Scientific

Analysis of clarithromycin in dental pulp with and without inflammation

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Abstract

Objectives: To establish the possibility of measuring the presence of clarithromycin in dental pulp after oral administration, and to measure the concentrations achieved in healthy and inflamed pulp. **Methods and materials:** 72 samples were obtained from patients either requiring extraction of impacted teeth or with pulpal pathology. Following extraction, dental pulps were obtained and the concentration of clarithromycin measured, using high-performance liquid chromatography and mass spectrometry (HPLC-MS). In each case, the time elapsed after the last dose of antibiotic was recorded. **Results:** Measurements at different time intervals detected concentrations ranging between 0.3 and 1.3 ng/mg in healthy pulp tissue and between 0.24 and 4.4 ng/mg in inflamed tissue. **Discussion:** The minimum inhibitory concentration of clarithromycin is estimated to be 2 to 4 times lower than that of erythromycin, which is 0.001 µg/µl, thus the data obtained allow us to establish the activity of clarithromycin in dental pulp. **Conclusions:** Clarithromycin is detected in dental pulp by HPLC-MS, finding concentrations above the minimum inhibitory concentration in both healthy and inflamed pulps. **Clinical significance:** Clarithromycin is useful in the treatment of infections affecting dental pulp.

Keywords: Clarithromycin, dental pulp, pulp pathology.

Short title: Clarithromycin pulp concentrations.

Introduction

Dental pulp tissue responds to attacks by triggering an inflammatory reaction,[1] microbial infection being the main pulp irritant.[2]

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Due to the polymicrobial nature of pulpal and periapical infections, prescribing broad spectrum antibiotics is more appropriate, except in cases refractory to treatment. In these cases an antibiogram can be a more selective treatment against the infecting microorganism.[3,4]

With biomechanical preparation of root canals and the use of irrigants, reduction or elimination of microorganisms is attempted.[5] The use of antibiotics in root canal treatment is usually limited mainly to the prevention of the local spread and impact of hematogenous dissemination which may occur during instrumentation. For antibiotic prophylaxis, the guidelines of the American Heart Association recommend a dose of 2 g of amoxicillin for adults. In patients allergic to penicillin clindamycin,

Table 1 Clarithromycin concentrations in healthy and inflamed pulp.

	Hours after collection	Number of Observations	Mean (ng/mg)	Standard Deviation (ng/mg)
Healthy Pulp	1	1	1.300	-
	2	5	1.0720	1.0005
	4	3	0.2287	0.1472
	8	2	0.4450	0.1909
	12	7	0.2767	0.1644
	24	4	0.2250	0.1297
	36	2	0.3750	0.3041
Inflamed Pulp	2	3	0.3733	0.3630
	12	6	4.4000	4.7692
	24	1	0.3300	-
	72	1	0.2400	-

cephalosporins and the latest macrolides, such as clarithromycin, are recommended.[6]

We hypothesized that clarithromycin is able to reach dental pulp. Therefore, the objective of our study was to establish the concentration of clarithromycin in pulp.

Material and Methods

The samples (n=72) were obtained from 40 patients, 16 men and 24 women (age 18-48 years), through consecutive sampling at the Stomatology Service of the Universitary General Hospital de Valencia (Spain); 47 were used to assess clarithromycin levels and 25 to calibrate the technique (10 of these last samples did not contain clarithromycin). Inclusion criteria were: age 18-50 years, pulpal and/or periapical pathology and included teeth (in all cases, teeth indicated for extraction). Exclusion criteria were: liver or kidney disease; digestive diseases with malabsorption, gastroduodenal ulcer or gastritis, blood disorders and/or anticoagulant therapy; allergy to any medicine or food, pregnancy or lactation, drug or alcohol addiction, no fixed address; mental disorders, intake of any medication 72 hours before commencing the study. Once the study began, the following also became grounds for exclusion: non-completion of questionnaire, a request by the patient to leave the study or the appearance of any side effect. All participants were asked to give informed consent prior to the study. The study followed the regulations laid down in national law and the Declaration of Helsinki (Edinburgh revision, 2000). The study was authorized by the Ethics Committee for Clinical Research of the University General Hospital (Valencia, Spain).

After establishing the diagnosis and indication for tooth extraction, patients were treated, before surgery, with 250 mg of clarithromycin (Bremon®, Laboratorios PENSA

Esteve, Barcelona, Spain) every 12 hours for a minimum of four days. Clarithromycin (6-O-methyl-erythromycin A) is a semisynthetic macrolide derivative of erythromycin A, with a broad spectrum of antibacterial activity.[7,8] Pulps were obtained (inflamed or not) from the extracted teeth, which were systematically distributed into 8 subgroups according to the time elapsed after the last dose of antibiotic and time of sample collection (group 1: first hour after last administration, and successively to Group 8: eight hours after last administration). Pulps not treated with antibiotics, necessary for calibration of the analysis, were also collected. The pulps obtained were placed in Eppendorf tubes, weighed on an analytical balance (BP 210S, Sartorius, Goettingen, Germany) and frozen (Nuaire -85 ° C Ultraflow Freezer NU-6511E, Plymouth, MN, USA) at -85 ° C until analysis. Of the 72 samples collected, 47 were used for the measurement of clarithromycin and 22 to calibrate the technique.

Clarithromycin in the dental pulp was detected and measured by high-performance liquid chromatography in reverse phase, coupled to mass spectrometry with electrospray ionization at atmospheric pressure (HPLC-MS) (9). An HPLC Agilent 1100 Series (Agilent, Palo Alto, CA, USA), a Zorbax Eclipse XDB-C8 column (Agilent, Palo Alto, CA, USA) of 4.6 x 150 mm with a particle size of 5 µm was used. The mobile phase was a mixture of acetonitrile/formic acid 0.1% (70/30). The column temperature was 40 °C and the flow 0.5 ml/min. The retention time (rt) was 2.4 minutes. The EIC (extracted ion chromatography) mode was used with an m/z value of 748.5 using the MS Agilent 1100 Series LC/MSD SL (Agilent, Palo Alto, CA, USA).

Since the samples were weighed in mg on collection, and knowing that the extracts were redissolved in a final volume equal to 500 µl, the results expressed in ppb (pg/µl) were changed to ng/mg of clarithromycin in pulp.
A descriptive analysis of data obtained is presented.

Results

From the 47 selected specimens, 12 were invalidated for presenting values lower than 1 ppb (pg/µl). Of the 35 samples finally tested, 24 corresponded to healthy pulp, while 11 were from inflamed pulp. In both cases, clarithromycin was detected in the post-extraction time spans studied (Table 1).

Discussion

The presence of polymicrobial flora with obligate anaerobes has been described in orofacial infections,[10-12] meaning that the microbial spectrum of oral infections is restricted, which in turn implies that of the hundreds of antibiotics available, only a few are sufficient to meet the needs arising from bacterial infections in dentistry.

The same protocol used to prevent bacterial endocarditis can be applied to reduce bacteremia subsequent to instrumentation in canals with symptomatic infection;[13] in this regard, instrumentation of root canals in a coronal-apical direction reduces the risk extrusion of necrotic tissue and bacteria toward the periapex,[14,15] significantly reducing bacteremia.

Because antibiotics are distributed around the body in a non-homogeneous way, the concentrations in plasma do not always accurately predict the concentration at the site of infection. Hence the importance of conducting research that, through in situ sampling, can determine the tissue concentrations of antibiotic reached using normal clinical doses. Although several studies have been made on what is called tissue pharmacokinetics,[16,17] this field can be considered to be still in the development phase, especially taking into account the more-recently introduced antibiotics, for which there is little information in this regard.

In our study, the maximum weight of the samples did not exceed 85 mg. Our quantification limit was 3 pg/µl similar to 2,95 obtained inthe study by van Rooyen et al.[9] for the determination of clarithromycin in human plasma, while for Lerner et al.[18] in their study of bioequivalence of clarithromycin in healthy volunteers it was 5 ng/ml (pg/µl), and for Benninger et al.[19] 11 ng/ml (pg/µl).

Macrolide antibiotics are a large group of antibiotics, derived mainly from Streptomyces spp., which have a common macrocyclic lactone ring to which one or more sugars are joined. All are weak bases slightly soluble in water; their properties are very similar and they generally have a low toxicity and a spectrum of antimicrobial activity with cross-resistance among members of the group. Macrolides are bacteriostatic or bactericidal depending on the concentration and type of microorganism, and act by interfering with bacterial protein synthesis. The antimicrobial spectrum is similar to the penicillins, but they are also active against other organisms such as Legionella pneumophila, Mycoplasma pneumoniae and some rickettsia and chlamydia.[20-23] Clarithromycin has a similar antibacterial spectrum to erythromycin, except that it has a stronger action against anaerobic bacteria.

The ability of a drug to achieve high concentrations, above the minimum inhibitory concentration for the infecting organism at the site of infection is very important, not only for their clinical efficacy, but also for the prevention of resistance.[24] In the case of clarithromycin, this concentration is not fully established,[25] although it is considered to be two to four times lower than that of

erythromycin.[26] The minimum inhibitory concentration of erythromycin may be as low as 0.001µg/µl (1pg/µl) for sensitive strains of S. Pneumoniae and Mycoplasma pneumoniae, while the minimum inhibitory concentration for these and other microorganisms more sensitive to erythromycin range from 0.01 to 0.25 µg/µl. In general terms, antibiotics with minimum inhibitory concentrations of up to 0.5 µg/µl are considered sensitive, and those between 0.5 and 2 µg/µl, moderately sensitive.[8] Macrolides (and related drugs such as lincosamides and streptogramin) have a consistent postantibiotic effect, in that the antibacterial activity continues after the concentration falls below the minimum inhibitory concentration,[8] then, levels of clarithromycine measured in our study are higher than it minimum inhibitory concentration. Macrolide antibiotics (erythromycin and clarithromycin), beta-lactams (penicillins, cephalosporins) and clindamycin belong to a group of time-dependent antimicrobials (as opposed to those that are concentration-dependent) and are characterized by maintaining slightly higher concentrations than the minimum inhibitory for as long as possible. In this group of antibiotics, concentrations 4-5 times higher than the minimum inhibitory are not generally translated into a greater bactericidal effect. Therefore, it is important to administer these antibiotics according to the pharmacodynamic characteristics, in relation to both dosage and the number of doses.[27]

Conclusions

With the methodology used, it has been possible to detect clarithromycin in both healthy and inflamed pulp. The established dosage for clarithromycin allowed effective therapeutic concentrations to be achieved in inflamed dental pulp. The drug concentrations were maintained over time, but with a downward trend in both healthy and inflamed pulp tissue samples.

Although other factors (age, medical history, previous pathologies) should be taken into account in determining the clinical use of clarithromycin in odontogenic infections, the present study confirms that clarithromycin has valid characteristics for use in infectious oral disease, although additional clinical and pharmacological studies are advisable to better define the clinical use of this antibiotic.

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