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Evaluation of Ciprofloxacin In saliva For the Healthy Volunteers

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Abstract

The use of antibiotics as an adjunctive therapy in the treatment of periodontal diseases is of special interest to dental practitioners. In addition to using an appropriate antibacterial agent, clinicians may find it useful to determine the local and systemic concentrations of antibiotics in infected periodontal sites to reduce the levels of bacteria. The purpose of this study is the determination of the ciprofloxacin concentration in saliva; as well as investigates the efficiency of triethylamine and acetonitrile mobile phase in separation of ciprofloxacin in saliva.

Ten Subjects were given orally a single dose of 500-mg. ciprofloxacin, then 0.5 ml saliva samples were collected into centrifuge tubes (0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 hrs) after dosing, samples were centrifuged and the separated were injected to HPLC using triethylamine and acetonitrile mobile phase after adjacent the pH at 3.0 ± 0.1 with phosphoric acid at flow rate 1.5 ml/min.

Ciprofloxacin tablets used in this study within the specification and meet the USP requirements in weight variation, disintegration time, dissolution and assay. The obtain result from HPLC method recorded saliva concentration of ciprofloxacin within one day run ranged from 0.062 to 2.0298 mg. / L while day to day run from 0.071-2.030 mg/L. The other pharmacokinetic parameters are: the mean concentration of ciprofloxacin 4.233 µg/ml, C max 3.285, AUC 0-∞ 20.388 µg. h/ ml, and T^{max} 1.203.

The results of this clinical study show that the detection of ciprofloxacin in saliva represents the distribution of the drug in saliva and indicated the suitability for ciprofloxacin in dentistry treatments, and The HPLC method provided successful method for mentoring the ciprofloxacin in saliva with a detection limit reach 1.2 ng/ ml. The analysis method are sensitive, reproducible, low coast and efficient for low concentration

Key word: Ciprofloxacin, Bioavailability of ciprofloxacin, Saliva, Concentration of ciprofloxacin in saliva.

Introduction

Saliva could be the key to diagnosing disease in the future, and many researches are focusing on the potential of this common bodily fluid for detecting disease without the need to draw blood or perform other invasive procedures. (1)

Chronic periodontitis is an infectious disease resulting in inflammation within the supporting

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tissues of the teeth, progressive attachment loss and bone loss. It is characterized by periodontal pocket formation, recession of the gingivae, or both [2]. The disease typically is associated with the presence of microbial plaque . Progression of the disease is related to the colonization of micro-organisms in the gingival crevice, including *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis.* [2]

In periodontal and other types of bacterial infections, polymorphonuclear leukocytes, or PMNs, migrate to the infection site, phagocytose the bacteria and attempt to kill them with reactive oxygen metabolites and microbicidal proteins. Although PMNs are highly effective in defending against bacterial infections, some pathogens are difficult to kill. [3,4]

Conventional periodontal therapy, with its focus on mechanical debridement of bacterial plaque, usually prevents the progression of periodontal breakdown. In some cases, however, the disease progresses despite periodontal debridement. These types of periodontal infections usually are related to increased levels of subgingival bacteria. The use of antibiotics as an adjunctive therapy has been of particular interest to clinicians and researchers in the treatment of periodontal diseases. It is important that the clinician use an appropriate antibacterial agent when treating patients with periodontal disease. [5]

Although tetracycline and metronidazole families have been studied widely in periodontal research, but there's no any report in the literature regarding ciprofloxacin concentrations in GCF of patients with periodontitis. Because the inflamed periodontium is densely infiltrated by PMNs, hypothesized that systemic ciprofloxacin may reach higher levels in GCF than in serum. [6-8]

Ciprofloxacin hydrochloride tablets and Ciprofloxacin Oral suspension are synthetic broad spectrum antimicrobial agents for oral administration [9]

Its chemical structure is a film-coated tablets are available in 100 mg, 250 mg, 500 mg and 750 mg (as ciprofloxacin HCl equivalent) strengths [10] .

Ciprofloxacin given as an oral tablet is rapidly and well absorbed from the gastrointestinal tract after oral administration.[11] The absolute bioavailability is approximately 70% with no substantial loss by first pass metabolism. Ciprofloxacin, a widely is a moderately potent and selective inhibitor of CYP1A2-mediated drug metabolism [12]. Ciprofloxacin has been found to impair the elimination of theophylline caffeine, clozapine, and ropivacaine [13-15].

Ciprofloxacin maximum serum concentrations and area under the curve are shown in the chart for the 250 mg to 1000 mg dose range [16-18]. A 750 mg oral dose given every 12 hours has been shown to produce an AUC at steady-state equivalent to that produced by an intravenous infusion of 400 mg given over 60 minutes every 8 hours [19-21]. A 750 mg oral dose results in a C^{max} similar to that observed with a 400 mg I.V. dose. [22].The binding of ciprofloxacin to serum proteins is 20 to 40% which is not likely to be high enough to cause significant protein binding interactions with other drugs. After oral administration, ciprofloxacin is widely distributed throughout the body [23-24]. Drug pharmaco-kinetics in preterm babies differs from other age groups because of higher extracelluar fluid volume, immature renal and hepatic functions at birth, and post-natal maturation of these organs [25-26].

The aims of this study are the evaluation of the ciprofloxacin hydrochloride concentration in saliva, and estimate the pharmacokinetic

parameters after single dose of 500 mg. The low information about ciprofloxacin hydrochloride concentration and the pharmacokinetic parameters in saliva encourages us to find the salivary concentration after single dose of 500 mg. as well as investigate the benefit of applied this drug in dentistry treatments. Until the time of the publishing this research no similar information were collected.

Materials and Methods

Chemicals: Reagents and Solutions:

All reagents used in this study were from Fluka or BDH companies which suitable for HPLC analysis. Hydrochloric acid was from Merck (Germany). Ciprofloxacin tablets were obtained from Arab pharma manufacturing / the Arab pharmaceutical Manufa (ciprofloxacin hydrochloride in tablets - 500 mg.). The liquid chromatograph is equipped with a 278-nm detector and a 4.6-mm × 25-cm column that contains packing L1 (particle size 5 μm) and it's operated at 30 ±1 ºC. Mobile phase — Prepare a filtered and degassed mixture of 0.025 M phosphoric acid, previously adjusted (with triethylamine) to a pH of 3.0 ± 0.1, and acetonitrile (87:13) at flow rate 1.5 ml/min. [27].

Chemicals: Standard Solutions:

A stock solution of ciprofloxacin (1 mg mL−1) was prepared in distilled water. Standard solutions then was prepared in distilled water in a concentrations range of 0.1-3.0 mg / L $^{-1}$ ciprofloxacin hydrochloride.

Sample Collection:

Ten patients participated in study. Subjects were administered a 500-mg single oral dose of ciprofloxacin. All volunteers were who had not received antibiotic and anti-inflammatory drugs or any other medication. No subject had a history of evidence of hepatic, renal, gastro-intestinal, hematological disease, acute or chronic disease or drug allergy according them medical history. The subjects were instructed to abstain taking any medication at least 2 days before salivary collections.

Subjects were given orally a single dose of 500-mg tablets in a randomized fashion with 200 ml of water. Food and drinks were not allowed for 4 hours after dosing to all volunteers. Approximately 0.5 ml saliva samples were collected into centrifuge tubes 0 hr and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 hr after dosing. The centrifuge samples were centrifuged at 3000 rpm for 15 min.

In-vitro Studies:

Weight variation, content uniformity, assay and dissolution studies were all carried out according to USP procedures. Samples were assayed by UV spectrophotometer at 277nm. [27].

Statistical Analysis:

For the purpose of bioequivalence analysis, AUC 0-∞, and C max were considered as primary variables. A difference between two related parameters was considered statistically significant for a P-value equal to or less than 0.05. The 90 % confidence intervals of the ratio of pharmaco-kinetic parameters of test to reference products. All Statistical analyses were performed by using SPSS 10.

Chromatographic Conditions:

A reversed phase HPLC method was developed to quantities saliva levels of ciprofloxacin. The apparatus was a waters HPLC system (Waters, Ireland), consisting of a model intelligent solvent delivering pump, a computerized system controller, and a SPD-6AV UV detector. The liquid chromatograph is equipped with a 278-nm detector and a 4.6-mm × 25-cm

column that contains packing L1 (particle size 5 μm) and it's operated at 30 ±1 ºC. Mobile phase — Prepare a filtered and degassed mixture of 0.025 M phosphoric acid, previously adjusted (with triethylamine) to a pH of 3.0 ± 0.1, and acetonitrile (87:13), at flow rate 1.5 ml/min. the retention time of ciprofloxacin was 3.4 min.[28]. Quantitation was achieved by measurement of the peak area ratios of the drug to the internal standard [29-30].

Results

The tablets used in this study were within the specification and complied with the USP requirements table (1). The tablet dissolution reaches more than 90 % at the end of the dissolution time figure (1). This method is highly sensitive, with lower limit of quantitation of ciprofloxacin HCl reach 1.2 ng/ml. The coefficient of variation for within - day was less than 6 % while that of day-to-day-run was less than 9% table (2). The calibration curve was linear in saliva with a regression of r=0.9887. The obtained concentration of ciprofloxacin and the pharmacokinetic parameters in saliva are listed in tables (3) and (4) respectively. The resultant chromatograms from the HPLC with ciprofloxacin showed that the HPLC method developed is selective as it provided a good resolution of ciprofloxacin there was no interference from endogenous materials. The concentration changes with time are plotted in figure (2). This allows for the analysis of about 3 samples per hour. Despite the fact that many modern HPLC methods have been described for the assay of ciprofloxacin, there is justification for investigating newer methods, especially where they are more cost-effective and there is an appreciable reduction in the procedural time compared to older methods. It may be applied to both research and therapeutic drug monitoring as demonstrated by the concentration time curves pharmacokinetic curve in Figure (2). In conclusion, the HPLC assay method achieved in this study using a reversed-phase system was found to be simple, rapid, not expensive and sensitive for assaying ciprofloxacin concentration as low as 1.2 ng/ml in saliva.

Discussion

In vitro studies:

All products met the pharmacopoeia specifications for specification, weight variation, assay table (1) and dissolution profiles formulations which were studied are shown in figure (1). The dissolution test revealed that after 30 minutes 88 ± 1.73 of stated ciprofloxacin was released from tablets (n = 6). Therefore, both formulations met the USP dissolution specifications (1) stating that not less than 80% of drug content should be released within 30 minutes.

HPLC assay:

HPLC analysis of blank saliva showed no interference of its compounds with ciprofloxacin. However well resolved peak is observed at 3.4 min. with minimum detection limits of 1.2 ng/ml. This result predicted acceptable method for ciprofloxacin analysis in saliva. Ciprofloxacin was possible to be detected in about 40 min of administration the ciprofloxacin tablet.

All chromatograms were free from any interference at the retention times of ciprofloxacin, and compound was eluted as completely and appeared separate resolved peaks without peak tailing in such a way that it was possible to calculate peak height or peak area of standard curves. Linear relationships were found when the

peak area ratios of ciprofloxacin was plotted versus the ciprofloxacin saliva concentration ranging from 0.062 to 2.030 mg. L (r2 = 0.9889).The results of within- and between-day variability are presented in table (1). With % coefficient of variation were within 2.6 to 5.26. Results of coefficients of variation and percent errors indicate that method is reproducible within day and between days. The limits of quantitation and detection of the method were 1.2 µg/mL (almost 10 times greater than intercept obtained from standard curve). Ciprofloxacin was measurable at the first saliva sampling time (0.25 h) and after 6 half-lives in all volunteers. The described method utilizes no organic extraction which makes the method rapid and simple [31] this method provided direct evaluation of the ciprofloxacin without internal standard study [32].

In vivo studies:

Ciprofloxacin was well tolerated by the subjects and unexpected incidents that could have influenced the outcome of the study did not occur. All volunteers who started the study continued to the end and were discharged in good health. The short time of analysis, simplicity, and sufficient sensitivity makes the method particularly useful for pharmacokinetic and bioequivalent studies of ciprofloxacin even following oral single dose (one tablet) of the drug rather than two tablets. Furthermore there was no significant difference with regards to periods and sequences (P> 0.05). In summary, a rapid, practical and sensitive HPLC method is described for determination of ciprofloxacin in human saliva. Without extraction or internal standard achieving for ciprofloxacin tablets and its level made it suitable for the dentistry treatments.

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Appendix

Table 1: Tablets specifications

No.	Test	No. of tabs	Result
1	Wt. variation	20	3.6%
2.	Disintegration time	6	5.0 min.
3.	Specification	6	Comply with USP
4.	Dissolution	6	88%
5.	Assay	6	101%

Table 2: Concentration of ciprofloxacin in saliva.

Parameters	Concentration mg/L	% coefficient of variation
Within day run	0.0620 1.237 2.0298	2.6 3.18 5.26
Day to day run	0.071 1.237 2.030	3.265 6.258 8.236

Table 3: Concentration changes within one and day to day variability.

Concentration (µg/ml)	Within one day			Between days variability		
Mean	Mean	SD	%CV			%CV
0.180	0.185	0.006	3.255	0.181	0.021	2.554
2.885	2.920	0.102	2.842	2.621	0.016	3.847
3.254	3.440	0.219	2.842	3.077	0.214	4.587
3.564	3.482	0.204	3.647	3.168	0.314	4.872
3.658	3.674	0.307	3.845	3.381	0.316	7.265
7.256	7.045	0.422	7.254	6.822	0.384	7.542
7.525	7.358	0.416	7.258	7.425	0.488	8.014
8.024	7.659	0.671	6.254	7.650	0.764	8.026

Table 4: Pharmacokinetics parameters.

Parameters	Test	Ref.	Test/ref.	P-value
Concentration (mean) (µg/ml)	4.233	4.526	0.935	0.325
(C^{max}) (µg/ml)	3.295	3.478	0.947	0.255
AUC 0-∞(µg. h/ml)	20.388	21.158	0.964	0.473
T^{max}	1.203	1.188	1.013	0.135

Figure 1: Dissolution of ciprofloxacin tablets.

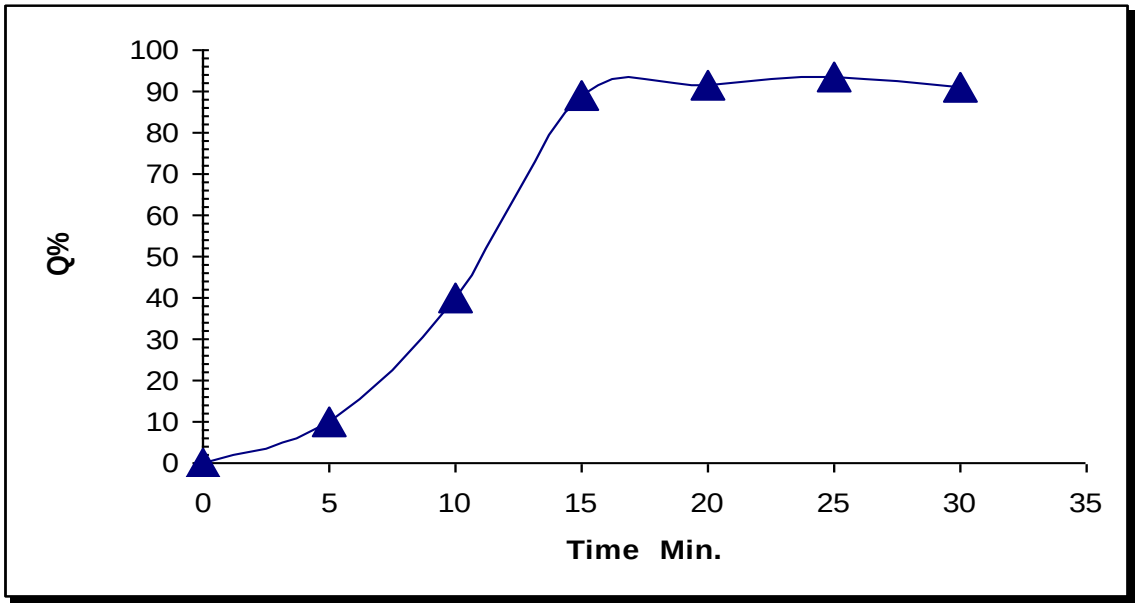

Figure 2: Changes of salivary ciprofloxacin with time