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IN VITRO RELEASE OF TETRACYCLINES IN PRESENCE OF H_2-RECEPTOR ANTAGONISTS

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There are a number of drug interactions reported for tetracyclines with many other drugs. In present study *in vitro* release of five different tetracyclines i.e., tetracycline, demecloctcycline, oxytetracline, methacycline and doxycycline in presence of cimetidine and ranitidine has been studied by USP XX dissolution method.
The release of almost all tetracyclines was depressed in presence of cimetidine and ranitidine while in some cases the value were greatly increased due to the formation of charge transfer complexes of the antibiotic with H_2-receptor antagonists, which gave absorbance at same wavelengths but with much higher molar absorptivity. An attempt was made to elucidate the mechanism of this effect.

Keywords: Tetracycline, H_2-receptor antagonists, drug interactions.

INTRODUTION

The bioavailability of drugs at their site of action can be enhanced or reduced by interaction with other drugs. Several studies concerning the biochemical and pharmacological effects of antimicrobial agent when given with other drugs are reported in the literature (Simonetti *et al.,* 2004; Potenski *et al.*, 2003; Aumais *et al*., 2004; Hu *et al*., 2002; Contracept Technol Update 1987; Jones & Ward 2002). The type of interactions involved competition for renal tubular excretion, displacement from carrier sites, chelation, decreased protein synthesis, increased tissue toxicity (Patient guide 1992; Schmidt & Dalhoff 2002), acid-base neutralization and many others. Such interactions may be proceeded by decreased dissolution and absorption of drug to affect the overall bioavailability.

Tetracycline, demeclocycline, oxytetracycline, methacycline and doxycycline contain octahydronapthacene skeleton and are important members of broad-spectrum antibiotics. All these tetracyclines are useful in the treatment of clinical and veterinary infections and are prescribed against many Gram-positive and Gram-negative infections. Their actions are primarily bacteriostatic rather than bactericidal. Tetracyclines are capable of forming stable chelate complexes with metal ions resulting in incomplete, irregularly and decreased absorption from G.I tract. Absorption is most active in the stomach and upper small intestine and is greater in the fasting state. It is much less complete from the lower portions of the intestinal tract and is negligible from the colon. The soluble salts of divalent and trivalent cations with which they form stable complexes and to variable degree and even milk and food diminish the degree of absorption (Khan & Musarrat 2003; Jiang & Wang 2004; Georgopoulos *et al.,* 2002; Deppermann 1989; Midolo 1999; Flockhart 2000; Gugler & Allgayer 1990).

Cimetidine is a histamine H_2-receptor antagonist that inhibits the secretion of basal and gastric hydrochloric acid secretion and reduces the output of pepsin. Cimetidine, an imidazole derivative is widely used in the treatment of duodenal and gastric ulceration, reflex esophagitis and for the management of Zollinger-Ellison syndrome. Ranitidine is a relatively new histamine H_2-receptor antagonist that contains a furan ring structure. The drug has been used in the treatment of duodenal and gastric ulceration. Ranitidine is as effective as cimetidine and has the advantage of less frequent dosing and fewer side effects. Ranitidine appears to be the drug of choice in the treatment of the Zollinger-Ellison syndrome because of its increased potency and lesser effect on endocrine functions as compared to cimetidine. Significant clinical consequences are those with cimetedine (Delgado 2003) because it interferes with hepatic cytochrome P-450. Aluminum and magnesium hydroxide containing antacids have been shown to further decrease the bioavailability of cimetidine and ranitidine (Mclnnes & Brodie 1988; Gugler & Allgayer 1990; Mangini 1982). Keeping in view the potential drug interactions (Rogers 1980; Garty & Hurwitz 1980, Garty & Hurwitz 1980[a]) of tetracyclines, present studies aimed to examine the effects of H_2-receptor antagonists like cimetidine and ranitidine on the dissolution characteristics of tetracycline, methacycline, demeclocy-cline and oxytetracycline in the form of their hydrochloride while doxycycline as a hyclate. The mechanism of interaction between the antibiotics and these H_2-receptor antagonists has also been discussed.

EXPERIMENTAL

Materials

Demeclocycline, doxycycline, methacycline, tetracycline and oxytetracycline reference standards were gift from Pfizer Laboratories (Pvt.) Ltd. Cimetidine and ranitidine

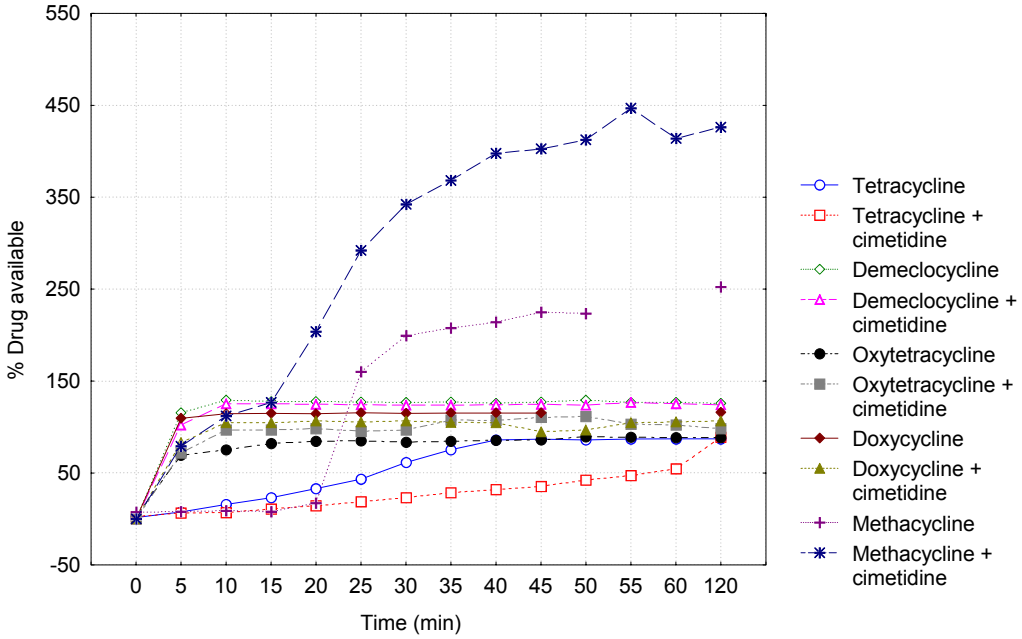

Fig. 1: Availability of tetracyclines in presence of cimetidine.

reference standards were gifts from SKF and MSD labs Pakistan Ltd. Demeclocycline hydrochloride (Ledermycin) capsule (Lederle (Pakistan); 150 mg), doxycycline hyclate (Vibramycin) capsules (Pfizer (Pakistan); 100 mg), methacycline hydrochloride (Rondamycin) capsules (Pfizer (Pakistan); 150 mg), tetracycline hydrochloride capsules (Karka, Italy; 250 mg), and oxytetracycline hydrochloride (Terramycin) capsules (Pfizer (Pakistan); 250 mg) were purchased from the market at the time of study. All of these had expiry time of more than one year at the time of study. Hydrochloric acid and methanol and all other chemicals used were of analytical grade.

Equipment

The dissolution equipment (Pharmacopoeia of the United State XX) was manufactured to the B.P. 2003 standards (British Pharmacopoeia 2003), which included the dissolution motor and variable speed controller with a stainless steel basket assembly. The top of the basket was modified and replaced by a conical head in order to eliminate air entrapment during dissolution, which is consistent with the present apparatus description. The dissolution vessel was flat bottomed glass vessel with an internal diameter of 100 mm and with a capacity of 1-liter dissolution fluid. The variable speed motor was modified to reduce unwanted vibrations by the incorporation of 1000 230-µF capacitor in the speed control circuit and was maintained within ± 0.5% of the required speed. The rotation speed of the basket assembly was fixed at 100 ± 0.5rpm throughout the experiment. The dissolution assembly was immersed in a water bath maintained at 37 ± 0.1°C.

The absorbances of various tetracyclines, withdrawn at periodic intervals were measured after appropriate dilutions of the dissolution medium, on a Shimadzu UV-1201 UV-Visible spectrophotometer.

Preliminary studies

All the antibiotics and H_2-receptor antagonists were assayed for their drug content. Absorbance of tetracycline hydrochloride was measured at 353 nm and demeclocycline hydrochloride at 319 nm in 0.1N HCl. Oxytetracycline hydrochloride was determined by measuring the absorbance of appropriately diluted solutions at 269, 351 and 353 nm, using 0.1 N HCl as blank. For the assay of methacycline hydrochloride stock solution was prepared in 0.1N HCl, while aliquots were diluted in methanolic HCl (0.01N) and absorbance were measured at 243, 340 and 345 nm, against reagent blank. For the assay of doxycycline hyclate stock solution was prepared in 0.1N HCl, while aliquots were diluted in methanolic HCl (0.01N) and absorbances were measured at 269, 351 and 353 nm, against reagent blank. Absorbance at 351 nm was used for further calculations.

Procedure for dissolution studies

Dissolution profiles were obtained for various tetracyclines capsules on the dissolution apparatus as detailed above. The dissolution fluid was 900 ml of 0.1N HCl, samples were withdrawn periodically with an interval of 5 minutes for 60 minutes, and a final reading was taken after 120 minutes. The samples were diluted with HCl 0.1 N or 0.01 methanolic HCl as required and then absorbance were measured at their specific wavelengths. The volume of dissolution fluid was maintained by adding an equivalent

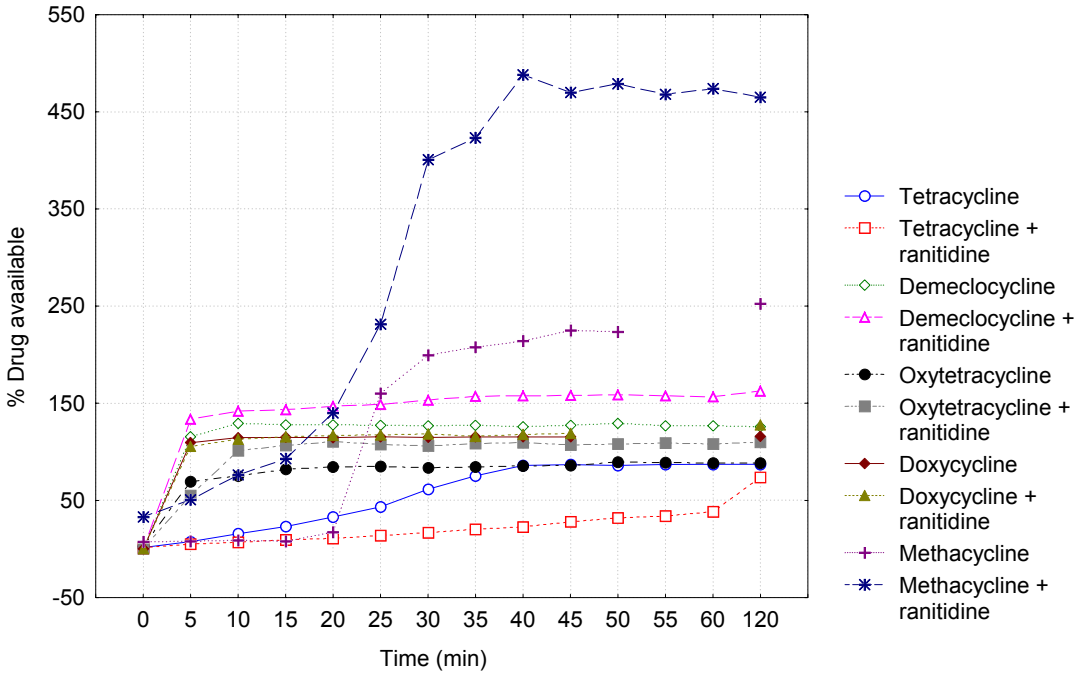

Fig. 2: Availability of tetracyclines in presence of ranitidine.

amount of dissolution fluid withdrawn, which had previously been maintained at the same temperature in the same bath.

Procedure for interaction studies

In the drug interaction studies, each of the tetracycline derivatives were separately interacted with each of the H_2-receptor antagonist. One capsule of each of the tetracycline derivatives, was placed in dissolution basket and either cimetidine or ranitidine was dropped in the beaker. Five minutes after the dissolution has started, aliquots were drawn similarly and concentration of the antibiotic in solution was determined by the standard method. Data shown in figs. 1 and 2 are the average of at least three runs; the results were satisfactorily reproducible.

Determination of tetracyclines, doxycycline, methacycline and demclocycline

Five ml of the sample was drawn at specified time intervals and was diluted to 50 ml with HCl 0.1 N. The amount was determined by measuring absorbance at 353 nm for tetracycline, 319 nm for demeclocycline, 243 nm for methacycline and 349 nm for doxycycline solutions, using HCl 0.1 N as the blank.

Determination of oxytetracycline

1 ml of the sample drawn at specified intervals was diluted to 25 ml in a volumetric flask with HCl 0.1 N. The amount of oxytetracycline was determined by measuring absorbance at 353, 351 and 269 nm of sample solutions, using HCl 0.1 N as blank.

RESULTS AND DISCUSSION

It has already been established that antacids, containing hydroxide or carbonate salts of aluminum, calcium or magnesium, widely used for the symptomatic treatment of hyperacidity, depress the absorption of orally administered tetracyclines. It is generally considered that tetracyclines form chelates with these divalent and trivalent cations. Some of these agents also have mild anti-ulcer effect, which is supposedly via H_2-receptor antagonistic mechanism. It has long been established that antihistamines act via different types of receptors usually designated as H_1, H_2, H_3 and H_4 etc. H_2 receptors are usually responsible for the gastric acidity and drug acting on these receptors block the acid secretions. In present studies, we selected H_2-receptor antagonists, cimetidine and ranitidine, for their interactive study with tetracyclines. Present study mainly deals with the *in vitro* availability of these antibiotics in presence of these antagonists.

The present work comprised of independent interaction studies of cimetidine and ranitidine with each of these antibiotics. Results of the *in vitro* availability studies of these antibiotics alone and in presence of cimetidine and ranitidine are given in table 1, and are plotted in figs. 1 and 2. It was found that the absorption of nearly all tetracyclines

Table 1
Dissolution behavior of tetracyclines

Antibiotic Time (min)->	Percent drug dissolved													
	0	5	10	15	20	25	30	35	40	45	50	55	60	120
Tetracycline HCl	1.51	7.57	13.75	23.02	33.02	45.33	61.51	75.45	84.06	87.96	89.92	91.27	92.84	99.09
Tetracycline HCl + cimetidine	2.42	6.66	7.27	10.9	14.24	19.09	23.33	28.78	31.81	36.45	42.42	49.87	54.84	89.09
Tetracycline HCl	1.51	5.14	6.96	9.39	10.9	13.63	16.96	20.3	23.72	28.18	31.81	34.64	37.48	73.63
Oxytetracycline	2.4	25	70	83	88.8	93.6	94	94	94.8	96.2	97.6	98.4	98.8	99.2
Oxytetracycline + imetidine	0	65.66	87.33	88.33	88.66	88.66	90	98	98.33	100.6	102	94.33	93.33	90
Oxytetracycline + ranitidine	1	51.66	94	98	98.66	99	99	99	99.33	99.33	99.6	100	100.6	101.33
Doxycycline	0.23	96	100.5	100.5	100.8	100.8	100.8	101.1	101.1	101.1	101.4	101.4	101.6	101.7
Doxycycline + cimetidine	0	73.14	92	92	92.28	92.57	93.42	93.71	93.42	92.57	92	90	87.14	79.71
Doxycycline + ranitidine	0.57	92.85	99.14	101.1	102.5	102.8	103.1	103.4	103.5	103.7	103.8	104	104.3	104.28
Demeclocycline	0	90	98.06	98.06	98.7	98.7	98.7	99.03	99.03	99.03	99.35	99.67	100.6	100.6
Demeclocycline + cimetidine	0	79.67	96.45	96.45	96.45	96.77	96.77	96.77	97.09	97.41	97.74	97.74	97.74	98.7
Demeclocycline + ranitidine	0	104.2	110.6	111.9	114.5	116.1	119.3	122.2	122.5	122.9	122.9	123.2	123.5	126.7
Methacycline	3.65	4.44	6.28	10.44	15.24	27	48.41	75.09	92.3	101.4	104.6	107.2	108.2	108.3
Methacycline + cimetidine	3.65	5.12	7.8	11.15	16.9	30.15	51.9	57.3	58.36	59	59	59.26	59.33	59.36
Methacycline + ranitidine	0.63	9.2	21.58	29.68	31.74	35.23	40.15	51.11	73.8	74.7	73.25	72	72	71.85

were depressed in presence of these H_2-antagonist. It is inferred that these H_2-antagonists form a complex with these tetracyclines, which depresses the absorption of the orally administered tetracycline.

It is evident from the results given in table 1 that availability of tetracycline hydrochloride was smoothly increasing when studied alone but when ranitidine was added to the dissolution medium along with the antibiotic, the dissolution was very much retarded as is clear in fig. 1. After 30 minutes 75.45% of the tetracycline hydrochloride was available but in presence of ranitidine only 20.3% of the drug was available. Similarly after one hour only 37.48% drug was dissolved when ranitidine was present while 92.84% when tetracycline hydrochloride alone was present. At infinite time, 99.05% of drug was present in the dissolution beaker while only 73.63% in presence of ranitidine (fig. 2).

From the results of studies of tetracycline hydrochloride with cimetidine, it was observed that in one hour the availability of tetracycline HCl was depressed by 32.43 % i.e., after 60 minutes, only 54.84 % drug was dissolved as compared to tetracycline HCl alone, which was 87.27 %. At infinite time, the percentage dissolved was 89.09 in presence of cimetidine as compared to 87.27 when given alone. These studies reveal that the availability of tetracycline was depressed only to a slight extent in presence of cimetidine.

When the availability of doxycycline was studied in presence of ranitidine and cimetidine, it was observed that

the absorption was increased instead of decreasing in presence of ranitidine. This increase in absorption may be due to the summation of absorption of ranitidine at this wavelength. When cimetidine was introduced along with doxycycline, slight decrease (~10%) in the concentration of the drug was observed. When cimetidine was present along with doxycycline, 115.9 mg/L of doxycycline was available as compared to reference drug, which was 106.5 mg/L present at infinite time.

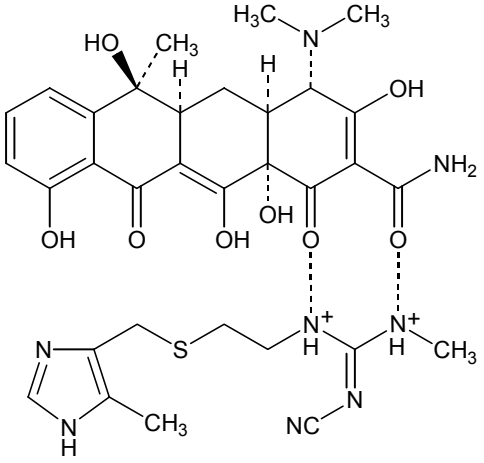

Fig. 3: Proposed structure of tetracycline–cimetidine charge transfer complex

The absorbance of oxytetracycline was measured at 269 nm and 353 nm. At both wavelengths the absorbance was increasing from time to time and was maximum at 120 minutes i.e., about 88.7% of the drug was available. When ranitidine was introduced along with oxytetracycline HCl, the absorbance value was very much increased, even more than the increase observed in case of doxycycline with ranitidine. After 20 minutes the percentage dissolved was 110.52 when ranitidine was present with oxytetracycline and the percentage dissolved was only 84.78 when oxytetracycline was given alone. Similarly, the absorbance value was also increased when cimetidine was introduced along with oxytetracycline. Maximum absorbance value was obtained after 50 minutes, where percent available was 111.67, after which time it was gradually decreased and after 120 minutes it was 98.54%. This value being more than the case, when oxytetracycline was given alone.

The absorbance of demeclocycline was taken at 319 nm. It was observed that only after 5 minutes 173 mg/L of the drug was present in dissolution beaker, which gave 115.5% drug concentration. After 10 minutes 129.2% of the drug was present and after that time there was increase in the drug content but a slight decrease was seen which could be due to binding of the drug molecules with the binders of the drug. At infinite time, 125.8% of the drug was present in the dissolution beaker, when the dissolution of demeclocycline was studied along with cimetidine it was seen that there was no change in the absorbance values, and nearly same concentration of the drug as when demeclocycline was studied alone, was present. There was a very slight decrease in the absorbance values, when the drug was studied in presence of cimetidine. The % dissolved after 5 minutes was 102.3 where as when demeclocycline was studied alone it was 115.5 and at infinite time the % dissolved was 124.2% as compare to demeclocycline alone which dissolved 125.8%. The maximum percentage dissolved at 55 minutes was 126.7%.

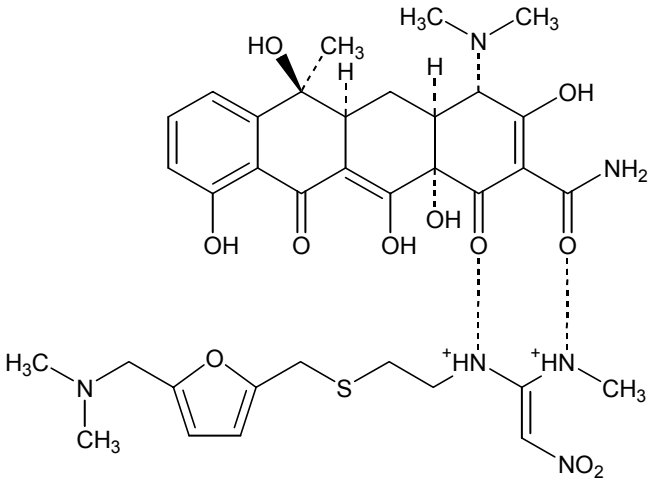

Fig. 4: Proposed structure of tetracycline– ranitidine charge transfer complex

When dissolution of demeclocycline was studied in presence of ranitidine, the results indicate a continuous increase in the absorbance values till infinite time. These values were more as compared to demeclocycline studied alone. More than 100% drug was present only after 5 minutes, and after 30 minutes more than 150% of the drug (153.2%) was available in the dissolution beaker. At 60 minutes 156.9% and at infinite time 162.7% of the drug was available in the dissolution beaker. This prominent increase in the drug content can be due to the formation of a charge transfer complex of demeclocycline with ranitidine.

The absorbance of methacycline was measured at 243 nm and the results observed were very much different from that of other tetracyclines. The amount of methacycline when studied with cimetidine at 30 minutes was 342.11%, at 60 minutes, it was 413.98, and at infinite time, it was 426.34%. These amounts are much higher as compared to methacycline reference drug alone. When methacycline was studied in presence of ranitidine at 30 minutes 400.97% of the drug was present in the dissolution beaker and only after 10 minutes i.e., at 40 minutes 488.13% of the drug was present, which was the maximum content of the drug present. After that time, there was a slight decrease in the drug content and at 60 minutes 473.82% and at infinite time 465.04% was present.

Change in availability of drugs in presence of each other is evident of the fact that there is strong interaction between two drug classes. The interaction is attributed to the formation of charge transfer complexes on acidic centers of tetracyclines. Predicted charge transfer complexes structure of tetracycline with cimetidine and ranitidine are presented in figs. 3 and 4.

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