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RESEARCH ARTICLE

Sucralfate Suspension Reduced Absorption of Oral Ciprofloxacin Hydrochloride in Rabbit

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ABSTRACT

One of the most frequent side effects due to the use of ciprofloxacin is a gastrointestinal disorder. Generally, in order to overcome the side effects of the Gastro Intestinal Track (GIT), the drug is given after meals (provided 15-30 minutes after meals). Unfortunately the present of food in gaster can significantly decrease maximum concentration (C_{max}) of ciprofloxacin. The aim of this study was to determine the effect of sucralfate suspension that containing alumunium (Al^{3+}) on the absorption of oral ciprofloxacin HCl. The effects of 0.47 mL/kg body weight doses of sucralfate suspension which is containing polyvalent cations, aluminum sucrose octa sulfate on the absorption of oral ciprofloxacin HCl before a single 23 mg/kg body weight doses were investigated in 6 rabbit subjects, randomized, cross over and single blind study. The 6 rabbits were enrolled in two studies. Each subject got single ciprofloxacin HCl administration as a control treatment. Treatments that were evaluated included the administration of sucralfate with single dose of ciprofloxacin HCl concomitantly (treatment 1) and the administration of sucralfate 2 hours before ciprofloxacin HCl administration (treatment 2). The absorption parameters of ciprofloxacin HCl were determined by spectrofluorometric method using time to reach maximum concentration (t_{max}), C_{max} and area under curve (AUC) parameters. In control treatment, the average value of C_{max}, t_{max}, and AUC_{0-360} were 1.34 µg/mL ± 26.15%, 160.78 minutes ± 5.85% and 337.06 µg minutes/mL ± 14.40%. In treatment 1, the average value of C_{max}, t_{max}, and AUC_{0-360} were 0.68 µg/mL ± 15.49%, 420.66 minutes ± 25.49% and 277.13 µg.minutes/mL ± 12.25%, and in treatment 2 were 0.95 µg/mL ± 18.54%, 284.93 minutes ± 15.44% and 309.75 µg.minutes/mL ± 11.71%. Statistical analysis used in this study was one-sided paired t test ($\alpha = 0.05$). On the basis of these findings, ciprofloxacin HCl and sucralfate should not be administered concomitantly, but normal kinetics are restored by administering the drug 2 hours before ciprofloxacin HCl. *Andrographis paniculata* extract suppressed cancer cell growth by decreased cell proliferation and increased apoptosis.

KEYWORDS

Ciprofloxacin, Sucralfate, Spectrofluorometric, Interaction of Fluoroquinolone

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INTRODUCTION

Several initial reports indicated that alumunium-containing antacids significantly impair the absorption of ciprofloxacin HCl form gastrointestinal tract. A retrospective study among 54 patients of ICU patients who received

ciprofloxacin therapy for 3 months showed there were 41 patients (76%) received antacids or sucralfate therapy simultaneously to cope with stress ulcer. Then antacid therapy was discontinued or replaced with H2 receptor antagonists which were reported not significantly interact with ciprofloxacin in 5 patients[1]. A study conducted by Garrelts in healthy subjects reported that a decrease in bioavailability (AUC_{0-12}) of 87.5% when 500 mg ciprofloxacin given together with 1 gram sucralfate administration after the previous day given 1 g sucralfate four times a day (30 minutes before meals and at bedtime). The maximum concentration of ciprofloxacin achieved in serum also decreased by 10 times compared to when ciprofloxacin was given alone. Other study also suggested that the bioavailability of ciprofloxacin 750 mg decreased respectively by 7%, 20% and 95% when given sucralfate consecutive 6 hours before, 2 hours before and at the same time[2].

Fluoroquinolone antibiotics have broad-spectrum antimicrobial activity, very good bioavailability, good penetration into the tissue, long serum half-life and generally only a few side effects in its use. One of the most frequent side effects that arise due to the use of ciprofloxacin is gastrointestinal tract disorder[3]. Therefore, it is possible to use combination therapy of ciprofloxacin with sucralfate together, especially for peptic ulcer patients. Sucralfate which contains about 200 mg of aluminum hydroxide component ($AlOH_3$) per gram is capable of forming an insoluble chelate between these cations with 3-carboxyl and 4-keto group of quinolone antibiotics that will decrease its absorption[2]. This study will try to prove the interaction between ciprofloxacin with sucralfate on absorption phase when used in combination therapy using rabbits as animal research subjects. Absorption parameters are to be determined in this study, including C_{max}, t_{max}, and AUC_{0-360}, which indicates the availability of ciprofloxacin in the blood.

MATERIALS AND METHOD

Ciprofloxacin used in this study were in the form of salt, a standard ciprofloxacin HCl powder and sucralfate suspension containing 500 mg of aluminum sucrose sulfate per 5 mL. A total of 6 rabbits have given 3 treatments, a single administration of ciprofloxacin HCl, concomitant sucralfate administration with ciprofloxacin HCl and sucralfate administration 2 hours before ciprofloxacin HCl treatment. This study was conducted using a cross-over model with washing period for 7 days to ensure the drug is eliminated perfectly from the plasma of the rabbit.

The method to analyze the plasma levels of ciprofloxacin is spectrofluorometry. Validation parameters defined in this study include linearity, precision, limit of detection/limit of quantification (LOD/LOQ), and accuracy. In the determination of linearity proved the existence of a linear relationship between increasing levels of ciprofloxacin HCl standard solution with an intensity that read by spectrofluorometer, it can be seen from this regression $y = 10.79\ x + 0.974$, with $r = 0.997$ ($r_{table} = 0.666$; with $df = 7$; $\alpha = 0{,}05$). These results meet the requirements $r > r_{table}$. On the determination of precision obtained %KV = 1.52 ± 0.29%, which also meets the requirements of KV ≤ 2%. Determination of accuracy was done by setting the ciprofloxacin levels that can be recovered from plasma matrix. Percent recovery obtained was 89.21 ± 14.02%, meet the requirements of % recovery range is 80-120% and ≥ 70% in other literature. LOD and LOQ were successfully determined is equal to 0.003 mg/mL and 0.01 mg/mL.

Research conducted using a cross-over model. The order of treatment 1s not the same among each subject, selected at random. It aims to minimize the variation in each subject. The treatment of research object can be seen in table 1. Dose conversion factor used are 0.07, obtained from the dose conversion of 70 kg human and 1.5 kg rabbit[4]. Based on these conversion factors, the dose of ciprofloxacin HCl and sucralfate for each rabbit can be determined, 23 mg/kg body weight and 0.47 mL/kg body weight.

Table 1: Treatment of the Research Object

Subject	Week 1	Week 2	Week 3
1	C	T_1	T_2
2	T_1	T_2	C
3	T_2	C	T_1
4	C	T_1	T_2
5	T_1	T_2	C
6	T_2	C	T_1

C: Oral single use of ciprofloxacin 23 mg/Kg body weight of the rabbit.

T_1: Concomittant administration of sucralfate and ciprofloxacin HCl at intervals of 5-10 minutes.

T_2: Concomittant administration of sucralfate and ciprofloxacin HCl at intervals of 2 hours.

After the rabbits were fasted overnight, then weighed and sheared the ear's fur, the sucralfate suspension preparation with dosage 0.47 mL/kg body weight administered orally. A ±2 mL solution of 23 mg dose of ciprofloxacin HCl/kg body weight administered orally. Heparin solution was prepared in the injection syringe 0.02 mL/mL of rabbit blood samples. Samples of blood were taken from the vena marginalis ± 1 mL at minute 0, 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360[5]. Samples of blood were centrifuged at 2000 rpm for 15 minutes and then the plasma separated and stored at -20 °C until analyze.

Sample extraction (Spectrofluorometry method with modification)[6] Two hundred and fifty µL plasma samples added by five hundred µL of 15% TCA were homogenized 1 minute and centrifuged at 4000 rpm for 5 minutes. Five hundred µL of supernatant was taken and then put in venoject which already calibrated 10 mL. The supernatant then added by 500 µL standard solution of ciprofloxacin HCl 1.0 mg/mL. The solution was extracted three times with 2.5 mL each of chloroform. Chloroform phase was taken then evaporated over a water bath. The pellet then added by 1.0 mL of ammonium molybdate 7.5 mg/mL, ± 3 mL citrate phosphate buffer (pH = 3.5) and methanol up to the mark. The solution centrifuged at 2000 rpm for 5 minutes. Supernatant was subsequently measured at maximum excitation and emission wavelength of Fluorescence Spectrophotometer Hitachi F-4000.

RESULT

After obtaining the absorption parameter data of 6 subjects research, statistical analysis was then performed using one-sided paired t test (α = 0.05) to determine whether the absorption parameter has a mean significantly decreased in conjunction with sucralfate administration of ciprofloxacin HCl and administration sucralfate 2 hours before ciprofloxacin HCl treatment when compared to the control condition.

Based on statistical analysis of paired t test (α = 0.05), concomitant administration of sucralfate suspension with ciprofloxacin HCl influence on its absorption. Pharmacokinetic parameters which were decreased and significantly affected were $AUC_{0\text{-}360.}$ An average % of decrease in $AUC_{0\text{-}360}$ in concomitant use was 16.32% to the control group. Administration sucralfate suspension with a lag time of 2 hours did not decrease $AUC_{0\text{-}360}$ significantly with an average % of decrease in $AUC_{0\text{-}360}$ was equal to 6.66% to the control group. While the results of statistical tests to t_{max} showed no significant difference between the control condition with the treatment 1 and treatment 2.

DISCUSSION

Several important interactions of fluoroquinolones with other drug have been reported in the literature. The absorption of all fluoroquinolones were almost entirely inhibited by concomitant administration of di- and trivalent cations, such as aluminium contained in sucralfate. The most plausible explanation for this interaction is the formation of ciprofloxacin-aluminum chelates[2].

In previous study, Myers and Blumer[7] using HPLC method with fluorescence detector to separate the metabolites of ciprofloxacin, showed that ciprofloxacin has two maximum excitation wavelengths, 280 nm and 328 nm, with maximum emission wavelength 446 nm. Based on these results, it can be seen that the qualitative analysis of ciprofloxacin with spectrofluorometry method in this study was selective. Qualitatively, the spectroflurometry analysis of a compound indicated with a Stokes shift[8]. Stokes shift of ciprofloxacin based on the research of Myers and Blumer[7] is approximately 166 nm (280 nm excitation and 446 nm emission). In this study, stokes shift was 162 nm (278 nm excitation and 440 nm emission). This result is specific to ciprofloxacin because the difference was ≤ 5 nm with a setting on the instrument band 5 nm on the appliance.

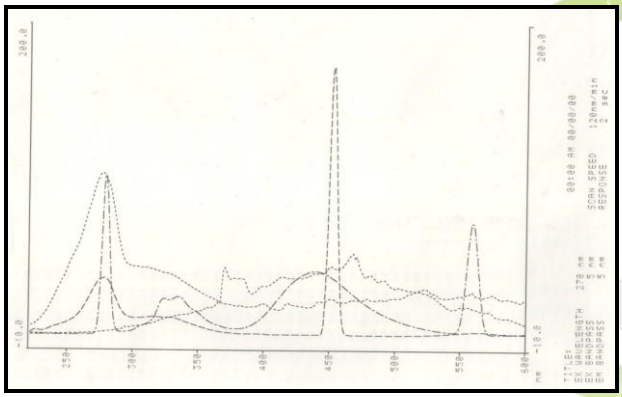

Figure 1: Determination of the maximum wavelength of ciprofloxacin HCl in rabbit plasma samples

The method to analyze the plasma levels of ciprofloxacin in this study was spectrofluorometry. The basic principle of the analytical method is the formation of a chelate between chelating agents with ciprofloxacin. Chelate formed will result in fluorescence intensity that can be measured using a spectrofluorometer. A chelating agent is in the form of molybdenum metal compound, ammonium molybdate. Maximum excitation and emission wavelength obtained at 278 nm and 442 nm for ciprofloxacin standard solution. Whereas for ciprofloxacin in plasma samples we used the maximum excitation wavelength 278 nm and maximum emission 440 nm. At these wavelengths, the validation process was done.

The sampling of rabbit plasma performed for 360 minutes (6 hours). The ideal time for sampling according to the literature is 3-5 x $t_{1/2}$. According to the results of previous study, half-life ($t_{1/2}$) of ciprofloxacin HCl in rabbit blood serum was 1.85 ± 0.25 hours at a dose of 25 mg/kg body weight and administered via the intravenous bolus[9]. Based on that literature, the time of rabbit plasma sampling was 4.8 to 6.3 hours. However, there were no data from previous studies regarding $t_{1/2}$ oral ciprofloxacin HCl in rabbits. Therefore, the sampling time in this study was determined based on the theory of interaction between ciprofloxacin HCl with sucralfate suspension occurs in the absorption phase and the rabbit gastric emptying time is 3-6 hours[10]. Bioavailability of ciprofloxacin were described by AUC was also calculated from minute 0 to minute 360.

In the single administration of ciprofloxacin HCl (without any influence of sucralfate), the average C_{max} calculation of 3 rabbits was 1.34 mg/mL ± 26.15%, the average t_{max} calculation was 160.78 minutes ± 5.85% and the average of AUC_{0-360} of 6 rabbits at 337.06 µg.menit/mL ± 14.40%. It was difficult to compare C_{max}, t_{max} and AUC_{0-360} in this study with those obtained by the literature because the dose of ciprofloxacin HCl that given to each rabbit among the studies was different. Based on research conducted by Bashir et. al.[11], the ciprofloxacin C_{max} in rabbit plasma was 0.26 ± 0.04 mg/mL at a dose of 20 mg/kg, t_{max} was 0.98 ± 0.07 hours and AUC_{0-12} was 3.3 ± 0.05 µg.hour/mL. The differences of the result cause by the different dose of ciprofloxacin HCl that given to the samples**.** In the above study, the doses of ciprofloxacin HCl was smaller than those in this study and the rabbit's weight that used in the above study were also smaller than used in this study. The difference can also be caused by several factors, like rabbit physiological condition (species and food) and the differences of analytical methods with different sensitivities.

Table 2: Average Plasma Concentration of Ciprofloxacin HCl in Rabbit Plasma at 0 - 360 minutes

Minutes	**Plasma Concentration (µg/mL)**					
	Control	**AUC**	**Treatment 1**	**AUC**	**Treatment 2**	**AUC**
0	0,00		0,00		0,00	
15	0,75	5,64	0,61	4,58	0,65	4,84
30	0,88	12,23	0,70	9,79	0,72	10,20
45	1,15	15,20	0,80	11,18	0,79	11,29
60	1,08	16,71	0,85	12,34	0,89	12,60
90	1,16	33,55	0,90	26,25	0,96	27,78
120	1,04	32,95	0,93	27,48	0,95	28,60
180	0,98	60,55	0,80	51,95	0,95	56,90
240	0,94	57,65	0,78	47,50	0,92	56,20
300	0,85	53,80	0,72	45,00	0,85	53,25
360	0,77	48,70	0,65	40,85	0,75	48,20
∑	336,98		276,90		309,85	

Table 3: Summary of the Calculation on Absorption Parameters Subject N1-N6 in Treatment 1

Subject	**Control**			**Treatment 1**			**% decreased C_{max}**	**% decreased AUC**
	C_{max} (µg/m)	**t_{max} (minutes)**	**AUC_{0-360} (µg/mL.minute)**	**C_{max} (µg/ml)**	**t_{max} (minutes)**	**AUC_{0-360} (µg/mL.minute)**		
N1	1.19	172.70	411.13	0.82	412.56	248.00	31.09%	39.68%
N2	0.73	266.82	286.14	0.89	208.87	298.89	-21.92%	-4.45%
N3	1.00	149.69	310.44	0.64	293.56	251.12	36.00%	19.11%
N4	0.59	508.24	294.28	0.73	201.02	256.74	-23.73%	12.76%
N5	1.82	159.95	366.85	0.57	555.85	273.18	68.68%	25.53%
N6	0.89	333.87	353.51	1.01	191.85	334.84	-13.48%	5.28%
Mean	1.34	160.78	337.06	0.68	420.66	277.13	45.26%	16.32%
KV	26.15 %	5.85%	14.40%	15.49%	25.49%	12.25%	36.86%	95.09%

Table 4: Summary of the Calculation on Absorption Parameters Subject N1-N6 in Treatment 2

Subject	Control			Treatment 2			% decreased C_{max}	% decreased AUC
	C_{max} (µg/mL)	t_{max} (minutes)	AUC_{0-360} (µg/mL.minutes)	C_{max} (µg/mL)	t_{max} (minutes)	AUC_{0-360} (µg/mL.minutes)		
N1	1.19	172.70	411.13	0.80	271.28	297.70	32.77%	27.59%
N2	0.73	266.82	286.14	0.73	363.61	290.56	0.00%	-1.54%
N3	1.00	149.69	310.44	1.20	239.20	365.21	-20.00%	-17.64%
N4	0.59	508.24	294.28	0.81	165.54	270.87	-37.29%	7.96%
N5	1.82	159.95	366.85	0.86	344.31	290.96	52.75%	20.69%
N6	0.89	333.87	353.51	1.03	201.43	343.22	-15.73%	2.91%
Mean	1.34	160.78	337.06	0.95	284.93	309.75	21.84%	6.66%
KV	26.15%	5.85%	14.40%	18.54%	15.44%	11.71%	140.52%	242.89%

Average C_{max} and t_{max} calculation can only be counted towards the subject N1, N3 and N5 because the results of these calculations deviate in 3 other subjects. It can be caused by the slope of log concentration versus elimination time varying so that the speed of elimination will also vary. While it can be seen that the administration of sucralfate suspension in conjunction with ciprofloxacin HCl decreased AUC_{0-360} significantly to the control condition, while administration sucralfate 2 hours before administration of ciprofloxacin HCl did not decrease AUC_{0-360} significantly.

In treatment 1, (sucralfate administered with ciprofloxacin HCl), C_{max} obtained decreased when compared with controls in each subject (N1, N3 and N5). But the decrease was not significant when analyzed statistically using one-sided paired t test ($\alpha = 0.05$, $df = 2$). Research conducted by Garrelts, et. Al[12] in 8 healthy subjects showed C_{max} of ciprofloxacin in control conditions was 2.0 ± 0.5 mg/mL while on treatment condition decreased to 0.2 ± 0.1 mg/mL. From the results, it can be concluded that sucralfate has a tendency to decrease the peak levels of achievement of ciprofloxacin HCl in rabbit blood plasma.

In treatment 2 (sucralfate was given 2 hours before administration of ciprofloxacin HCl), C_{max} obtained was decreased in 2 subjects (N1 and N5) and increased in 1 subject (N3) when compared with controls. There was no research on the effect of 1 g sucralfate if given 2 hours before the administration of 500 mg ciprofloxacin HCl. But it has recommended that administration of oral ciprofloxacin HCl preferably 2 hours before or 6 hours after products containing antacids and other metal ions[13]. Statistical analysis of the results stated that C_{max} reduction in treatment 2 was non-significant difference when compared with controls. From the analysis, it can be concluded

that sucralfate still have a tendency to decrease the peak levels of ciprofloxacin HCl achievement in the treatment 2 group, although the decreasing trend is lower when compared to the treatment 1.

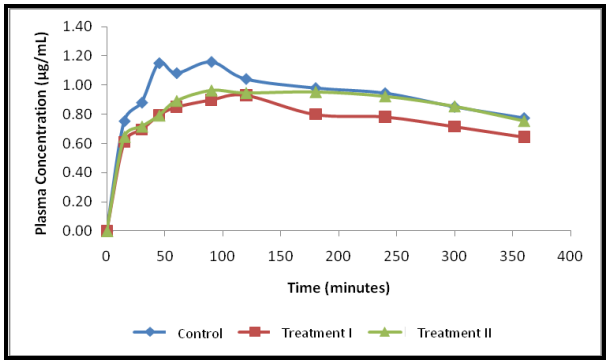

Figure 2: Curve of average levels of ciprofloxacin HCl versus time in rabbit blood plasma

Results of statistical analysis using two-sided paired t test ($\alpha = 0.05$, $df = 2$) to t_{max} showed no significant difference between the control condition with the treatment 1 and treatment 2. Research conducted by Garrelts, et. Al[12] as a reference, the t_{max} results obtained in the control group was 1.4 ± 0.3 hours and the treatment group 1.3 ± 1.2 hours. Based on these results, time to reach peak level in the plasma (t_{max}) relatively unaffected by sucralfate administration together with ciprofloxacin HCl. In this study, t_{max} both in treatment 1 and treatment 2 increased in 3 subjects (N1, N3 and N5) when compared with controls, instead decreased in 3 other subjects. Based on the t_{max} obtained in this study, it can be concluded that sucralfate relatively no effect on the time to reach peak levels of ciprofloxacin HCl in the rabbit plasma (t_{max}).

According to these result, the bioavailability of ciprofloxacin HCl impaired by concomitant dosing with sucralfate, but normal kinetics are restored by administering the drug 2 hours before ciprofloxacin HCl treatment.

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