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Chromatographic estimation of tetracycline in saliva

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Abstract

Tetracyclines have been widely used in dentistry, especially in treatment of periodontal diseases and other oral pathological lesions. They are effective in treatment because their concentration in the gingival crevice is 2-10 times that in serum. In addition, several studies have demonstrated that tetracyclines at a low gingival crevicular fluid concentration (2-4 mg/ml) are very effective against many periodontal pathogens. Tetracyclines are an extensively used group of antibiotic medicines. Clinical pharmacology of tetracycline in saliva was not clear until the time of this research achieving on normal subjects or patients required tetracycline treatment. The evaluation was achieved by using suitable method (efficient, low cost, and reproducible). The purpose of this study is the determination of the tetracycline concentration in saliva. The obtained results from HPLC method recorded saliva concentration of tetracycline ranged fom 0.08 to 0.23 µg. / ml. The HPLC method provided successful methods for monitoring the tetracycline in saliva with a detection limit reached to 0.05 ng/ml. Since the saliva is considered as the first line of immune system in the oral cavity, so it is important to suggest further study to know the therapeutic effect of tetracycline through saliva against certain oral lesions.

Key word: bio-bioavailability of tetracycline – concentration of tetracycline in saliva

Introduction

The tetracyclines, which were discovered in the 1940s, are a family of antibiotics that inhibit protein synthesis by preventing the attachment of aminoacyl-t RNA to the ribosomal receptor (A) site [(1)]. Tetracyclines are distributed in varying degrees into bile, liver, lung, kidney, prostate, urine, and cerebrospinal fluid, and synovial fluid, mucosa of the maxillary sinus, brain, sputum, and bone[(2)]. Tetracyclines cross the placenta and enter the fetal circulation and amniotic fluid. Following a single oral dose, peak plasma concentrations are achieved in two to four hours [(3)].

Tetracyclines are concentrated by the liver in the bile. They are excreted in both the urine and faeces at high concentrations in a biologically active form. Since renal clearance of tetracyclines is by glomerular filtration, its excretion is significantly affected by the state of renal function [(4)].

Maximum blood levels of the antibiotic are reached at about two hours after administration and are

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maintained at high levels for 6 to 8 hours. [5]

Tetracycline has been widely used in dentistry, especially in treatment of periodontal diseases. They have been frequently used in treating refractory periodontitis, including localized aggressive periodontitis. Tetracyclines have the ability to concentrate in periodontal tissues and inhibit the growth of Actinobacillus actinomycetemcomitance. In addition, they exert an anticollagenase effect that can inhibit tissue destruction and may aid bone regeneration [6].

Trials both in Britain & USA showed that tetracycline rinses were significantly reduced both the frequency & severity of aphthae (herpetiform) [7] , while in mild cases of herpetic gigivostomatitis, topical tetracycline suspension rinsed round the mouth several times a day, relieves soreness & may enhance healing by controlling secondary infection[8]. In recent years saliva has attracted much attention, in particular among people interested in the determination of drug concentrations, who suggest that saliva might be a substitute for plasma in the areas of pharmacokinetic studies and drug monitoring. However, from this point of view we designed this research to estimate the tetracycline concentration in saliva by high performance liquid chromatography (HPLC). The HPLC method was applied to capsules to investigate the ability of using this method for evaluation of tetracycline concentration in saliva. However no similar research was conducted to evaluate tetracycline concentration in saliva till the time of writing this research.

Materials and methods

Reagents and Solutions:

All chemical reagents used in this study were either from Fluka or BDH companies suitable for HPLC analysis. ammonium oxalate, dimethylformamide, dibasic ammonium phosphate ammonium hydroxide and zinc sulfate were from BDH (England). Tetracycline 250 mg caps were obtained from Dar Al-Dawa development & investigation.

Weight variation, content uniformity, assay and dissolution studies were all carried out according to USP procedures [9].

Ten clinically healthy Iraqi males were used in this study. Their age ranged from 25-28 years. Each person was given 250 mg. of tetracycline caps every 6 hours for one day. Subjects were given orally a single dose of two caps (each 250mg) in a randomized fashion with 200 ml of water. Food and drinks (other than water), which were allowed after 2 hours 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, 24, 36 and 48 hrs after initial dosing. The samples were centrifuged at 3000 rpm for 15 min; plasma samples were separated and kept frozen at -4 ◦C in coded glass tubes.

Chromatographic Conditions:

A reversed phase HPLC method was developed to estimate salivary concentration of tetracycline. 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. Chromatographic separation was performed using a μ Bondapak phenyl (300 ×3.9 mm, Waters, Ireland) column. Mobile phase: Mix 680 mL of 0.1M ammonium oxalate, 270 mL of

dimethylformamide, and 50 mL of 0.2 M dibasic ammonium phosphate. Adjust, if necessary, with 3 N ammonium hydroxide or 3 N phosphoric acid to a pH of 7.7. The liquid chromatograph is equipped with a 280-nm detector, a 4.6-mm × 3-cm guard column that contains 10-µm packing L7, and a 4.6-mm × 25-cm analytical column that contains 5 µm packing L7. The flow rate is about 2 mL per minute [10].

Sample Preparation

For protein precipitation 100 µl of 10 % zinc sulfate was added to 300 µl of saliva in a 5 ml test tube. Samples were vortexed, placed in refrigerator for 15 min and centrifuged at 3000 rpm for 15 min. Supernatant layer was separated of which 20 µL was injected into the column and peak areas were recorded [1,11] The tetracycline capsules used in this study were supplied by Dar Al-Dawa development & investigation, the dose was two 250 mg. giving orally.

Results

The assays, dissolution and other USP parameters are listed in (table 1). The separation chromatogram of tetracycline is plotted in (Figure 1), in which the retention time of tetracycline was 8.2 min. This allows for the analysis of about 3 samples per h. the obtained chromatogram recorded abroad peak of tetracycline. The retention times as well as the area under the curves are listed in (table 2). The method is highly sensitive, with the lower limit of quantitation of tetracycline at 0.05 µg/ml. The calibration curve was linear in saliva with a regressions of r=0.99986. The concentrations of tetracycline in saliva were ranged from 0.08-0.23 µg/ml (table3). Despite the fact that many modern HPLC methods have been described for the assay of tetracycline there is justification for investigating newer methods, especially where they are more cost and there is an appreciable reduction in the procedural time compared to older methods. It could be applied to both research and therapeutic drug monitoring the pharmacokinetic parameters are shown in (table 4).

Discussion

Tetracycline are effective in treating periodontal diseases in part because their concentration in the gingival crevice is 2-10 times that in serum [12-14].This allows a high drug concentration to be delivered into periodontal pocket. In addition, several studies have demonstrated that tetracycline at a low gingival crevicular fluid concentration (2-4mg/ml) are very effective against many periodontal pathogens [15-17].

Graykowski and his co-workers found that a tetracycline mouthwash(250 mg per 5ml), used four times daily for 5-7 days, for patients with recurrent aphthous ulcers produced a good response in nearly 70% of the patients tested by reducing size , healing time and relieving pain[18].

In this study, the determination of tetracycline concentration in saliva was done but its therapeutic effect in oral cavity against certain lesions (for instance bacterial , viral vesiculo-bullous lesions and mucosal stomatitis) is unknown, so further study is needed to evaluate the effect of tetracycline against certain oral lesions through saliva.

Systemic tetracycline can eliminate tissue bacteria that cannot be removed mechanically by scaling, polishing and root planing, therefore it has been shown that tetracycline can arrest bone loss and suppress Actinobacillus

actinomycetemcomitance level in conjunction with scaling, polishing and root Planing [19-20].

Studies that have been done before were directed comprehensively about estimation of the concentration of tetracycline in serum and in gingival crevicular fluid so there is no much attention paid to estimate the concentration of tetracycline in the saliva which is considered to be an important subject while saliva is regarded as the first barrier of the immunity in the oral cavity. Saliva is also considered as one of the components of the periodontal pocket so that saliva may enrich the pocket with additional amount of tetracycline and this may be beneficial in reduction of pathogenic microorganisms which are sensitive to tetracycline.

Tetracycline was well detected in saliva of all volunteers. This method is very simple for monitoring of tetracycline concentration in saliva. The short time of analysis, simplicity, [21-23] and sufficient sensitivity makes the method particularly useful for pharmacokinetic and bioequivalent studies of tetracycline even following oral single dose (1 caps/6 hrs.). All products met the pharmacopeia specifications for weight variation, assay, and. dissolution profiles (table 1). The dissolution test revealed that 91% of stated tetracycline was released from Dar Al-Dawa development & investigation caps (n = 6). Therefore, either formulation met the USP dissolution specifications stating that not less than 80% of drug content should be released at dissolution time [9].

HPLC chromatograms represent tetracycline. Tetracycline can easily detected after 2-5 hours of ingestion of tetracycline capsules .All chromatograms were free from any interference at the retention times of tetracycline, and both compounds were eluted as completely and appeared as one separate resolved peak with peak tailing in such a way that it was possible to calculate peak height or peak area of standard curves. The retention times for tetracycline 8.2 min. A linear relationship was achieved between peak area and tetracycline concentration. The retention time, tetracycline standard and tetracycline concentration in saliva was similar .The tetracycline concentration in saliva was ranged from 0.08-0.23 µg/ml. The use of external tetracycline standard to increase the accuracy of the assay whose availability is an important issue in HPLC assays and avoid interferences with other internal standard such as Epianhydrotetracycline that could effect the tetracycline concentration. [25] In conclusion, a rapid, sophisticated and sensitive HPLC method is described for determination of tetracycline in human saliva. The collected results predicted efficient method for monitoring of tetracycline in saliva and monitoring efficiency of tetracycline for dentistry treatments. .

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Table 1. The USP parameters of tetracycline capsules.

No.	Test	No of caps	result
1	Wt. variation	20	2.6 %
2.	Disintegration time	6	4.66 min.
3.	Specification	6	Comply with USP
4.	Dissolution	6	91%
5.	Assay	6	102 %

Table 2. The chromatogram of tetracycline separation

Replicate	Peak area	Retention time(minutes)
1	26670420	8.22
2	26770554	8.21
3	25982575	8.16
Av.	26474516.333	8.20

Table 3. The tetracycline concentration in saliva

Patients number	Concentration (µg/ml)
1	0.08
2	0.19
3	0.11
4	0.23
5	0.12
6	0.22
7	0.21
8	0.11
9	0.20
10	0.18

Table 4: The pharmaco-kinetic parameters

Parameters	Test (mean ± SD)	P- values
C *max*. µg. /ml	0.23. ±0.004	0.223
AUC *0*-∞ (µg.h /ml)	202.25±10.25	0.825
T*max*.(30hrs.)	6.58±0.42	0.894
T *1/2* (hr.)	9.12±3.26	0.851

figure 1 the tetracycline separation chromatogram in saliva.

8.2 minutes