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Penetration of moxifloxacin into rat mandibular bone and soft tissue

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Abstract

Objective. Based on its *in vitro* activity and spectrum of activity, the new 8-methoxyquinolone antibiotic moxifloxacin (MXF) seems suited for the antibiotic therapy of odontogenic infections. Penetration into the relevant tissue is another prerequisite for clinical efficacy. For this reason, the levels of MXF in plasma, soft tissue, and mandibular bone were determined in an animal model with Wistar rats. ***Material and methods.*** Samples of 49 rats were analyzed. Tissue samples were homogenized and proteins were precipitated. The pharmacokinetic evaluation was conducted based on non-compartmental analysis. ***Results.*** The concentration-time courses of tissues show a more plateau-shaped curve compared to plasma. Calculated AUC (area under the curve) ratios tissue:plasma were *M. masseter*:plasma = 2.64 and mandibles:plasma = 1.13. ***Conclusions.*** Administration of antibiotics is considered an important part of therapy during and/or after surgical procedures in the maxillofacial area. Because of the good penetration into bone and muscle tissues demonstrated in Wistar rats, MXF might be an option for clinical application in this indication.

Key Words: *Animal study, antibiotics, odontogenic infections, plasma and tissue concentrations*

Introduction

Odontogenic infections are bacterial diseases that result from necrotic pulps of teeth or invade the surrounding tissues via the periodontal margin. These infections penetrate soft tissues of the maxillofacial region and the alveolar bone causing submucosal infiltrates and abscesses. They are caused by a polymicrobial flora of aerobic, facultatively anaerobic, and anaerobic bacteria [1–4]. Bone infections are difficult to treat, therapeutic success depending greatly on the diffusion of antibiotics into the bone tissue.

Moxifloxacin (MXF) is an 8-methoxyquinolone antibiotic with a broad antibacterial spectrum that is *in vitro* effective against these pathogens. The antimicrobial activity depends on inhibition of DNA gyrase and there is a marked post-antibiotic effect [5]. It has also been shown to penetrate extravascular tissue effectively [6]. MXF has good *in vitro* activity against odontogenic pathogens compared with the activities usually administered [3] and also high efficacy in biofilms [7]. MXF has promising pharmacokinetic and pharmacodynamic properties [8–10], but no pharmacokinetic data were available for the orofacial region before this investigation. There are a few publications on the adjunctive [11], preventive usage of MXF [12] and a clinical pilot study on severe odontogenic abscesses [13]. The degree of penetration of an antibiotic into the infection site, particularly in bone infections, is an important factor in its therapeutic efficacy. The aim of this experimental study in Wistar rats was to determine the levels of MXF in mandibular bone, surrounding soft tissue (*M. masseter*), and plasma, and to investigate its potential suitability in the treatment of odontogenic infections.

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Material and methods

Animals

Male albino Hsd/Ola Wistar rats (aged approximately 6 weeks) weighing 190–300 g were housed under standard conditions of light and dark cycle with free access to food and water (Aytekinler, Konya, Turkey) during the study. The animals were obtained from the medical experimental research institute DETAM (Istanbul, Turkey). All experimental procedures were in accordance with the European Community Guidelines for the care and use of laboratory animals. The protocol was approved by the Ethics Committee of the Faculty of Medicine, University of Istanbul (Permission No. 102/18 September 2008).

Animal preparation and sample extraction

A total of 42 rats were divided by simple randomization (random number table) to 6 groups, each consisting of 7 animals for the 7 time-points (6 MFX groups, 1 control). A dose of 10 mg/kg MXF dissolved in water was administered orally per gavage. The animals were anesthetized with ketamine hydrochloride (10 mg/kg, i.m.; Ketalar, Pfizer, Istanbul, Turkey). The abdominal region was shaved and sterilized with iodine solution (Betaisodona, Mundipharma GmbH, Limburg (Lahn), Germany). A midline incision was made and the superior caval vein was punctured. Blood samples of $n=6$ MXF dosed animals per time-point were obtained 0.5 h, 1 h, 1.5 h, 2 h, 4 h, 8 h, and 24 h, respectively, after administration. Additionally, samples of 6 animals from the control group (just vehicles, no drugs) were obtained; 2 ml blood was collected into heparinized syringes (BD Vacutainer; Becton Dickinson & Co, N.J., USA). The anesthetized animals were killed by exsanguination for collection of tissues. The mandibles and *M. masseter* were removed, weighed using an analytical balance (Shimadzu UX 8200 S; Shimadzu Co Ltd., Kyoto, Japan), and deep frozen at -20°C. The mandibles and *M. masseter* with an average weight of 60 mg were prepared at a fixed ratio of four parts bone to three parts *M. masseter*. Saline 0.9% (Baxter S.A., Unterschleissheim, Germany) was added and the exact volume pipetted by preparing 200 µl of calibration probes. For this reason, the exact amount was not relevant. Plasma was obtained by centrifugation (Mikro 12–14 Hettich Zentrifugen, Tuttlingen, Germany) of the samples and stored below -15°C before further analysis. The MXF concentrations in tissue homogenate and plasma were determined by high performance liquid chromatography (HPLC) and fluorescence detection [14–16]. After the addition of 10 µl of an internal standard (BAY y 5658; Bayer AG, Germany) to 0.2 ml plasma or tissue homogenate, 0.2 ml acetonitile (Merck No. 30, Darmstadt, Germany) was added. Precipitated matrix proteins were removed by centrifugation (10 min at 1550*g*). The supernatant was diluted 4-fold with 0.067 mol/l disodium hydrogen phosphate buffer, pH 7.5 (Merck No. 6587, Darmstadt, Germany) and transferred to HPLC autosampler vials (Latek Labortechnik, Eppelheim, Germany). The HPLC separation was performed using a Hewlett Packard 1090A high performance liquid chromatograph with HP ChemStation software (Hewlett Packard Co., Düsseldorf, Germany) and a Jasco Model FP 920 S fluorescence detector (Jasco, Labor- und Datentechnik GmbH, Gross-Umstadt, Germany).

Data analysis

Pharmacokinetic evaluation was performed by non-compartmental analysis using validated software KINCALC version 2.50.2 (Bayer AG, 2001, Leverkusen, Germany). The areas under the concentration-time curves (AUC) were calculated on the basis of mixed linear-logarithmic trapezoidal rule.

Evaluated pharmacokinetic parameters were:

AUC (0–8 h) [µg·h/l]	area under the plasma concentration-time curve from 0 to 8 h
C_{max} [µg/l]	peak concentration (maximum plasma concentration)
t_{max} [h]	time to C_{max}
C_{max}/C_{8h} [%]	peak/trough ratio
$t_{1/2}$	rough estimation of elimination half-life

Results

Figure 1 shows the concentration-time course in different matrices. MXF concentrations above the lower limit of quantitation (LLOQ) were measured from 0.5 h up to 8 h after administration. The pharmacokinetic parameters following oral MXF administration are given in Tables I and II.

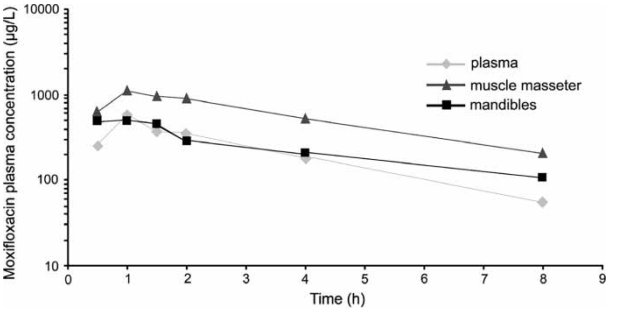

Figure 1. Concentration time course in plasma, *M. masseter* and mandibles at different intervals.

Table I. Mean concentrations in different matrices [μg/L or μg/kg] and pharmacokinetic parameters of moxifloxacin (10 mg/kg) after oral administration to male rats

		Plasma		*M. masseter*		Mandibles	
Time [h]	Matrix	Mean geom.	SD geom.	Mean geom.	SD geom.	Mean geom.	SD geom.
0.5		256	1.59	636	1.27	480	2.67
1		554	1.34	1098	1.71	504	1.53
1.5		380	1.66	956	1.49	450	1.59
2		348	1.74	902	1.49	289	1.40
4		188	1.62	520	1.57	200	1.58
8		56.2	1.39	211	1.34	104	1.67
24		<5.00	n.c.	n.c.	n.c.	<25.0	n.c.
Pharmacokinetic parameter calculated from geometric means							
AUC (0–8)	[μg·h/L, μg·h/kg]	1636		4325		1856	
C_{max}	[μg/L, μg/kg]	554		1098		504	
t_{max}	[h]	1.00		1.00		1.00	
C_{max}/C_{8h}	[%]	10.1		19.2		20.6	
$t_{1/2}$	[h]	2.28		2.89		4.08	

n.c. = not calculated.
Mean geom. = geometric mean.
SD geom. = geometric standard deviation, the 1s interval is represented by geom. mean/SD to geom. mean *SD.
*Regression to determine $t_{1/2}$.
Pharmacokinetic parameters for muscle and bone are given "per kg" instead of "per liter".

In 2 out of 17 samples of the control group, a MXF concentration close to the LLOQ (20 and 25 μg/l) was found. These concentrations are most likely due to a slight contamination of the samples and are not relevant for further evaluation of the results in Table I. The exposure in terms of AUC (0–8 h) amounted to 1636 μg·h/l (plasma), 1856 μg·h/kg (mandibles), and 4325 μg·h/kg (*M. masseter*). Calculated AUC ratios tissue:plasma were *M. masseter*:plasma = 2.64 and mandibles:plasma = 1.13.

Discussion

Odontogenic infections penetrate soft tissues of the maxillofacial region and the alveolar bone causing submucosal infiltrates and abscesses. They have various possible sources, including necrosis of dental pulp, marginal periodontitis, and pericoronitis [17]. Odontogenic infections can also occur due to dental extractions [12]. These infections are generally localized, but may proceed into medullary spaces or via fascial planes into deeper soft tissues, producing complex multi-space infections up to osteomyelitis [18]. Infections involving the lateral pharyngeal and retropharyngeal spaces are very serious and potentially life-threatening [19,20]. Antibiotics are indicated when there are clear signs of systemic involvement [17,21]. The clinical signs of systemic involvement are pyrexia, lymphadenopathy, difficulty in swallowing, and lockjaw. In a review of 141 cases of

Table II. Plasma concentrations [μg/l] and concentration ratios of moxifloxacin (10 mg/kg) after oral administration to male rats

Time [h]	Matrix	Concentration per rat [μg/L]					
0.5	Ratio	1.13	1.95	n.c.	3.16	5.52	n.c.
1	*M. masseter*	2.76	3.01	3.11	1.38	1.13	1.50
1.5	vs. Plasma	2.36	3.50	2.14	2.75	1.95	2.67
2		2.30	2.67	3.09	1.79	3.43	2.61
4		2.77	2.21	2.89	2.97	2.08	4.03
8		3.91	6.70	3.47	2.86	3.35	3.20
24		n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
						ari. mean all time-points	
0.5	Ratio	0.629	0.883	n.c.	n.c.	7.21	n.c.
1	*Mandible*	1.11	1.41	1.18	0.729	0.583	0.730
1.5	vs. Plasma	1.04	1.15	1.29	1.29	0.949	1.46
2		1.10	0.723	0.948	0.508	0.858	1.00
4		1.07	1.04	1.19	1.06	0.961	1.04
8		1.80	4.98	1.99	1.63	1.30	1.05
24		n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
						ari. mean all time-points	

n.c. = not calculated.
ari = arithmetic.
[ ] = not used, regarded as outlier.

jaw osteomyelitis, odontogenic infections were found to be the cause of 38% of mandibular and 25% of maxillary involvement [22]. Antimicrobial therapy is indicated when infection has perforated the cortex and has spread into the surrounding tissue [18]. There is also an indication in the case of endocardical prophylaxis, e.g. in prevention of bacteremia following dental extractions [12]. However, if infection develops in bone and in soft tissue, good antibiotic penetration is required. Bone is a vascular structure capable of responding to changes in the systemic circulation. Antibiotics in the systemic circulation reach the capillaries in bone and, depending on the chemical profile and molecular size, pass through the capillary walls to enter the fluid space.

Tissue penetration and distribution of antibiotics are important issues when establishing antibiotic therapies [23,24]. Free concentrations of antibiotics at the infection site are responsible for a bactericidal effect. Knowledge of the correlation between plasma levels and tissue concentrations is helpful when choosing the adequate drug and eventually the right dose. Based on *in vitro* activity, MXF seems suited for the therapy of odontogenic infections [3,25]. MXF pharmacokinetics have been studied mainly in the setting of respiratory tract infections [26]. The aim of this study, therefore, was to investigate MXF pharmacokinetics of orofacial structures in rats to predict whether this might be an option for clinical application in this indication.

The tissue concentrations in this study follow the plasma levels. Only in the elimination phase do tissue concentrations seem to decrease slightly more slowly than plasma concentrations (Figure 1). These data show a good penetration into muscle and bone tissue with enrichment in muscle tissue and approximately the same exposure in the alveolar bone. The standard antibiotic in odontogenic infections, clindamycin, has been shown in animal mandibular bone to reach three times the serum concentration after intravenous infusion [27]; morselized cancellous bone can act as a carrier of this drug [28]. Clindamycin has been reported to give high bone concentrations [29,30] and is regarded by some authors as a first-line antimicrobial in the treatment of odontogenic infections [17,20]. Clindamycin has been proposed by different authors as an alternative regime for patients with penicillin allergies or when penicillin therapy has failed [31]. The antibiotics most commonly prescribed by dentists in managing odontogenic infections are penicillin and amoxicillin [32]. Despite lower bone concentrations [24,30], penicillins remain effective against major pathogens and are still the drugs of choice in uncomplicated odontogenic infections [33–35]. There is no difference in susceptibility regarding amoxicillin/clavulanic acid and clindamycin [36]. In addition to its anti-infective properties, MXF has a high bioavailability [3] comparable to clindamycin, a good bone and tissue penetration [10–12,37], and a favorable safety profile [38]. When the bone is affected, amoxicillin/clavulanic acid, clindamycin, metronidazole, or fluoroquinolones (levofloxacin) are recommended [18,36]. Fluoroquinolones penetrate the tissues and cells very rapidly after administration and often achieve high tissue concentrations [39].

The tissue:plasma ratios calculated from the intraindividual concentrations and from the integral parameter AUC reveal a good penetration of MXF into muscle and bone tissue of Wistar rats. Similar ratios between plasma and various body tissues can be expected in clinical studies.

In rats, bone penetration of MXF is at least as good as that of clindamycin [24,27]. The predictivity of rats pharmacokinetics to humans is supported by a good allometric correlation of the clearance in rats and humans [15]. Assuming pharmacokinetics in this animal experiment are predictive for the human situation, MXF seems suited for the antibiotic therapy of odontogenic infections. Based on the experimental data, controlled studies have to evaluate the clinical efficacy of MXF compared to other antibiotics.

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