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RESEARCH ARTICLE **PHARMACOKINETICS AND DISPOSITION**

COMPARATIVE STUDY OF PHARMACOKINETIC BEHAVIOR OF ERYTHROMYCIN IN RATS USING LC MS/MS

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Abstract:
In an open randomised crossover study, the pharmacokinetics of erythromycin were compared to those of Neostigmine, Atropine, Metoclopramide, and omeprazole after a single dose in 12 wistar rats for oral and IV pharmacokinetic studies. Erythromycin was given at a dose of 50 mg/kg, and the oral and IV pharmacokinetics of Neostigmine (40 mg/kg), Atropine (10 mg/kg), Metoclopramide (12.5 mg/kg), and omeprazole (50 mg/kg) were examined. When taken orally, Erythromycin in conjunction with Omeprazole resulted in a substantial increase in Cmax (2.760.15) and AUC (7.29 0.554) when compared to the control group. Erythromycin in conjunction with Neostigmine (1.24 0.755), Atropine (0.56 0.085), and Metoclopramide (1.06 0.25), on the other hand, yielded less significant results.The effect of erythromycin on pharmacokinetic parameters was investigated in this study.
Key words: Erythromycin, Maximum concentration (Cmax), LC MS/MS, Omeprazole

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Introduction:

Erythromycin is a 14 membered macrolide antibiotic obtained from a strain of the actinomyces saccaropolyspora erythraea, formerly known as Streptomyces erythraeus. [3] It possesses broad spectrum antimicrobial activity. It is used in treatment of respiratory tract infections; erythromycin has good coverage of atypical organisms, including mycoplasma. Erythromycin is also used to treat infections produced by Chlamydia, syphilis, and gonorrhea. It possesses spectrum similar to or quit wider than that of penicillin, and is often used for patient who have an allergy to penicillin.

Erythromycin displays bactericidal activity, specifically at high concentrations, but the mechanism is not fully elucidated. Protein synthesis and, as a result, structure/function processes necessary for bacterial life or replication are blocked by binding to the 50s subunit of the bacterial 70s r RNA complex. [3,7] The acidity level of the GIT also can determine where and how much of drug absorbed. Weakly acidic drugs are usually best absorbed in an environment where the pH is lower than the pKa (negative log of the dissociation constant) of the chemical entity.[10] By being un-ionized, the product remains more lipid soluble, allowing it to pass past the lipid barrier that separates the GI tract from the systemic circulation.. Other hand, weakly basic drugs are absorbed best from a more alkaline condition, such as the found in the upper small intestine. Sometimes an acidic environment is necessary for a product to work. The chemical stability of medicines is also affected by GI pH. In such case, drugs altering the gastric pH could also alter the drug exposure.[15] GIT's high perfusion rate guarantees that once a medication has crossed the membrane, it is quickly eliminated from the absorption site, preserving the sink conditions and concentration gradient for ongoing drug absorption. Because oxygen and energy are required for delivery, blood flow is

particularly crucial for actively absorbed drugs. Taking all these factors into consideration, the present study was planned to evaluate the pharmacokinetic of erythromycin in rat by modifying the gastric motility, pH and intestinal transit.[14,18] Metoclopramide, Atropine, Neostigmine, and Omeprazole were used to alter gastric emptying, intestinal transit, and gastric pH in this investigation. Metoclopramide has been shown to have gastroprokinetic action, which means it can help with GI motor dysfunctions like gastritis, gastroparesis, and gastroesophageal reflux disease. Its action is ascribed to an antidopaminergic property. Metoclopramide also considerably increases blood flow in the duodenum and jejunum, respectively, by 67.3 and 29.7%. Atropine is antimuscarinic agent known to inhibit both gastric emptying and intestinal transit.[17]On the other hand, neostigmine is a potent acetyl cholinesterase (AchE) inhibitor and increases intestinal motility in human without affecting the gastric emptying. Omeprazole is the proton pump inhibitor drug, which is used as antiulcer drug. Omeprazole decreases the acid secretion in the stomach by inhibiting the H^+, K^+ ATPase.[6] In humans, erythromycin is known to increase the gastric motility by binding to motilin receptor; however this phenomenon is not seen in rat. This could be because of the absence of motilin receptors in rat. None of these medications have been found to interfere with the metabolism or clearance of erythromycin.

Material and methods:

Drugs and chemicals:

Erythromycin base, Neostigmine (Neon Lab) Atropine Sulfate salt (Sigma-Aldrich USA), Metoclopramide (Ipca Lab.Ltd. Gujarat), Omeprazole, 10 % Pharmasol, 10 % Methanol, Mili Q water.

Experimental animals:

Overnight fasted adult male Wistar rats weighing 200-250g were used. The animal were placed in air conditioned (18-22ºc) animal house kept relative humidity between 40% to 70% (except during the cleaning slot) when non-recycled filtered air was changed every 10 minutes or so. The artificial day/night cycle consisted of 12 hours of light followed by 12 hours of darkness, beginning at 7.30 a.m. [8] Animal had free access to standard pallet diet (Amrut laboratory animal feed, Sangali, Maharashtra) and water *ad libitum.*The experimental protocol was approved the Institutional Animal Ethics Committee IAEC and CPCSEA.

Preparation of solution:

Erythromycin solution: Erythromycin solution was made with 10% Pharmasol, 10 % Methanol and volume made up by Mili Q water.

Metoclopramide, Neostigmine, Omeprazole and Atropine solutions was made with Mili Q water.

Experimental procedure for oral pharmacokinetic study:

The rats were kept for overnight fasting in individual cages provided with SS bottom grills and water *ad libitum*. Erythromycin suspension at the dose of 50 mg/kg was administered at dose of 2.5 ml/kg body weight to the selected Wistar Rats (n= 6). Approximately 0.3ml aliquot of blood samples was collected from each animal from the retro-orbital plexus at 0.25 hrs, 0.5 hrs, 1 hrs, 2 hrs, 4 hrs, 6 hrs, 8 hrs, 10 hrs, 12 hrs and 24 hrs. Immediately after collection of the blood samples were kept at bacteriological incubator (Remi Instruments-RI12S) at 37ºC for 10 minutes. Blood samples were centrifuged immediately at 10000 rpm at temperature of 18-22ºC for 10 minutes by centrifuge machine, (Hermle Z 323K). The separated serum was pooled from 2 animals for each time point and stored at in a -60ºC freezer (Thermo Electron Corporation VXE490) until LC-MS analysis of Erythromycin were performed.

Experimental procedure for intravenous pharmacokinetic study:

Erythromycin at the dose of 50 mg/kg was administered by I.V. to the selected Wistar Rats (n= 6) via tail-vein. Approximately 0.3ml aliquot of blood samples was collected from each animal from the retro-orbital plexus at 0.083 hrs, 0.25 hrs, 0.5 hrs, 1 hrs, 2 hrs, 4 hrs, 6 hrs, 8 hrs, 10 hrs, 12 hrs and 24 hrs. Immediately after collection of the blood samples were kept at bacteriological incubator (Remi Instruments-RI12S) at 37ºC for 10 minutes. Blood samples were centrifuged immediately at 10000 rpm at temperature of 18-

22ºC for 10 minutes by centrifuge machine, (Hermle Z 323K). The separated serum was pooled from 2 animals for each time point and stored at in a -60ºC freezer (Thermo Electron Corporation VXE490) until LC-MS analysis of Erythromycin.

Analysis of serum sample on LC-MS/MS:

Stock solution: Standard solution of 1mg/ml Erythromycin was prepared in methanol and kept at 4-5ºC. Plasma calibration standards: The stock solution was serially diluted with rat plasma to 0btain solutions of the following strength 20, 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039 µg/ml.

Plasma sample processing (Acetonitrile precipitation method):

To 100 µl of plasma (either unknown plasma samples or plasma spiked with known amount of varying concentration of Erythromycin reference substance), in a 1.5ml eppendorf tube, 400 µl of Acetonitrile was added to precipitate proteins. The tubes were vortex-mixed for 30 seconds to bring about a complex extraction of erythromycin from proteins. The tubes were then centrifuged at 10000 rpm at 4-6ºC for 5 minutes. Upper supernatant was given for LC-MS/MS analysis.

Result and Discussion:-

The PK parameters were calculated by Non compartmental analysis using WIN NONLIN software package (Professional version 2.1, Pharsight, U.S) Erythromycin, Atropine, Neostigmine, Metoclopromide and omeprazole treated groups were compared with control using non parametric Mann-Whitney test. Erythromycin + Neostigmine, Erythromycin +Atropine, Erythromycin+ Metoclopromide and Erythromycin + Omeprazole treated group were compared with Neostigmine, Atropine, Metoclopromide and Omeprazole treated groups respectively using the same Statistical test. Graph Pad prism 4 was used to perform the statistical tests.

Our focus of the current topic is mainly on the patient related factors affecting bioavailability. These factors include gastric blood flow and gastric pH. Our inquiry was guided based on these considerations. Pharmacokinetic properties of Erythromycin (50 mg/ kg) were studied and its interaction with Atropine (10 mg/ kg, i.p.), Neostigmine (40 µg/ kg i.p.), Metoclopramide (12.5 mg/ kg, i.v.), Omeprazole (50 mg/ kg, p.o.) was examined to determine pharmacokinetic drug-drug interaction. Erythromycin in combination with neostigmine decrease the time to achieve maximum concentration (Tmax) .However no difference was seen in Cmax and AUC when compared with erythromycin treated group. Omeprazole, an antiulcer medication, elevated stomach pH while simultaneously improving intestinal transit.. Omeprazole exhibited significantly higher Cmax and AUC and lower Tmax. Thus we can say that. When erythromycin was combined with omeprazole, the rate and extent of oral absorption improved dramatically. This could be due to the neutralisation of stomach pH. Erythromycin did not affect the gastrointestinal motility which improved with omeprazole to 30%.

Table 1: Effect of Erythromycin (50mg/kg) in combination with Neostigmine, Atropine, Metoclopramide and Omeprazole on Cmax.

Groups	Cmax (mcg/ml)
Erythromycin 50mg/kg	1.19 ± 0.245
Ery 50mg/kg + Neostigmine 40µgm/kg	1.24 ± 0.755
Ery 50mg/kg + Atropine 10mg/kg	0.56 ± 0.085
Ery 50mg/kg + Metoclopramide 12.5mg/kg	1.06 ± 0.25
Ery 50mg/kg + Omeprazole 50mg/kg	2.76 ± 0.15*

Figure 1: Comparison of Cmax of Erythromycin 50mg/kg in combination with Neostigmine, Atropine, Metoclopramide and Omeprazole in Wistar rats.

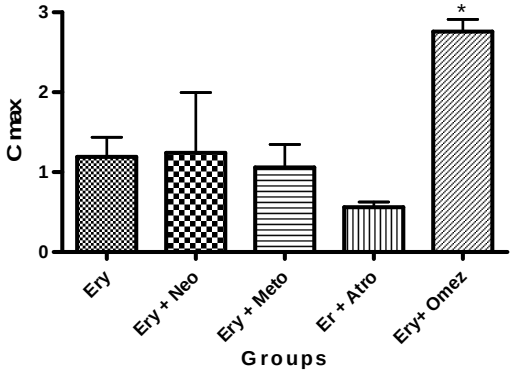


Table2: Effect of Erythromycin (50mg/kg) in combination with Neostigmine, Atropine, Metoclopramide and Omeprazole on AUC.

Groups	AUC last
Erythromycin 50mg/kg	3.31 ± 0.6
Ery 50mg/kg + Neostigmine 40µgm/kg	1.72 ± 0.065
Ery 50mg/kg + Atropine 10mg/kg	4.13 ± 0.865
Ery 50mg/kg + Metoclopramide 12.5mg/kg	2.39 ± 1.01
Ery 50mg/kg + Omeprazole 50mg/kg	7.29 ± 0.554*

Figure 2: Comparison of AUC of Erythromycin 50mg/kg in combination with Neostigmine, Atropine, Metoclopramide and Omeprazole in Wistar rats

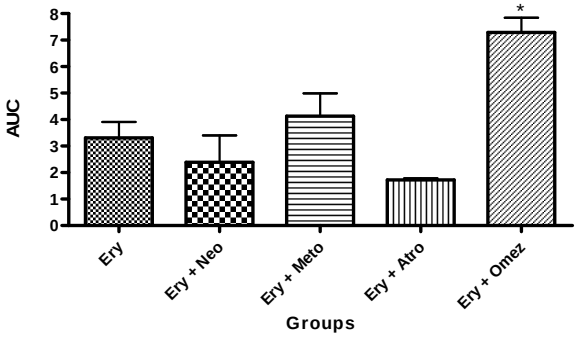
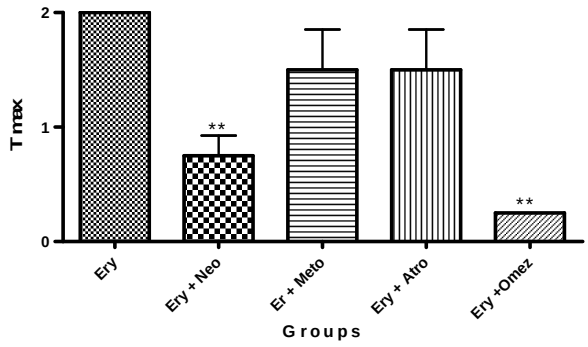


Table3: Effect of Erythromycin (50mg/kg) in combination with Neostigmine, Atropine, Metoclopramide and Omeprazole on Tmax.

Groups	Tmax
Erythromycin 50mg/kg	2.0 ± 0.00
Ery 50mg/kg + Neostigmine 40µgm/kg	1.50 ± 0.5
Ery 50mg/kg + Atropine 10mg/kg	1.50 ± 0.5
Ery 50mg/kg + Metoclopramide 12.5mg/kg	1.50 ± 0.5**
Ery 50mg/kg + Omeprazole 50mg/kg	0.25 ± 0.00**

Figure 3: Comparison of Tmax of Erythromycin 50mg/kg in combination with Neostigmine, Atropine, Metoclopramide and Omeprazole in Wistar rats.



Conclusion

Thus at the end we can conclude that alteration in gastric and intestinal transit i.e. reduction in gastrointestinal motility with atropine and increase in gastrointestinal motility with metoclopromide, neostigmine did not change the serum exposure of erythromycin. Changing the gastric pH with omeprazole, on the other hand, greatly enhanced the oral pharmacokinetics of erythromycin. If the improvement in pharmacokinetics is due solely to pH, or if another mechanism is at work, more research is needed.

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