

## Concentration of moxifloxacin in plasma and tonsillar tissue after multiple administration in adult patients

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**Objectives:** The antibacterial spectrum of moxifloxacin includes all the major respiratory pathogens, and its pharmacokinetics demonstrate high peak concentrations in plasma as well as at respiratory sites. Nevertheless, tonsillar tissue concentrations have never been investigated. In this study we determined the moxifloxacin concentrations in plasma and tonsillar tissue after the administration of three doses of moxifloxacin 400 mg to adult patients with chronic or recurrent tonsillitis undergoing tonsillectomy.

**Methods:** This was an uncontrolled, open-label, randomized, parallel group study including 35 patients assigned randomly to five groups of 7 patients each, depending on the time between the last dose of moxifloxacin and plasma and tissue sampling. Moxifloxacin was given orally once daily for 3 days; its concentrations were measured using a validated HPLC assay and fluorescence detection. Each sample was analysed twice and the mean value obtained used for the statistical analysis. Pharmacokinetic data were analysed by presenting descriptive statistics of moxifloxacin concentrations in plasma and tonsillar tissue.

**Results:**  $C_{max}$  occurred at 3 h in tonsillar tissue (mean 8.96 mg/L) and in plasma (mean 3.20 mg/L), the tissue/plasma concentration ratios (mean values) being constantly >2, ranging between 2.37 (after 2 h) and 2.93 (after 24 h), which indicates a prolonged maintenance of moxifloxacin concentration in tonsillar tissue compared with plasma. Variability among patients was present at 6 h, with the tonsillar tissue/plasma concentration ratio ranging between 0.8 and 3.4.

**Conclusions:** Moxifloxacin achieves a good penetration in tonsillar tissue, which compares favourably with that reported for other fluoroquinolones. The moxifloxacin concentrations we observed exceed the MICs for the usual respiratory tract pathogens.

Keywords: pharmacokinetics, fluoroquinolones, otorhinolaryngology

### Introduction

The primary objective of this study was to assess moxifloxacin concentrations in tonsillar tissue in comparison with those in plasma up to 24 h after three oral doses of 400 mg to adult patients with chronic or recurrent tonsillitis undergoing tonsillectomy.

### Patients and methods

This was an uncontrolled, open-label, randomized, parallel group multicentre study. The study protocol and the informed consent form were approved by an appropriate ethics committee.

A total of 35 patients undergoing tonsillectomy, meeting the inclusion/exclusion criteria, were randomly assigned to five groups

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(7 patients in each group), depending on the time between the last dose of moxifloxacin and the time of tissue sampling: group A, 2 h  $\pm$  30 min; group B, 3 h  $\pm$  30 min; group C, 6  $\pm$  1 h; group D, 12  $\pm$  2 h; group E, 24  $\pm$  3 h. The inclusion criteria were as follows: patients of both sexes between 18 and 65 years of age, body weight 40–85 kg, and patients willing and able to provide their informed consent. The exclusion criteria were as follows: hypersensitivity to quinolone derivatives, pregnancy or lactation, history of tendinopathy associated with fluoroquinolone therapy, syndromes of QTc prolongation, renal or hepatic impairment, concomitant therapy with antacids and sucralfate, and history of convulsions.

Eligible patients were randomized to one of the groups (2, 3, 6, 12 and 24 h) and instructed to take the study drug prior to surgery at a time dependent on the group to which they were assigned.

Moxifloxacin 400 mg was administered orally, as perioperative prophylaxis, once daily for 3 days, at the same time each day, with water, before or with food. The real time of initiation of treatment depended on the study group to which the patient was assigned.

Moxifloxacin was supplied by Bayer S.p.A. as 400 mg film-coated tablets, packaged in bottles labelled with the study number, batch number, expiry date and a statement indicating that it was an investigational drug.

Patients were randomized according to a predefined block randomization list generated at Bayer Vital (Germany).

For the samples, 5 mL of venous blood was collected in 5 mL ammonium lithium tubes, cooled (4°C) and centrifuged for 5 min at  $\sim$ 5000 g within 2 h from collection. Plasma was pipetted into appropriately labelled polypropylene sample tubes and stored at  $-80^{\circ}\text{C}$  until the time of the HPLC analysis, which was performed within 2 months.

Tonsillar tissue samples, removed during surgery from one tonsil, were rinsed with sterile saline solution and frozen in sealed stoppered glass tubes at  $-80^{\circ}\text{C}$  until the time of the analysis, which was performed within 2 months.

The plasma and tonsillar tissue concentrations of moxifloxacin were determined using a validated HPLC assay and fluorescence detection.<sup>1–3</sup>

After addition of 10  $\mu\text{L}$  of internal standard (ofloxacin) to 0.25 mL of plasma, 0.25 mL of acetonitrile was added. Plasma proteins were precipitated by shaking in an ultrasonic bath (Eurosonic 22), followed by centrifugation for 10 min at 1500 g (Labofuge 6000, Hereus Christ). The supernatant was then filtered on filter paper, diluted 3-fold with 0.067 M disodium hydrogen phosphate buffer (pH 7.5) and injected onto the column (50  $\mu\text{L}$ ). Every sample was extracted and tested twice. A 15% difference between the two analyses of each sample was regarded as the threshold for repeating the analysis of the same sample.

Tissue specimens were weighed, diluted in sterile normal saline (1:1, w/v), homogenized at 4°C (Polytron PT 10-35 Homogenizer, Kinematica, Lucerne, Switzerland) and centrifuged at 1200 g for 10 min, and the supernatant was stored at  $-80^{\circ}\text{C}$  until the HPLC analysis. The supernatant was then processed in the same manner as plasma samples.<sup>3,4</sup>

The calibration curve was plotted by spiking blank plasma into aqueous phosphate buffer (pH 7.5) with nine different concentrations in the range 5–1500 mg/L.

Quality control (QC) samples were prepared at three concentrations, covering the whole range expected for the unknown samples. QC samples were stored together with samples of the respective study at  $-80^{\circ}\text{C}$ . Two replicates of each QC sample were analysed together with calibration and unknown samples in the same analytical sequence. Calibration samples were freshly prepared prior to each sequence.

An HPLC model 2700 instrument (Bio-Rad) equipped with a fluorescence detector Series 200 FL Detector (Perkin Elmer) was used. The fluorescence detection was performed at an excitation wavelength of 296 nm and an emission wavelength of 504 nm for all samples. The sampler temperature was kept at 8°C. A Nucleosil 100 C<sub>18</sub> (5  $\mu\text{m}$  particle size, 250  $\times$  4.6 mm I.D.; Alltech, Lokeren, Belgium) capillary column preceded by a guard column (Nova Pak C<sub>18</sub>) was used for separation. The column was set at room temperature.

The mobile phase consisted of a solution of tetrabutylammonium hydrogensulphate (Sigma) (pH 3.0) in Milli-Q Plus water (10 g/L) (Millipore) with a gradient from 60% (0 min) to 0% within 7 min (pump A) and tetrabutylammonium hydrogensulphate and acetonitrile (Merck) 50:50 with a gradient from 40% (0 min) to 100% within 7 min (pump B) and a flow rate of 1.0 mL/min. Moxifloxacin and the internal standard were eluted at  $\sim$ 7 and 4 min, respectively.

Validation was performed according to the guidelines for development of bioanalytical assays in human biomatrices.<sup>3</sup>

Pharmacokinetic data were analysed by presenting descriptive statistics of plasma and tonsillar concentrations. Statistics were arithmetic mean, standard deviation, minimum value, maximum value and number of measurements.

The ratio between tonsillar and plasma concentration was computed for each patient and was analysed descriptively.

## Results

Patient enrolment was started on 16 November 2001, and the last visit was on 9 May 2002. A total of 35 patients were randomized from three centres (7 patients in each group): 33 (94%) completed the study and 2 (6%) terminated prematurely, one for adverse event and one for protocol violation.

Four more patients were excluded from the per protocol analysis for the following reasons: serum alanine transaminase and aspartate transaminase values outside the range of acceptance for including patients in the study (one patient); collection time of kinetic samples outside the planned window (one patient); and inadequate tonsil sample (two patients). In the end, 29 patients were included in the per protocol analysis.

Demographic and baseline characteristics appeared to be well balanced among time groups (Table 1).

Results in terms of concentration in plasma and in tonsillar tissue and the tissue/plasma concentration ratio are reported in Table 2.

All the collection times showed higher mean tissue concentrations of moxifloxacin than plasma mean concentrations. The mean ratio was on average always  $>2$  (range: 2.37–2.93). A peak in the ratio mean was evident corresponding to the third hour, the estimated peak for both plasma and tissue concentration curves.

## Discussion

This was a non-controlled, open-label, randomized, parallel group multicentre trial, primarily aimed at assessing the moxifloxacin concentrations in tonsillar tissue in comparison with those of plasma up to 24 h after three oral doses of 400 mg, performed in adult patients with chronic or recurrent tonsillitis undergoing tonsillectomy.

As far as the tissue/plasma concentration ratio is concerned, the results were fully consistent with data previously obtained in

## Plasma and tonsil pharmacokinetics of moxifloxacin

**Table 1.** Demographic and baseline characteristics (population meeting criteria for per protocol analysis)

	2 h	3 h	6 h	12 h	24 h	Total
Sex						
male	3	5	3	3	2	16
female	2	1	4	2	4	13
Age, years						
mean (SD)	25.6 (5.5)	27.2 (5.7)	26.3 (7.5)	25.5 (2.8)	29.4 (14.4)	26.8 (7.9)
min-max	20-34	19-34	18-40	21-29	19-58	18-58
Weight, kg						
mean (SD)	66.4 (10.5)	67.8 (13.3)	71.9 (12.3)	66.4 (11.4)	66.5 (10.3)	68.0 (11.1)
min-max	55-80	50-88	54-85	56-82	58-82	50-88
BMI						
mean (SD)	21.4 (2.2)	23.1 (3.1)	25.9 (5.7)	21.1 (2.7)	23.8 (2.0)	23.3 (3.8)
min-max	18.6-23.9	20.8-28.4	20.3-34.5	18.5-24.0	20.8-26.9	18.5-34.5

BMI, body mass index.

**Table 2.** Plasma and tonsillar tissue concentrations (mg/L) and tissue/plasma concentration ratio (population meeting criteria for per protocol analysis)

	2 h ± 30 min	3 h ± 30 min	6 ± 1 h	12 ± 2 h	24 ± 3 h
Plasma, mg/L					
mean (SD)	2.67 (1.26)	3.20 (0.86)	2.49 (1.24)	1.68 (0.20)	0.68 (0.26)
min-max	0.4-3.4	2.0-4.3	0.3-3.9	1.4-2.0	0.5-1.2
Tissue, mg/L					
mean (SD)	6.06 (2.86)	8.96 (1.47)	5.44 (3.16)	4.27 (1.01)	1.97 (0.68)
min-max	1.3-8.9	6.4-10.8	0.9-10.8	2.9-5.7	1.4-3.3
Ratio tissue:plasma					
mean (SD)	2.37 (0.36)	2.89 (0.50)	2.41 (0.80)	2.56 (0.56)	2.93 (0.27)
min-max	2.0-2.8	2.3-3.6	0.8-3.4	1.6-3.0	2.5-3.3

other tissues of the respiratory system.<sup>4,5</sup> In particular, tissue concentrations were on average 2- or 3-fold higher than plasma concentrations.

The time profile in tissue seemed to be very similar to that in plasma. In particular, the peak concentration in the tissue occurred at the same time (hour 3) as in the plasma, with a ratio of ~3 (2.89) in favour of the tissue mean.

After hour 3, the ratio seemed to increase, starting linearly from a value similar to that of hour 2, indicating a prolonged maintenance of moxifloxacin concentrations in the tissue compared with the plasma. However, a large variability among patients was present at hour 6, where the ratio ranged between 0.8 and 3.4. For the other collection times, the ratio was between 1.6 and 3.6.

The tissue concentration at 24 h was >1 mg/L in all patients.

These concentrations, for a period of 12-24 h following administration, were more than 10-fold higher than the MIC<sub>90</sub> values for a wide range of causative microorganisms, including atypical pathogens.<sup>6</sup>

Thus, moxifloxacin achieves a good penetration in tonsillar tissue, which compares favourably with the following tonsil/plasma mean concentration ratios reported for other fluoroquinolones: 1.5-1.9 for ciprofloxacin, from 1 to 8 h after oral or intravenous doses of 200-500 mg; 2.02-2.08 for levofloxacin, from 1 to 9 h after single oral doses of 100 or 200 mg; and 1.4 for

ofloxacin, 2 h after a single administration of an oral 200 mg dose.<sup>7,8</sup>

Taking into account the failure of penicillin to penetrate the tonsillar tissue, the possible internalization of *Streptococcus pyogenes* in the tonsillar crypts, the increasing macrolide resistance among streptococcal strains and the changing epidemiology of pharyngotonsillitis aetiology, now including *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*, the results of our study showing a high tonsillar concentration of moxifloxacin appear, in our opinion, of some interest.<sup>9,10</sup>

### Transparency declarations

None to declare.

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