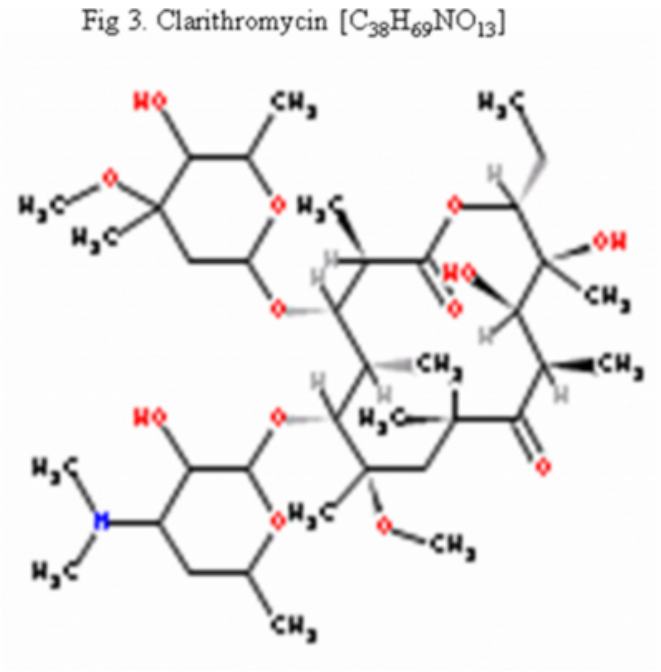


Figure 3

Fig 3. Clarithromycin [$C_{38}H_{69}NO_{13}$]



After oral administration sulfasalazine is biotransformed in the cecum by bacterial azoreductase into sulfapyridine and 5-amino salicylic acid. [15,16,17] The released sulfapyridine is immediately absorbed in the blood and provides a measure of OTT. [18] A strong correlation between plasma and salivary concentrations of sulfapyridine was shown in previous studies. [12,19,20]

MATERIALS AND METHODS

SUBJECTS

Eleven healthy, male subjects aged between 18-65 years, with no history of cardiac, renal, neurological, metabolic or gastrointestinal disorders and with no history of smoking, alcohol or drug abuse participated in the study after obtaining the informed consent. The study protocol was approved by Ethics committee of Nizam's Institute of Medical Sciences, Hyderabad, India. The subjects with history of intolerance to sulfas, gastrointestinal and biliary disorders and smokers were excluded from the study. They had not received any drug for a 2-week period before inclusion. The clinical examination and laboratory tests (erythrocytes, Hb, leukocytes, AST, ALT, creatinine and alkaline phosphatase) were normal.

STUDY DESIGN

In this randomized, open, placebo controlled, crossover study, the subjects ingested placebo or 2mg loperamide or 500mg clarithromycin as per randomization with 240ml water at 8PM the day before the study and at 8AM on the

day of the study. One hour later the volunteers were given 2g of sulfasalazine (4 tablets of sulfasalazine 500mg each) with 240ml of water. [12] Salivary samples were collected at 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 hours after sulfasalazine administration.

The salivary samples were centrifuged at 6000rpm for 10 minutes; the supernatant was pipetted into labeled storage vials and stored at -70 C for future analysis. Breakfast and lunch were served after 4h and 6h respectively. The volunteers were crossed over after one week washout period as per randomization. Safety assessment of the volunteers was done by recording any side effects during the study and recording of the vitals.

SAMPLE ANALYSIS

Saliva sulfapyridine analysis was derived from the Bratton and Marshall Method (diazotization). [21] An aliquot of 1ml saliva was acidified with 2ml of 20% trichloro acetic acid, cyclomixed and allowed to stand for 3minutes. The samples were centrifuged at 3000rpm for 5 minutes. To 2ml of clear supernatant, 200µl of 0.1% of freshly prepared sodium nitrite was added, cyclomixed and allowed to stand for 3 minutes to obtain aromatic diazonium salt. The excess acid was neutralized by adding 200µl of 0.2% ammonium sulfamate and allowed to stand for 2 minutes. The diazonium salt was revealed by 200µl of 0.1% n-naphthyl ethylene diamine and the absorbance was measured by using spectrophotometer at 540nm after 5-10 minutes. The appearance time in saliva was given by the time between sulfasalazine ingestion and the first positive sample.

STATISTICAL ANALYSIS

All the values were expressed as mean ± SD. For comparison between groups, ANOVA and Student's t-test were performed. Difference between groups were considered to be significant at $p<0.001$

RESULTS

Eleven healthy male human subjects with a mean age of 28.73 ± 6.8years, mean height of 165.95 ± 3.54cm and mean weight of 64.36 ± 10.91kg participated in the randomized, open, placebo controlled crossover study. The mean salivary sulfapyridine lag time with clarithromycin was 245.45 ± 56.63minutes, with placebo was 313.64 ± 49.05 minutes, with loperamide was >480 minutes (Table 1).

As compared to loperamide and placebo the mean sulfapyridine lag time was significantly reduced with clarithromycin ($p<0.001$) (Fig. 4).

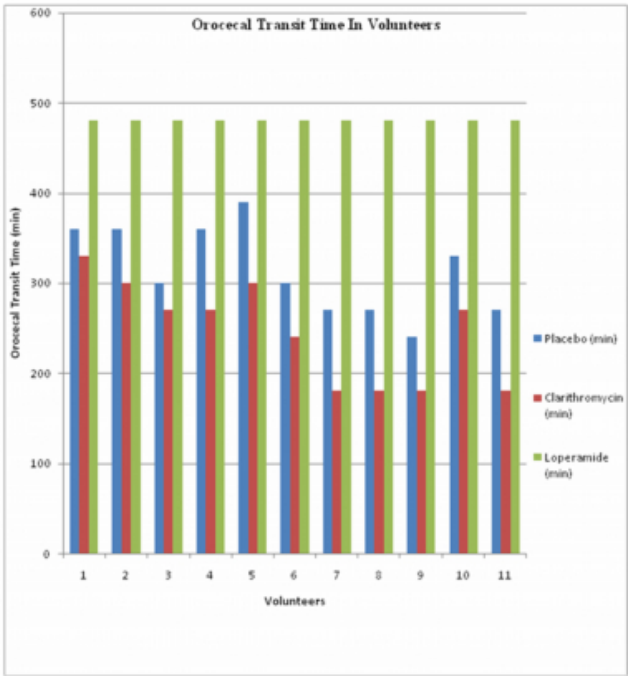
Figure 4

Table 1: Orocecal Transit Time of Placebo, Clarithromycin and Loperamide in Healthy Subjects.

Subject Code	Orocecal Transit Time In Minutes (OCT)		
	Placebo	Clarithromycin	Loperamide
1	360	330	480
2	360	300	480
3	300	270	480
4	360	270	480
5	390	300	480
6	300	240	480
7	270	180	480
8	270	180	480
9	240	180	480
10	330	270	480
11	270	180	480
Mean	313.64	245.45	480
SD	49.05	56.63	0
CV	15.64	23.07	0
SE	14.79	17.08	0
Placebo vs Clarithromycin		P<0.001	
Placebo vs Loperamide			P<0.001
Clarithromycin vs Loperamide		P<0.001	

Figure 5

Fig 4. Orocecal Transit Time in Healthy Volunteers (Saliva sulfapyridine appearance time after administering placebo/clarithromycin/loperamide).



DISCUSSION

Macrolide antibiotics like erythromycin and clarithromycin cause epigastric distress. [1] The various mechanisms of prokinetic action of erythromycin were widely studied. [2,3,4,5,6,7,8] Clarithromycin, a very close chemical analogue of erythromycin (Fig, 1 and 2) shares many actions of erythromycin. Oral clarithromycin enhances postprandial gall-bladder emptying in healthy volunteers. [9] Plasma motilin concentrations peak with the periodic contraction of

the gall-bladder, which occurs in the interdigestive period, synchronous with phase III of the migrating motor complex. [22,23]

Intravenous clarithromycin enhances interdigestive gastro duodenal motility of patients with functional dyspepsia and Helicobacter pylori gastritis. [10] Clarithromycin reduces fasting gall bladder volumes and enhances both fasting and postprandial gall bladder contractions in normal humans and also in those with gall stone disease. [11]

Standard methods for measurement of orocecal transit time are inconvenient or difficult to use in clinical practice. In the lactulose breath test, lactulose osmotic properties tend to decrease orocecal transit time and some subjects are not producers of hydrogen. [24,25] Scintigraphic studies involve exposure to radiation and the use of expensive and inconvenient equipment. Orocecal transit time measurement with pellets requires exposure to radiation, reflects only the solid phase of the aliment, and the passage from the small bowel to the colon is not easy to determine. [26] Kennedy et al [27] proposed evaluation of orocecal transit time by measuring sulfapyridine plasma appearance time. Dhote et al proposed the salivary sample method as a validated simplification of the plasma sulfasalazine-sulfapyridine test for the measurement of orocecal transit time. Salivary appearance of sulfapyridine is independent of acetylator phenotype as the appearance time is assumed to be independent of sulfapyridine plasma rates. [12] Sulfapyridine passive diffusion in saliva is independent of the salivary flow and of the pH variations. [28] As with the breath test orocecal transit time is inconvenient in cases of microbial small bowel overgrowth, but crossover studies in normal volunteers prevents this problem and the same was followed in the study. A high calorie meal slows orocecal transit time, [12] hence the test was performed with a standard meal.

CONCLUSIONS

The prokinetic action of clarithromycin is substantiated in this study as it significantly reduced OTT as compared to placebo and loperamide. Sulfapyridine lag time in saliva and hence OTT were significantly reduced by clarithromycin indicating its prokinetic effect like erythromycin. However, the clinical utility of this decrease in the lag time has to be evaluated in further studies before any therapeutic indication can be recommended.

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