Concentrations of Gatifloxacin in Plasma and Urine and Penetration into Prostatic and Seminal Fluid, Ejaculate, and Sperm Cells after Single Oral Administrations of 400 Milligrams to Volunteers

CHRISTOPH K. NABER,1 MICHAELA STEGHAFNER,2 MARTINA KINZIG-SCHIPPERS,3 CHRISTIAN SAUBER,3 FRITZ SÖRGEL,3 HANS-JÜRGEN STAHLBERG,4 AND KURT G. NABER2*

Department of Pharmacology, University of Essen, Essen, 1 Department of Urology, Hospital St. Elisabeth, Straubing, 2 IBMP, Institute for Biomedical and Pharmaceutical Research, Nürnberg-Heroldsberg, 3 and Grünenthal GmbH, Clinical Research Anti-Infectives, Aachen, 4 Germany

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Gatifloxacin (GTX), a new fluoroquinolone with extended antibacterial activity, is an interesting candidate for the treatment of chronic bacterial prostatitis (CBP). Besides the antibacterial spectrum, the concentrations in the target tissues and fluids are crucial for the treatment of CBP. Thus, it was of interest to investigate its penetration into prostatic and seminal fluid. GTX concentrations in plasma, urine, ejaculate, prostatic and seminal fluid, and sperm cells were determined by a high-performance liquid chromatography method after oral intake of a single 400-mg dose in 10 male Caucasian volunteers in the fasting state. Simultaneous application of the renal contrast agent iohexol was used to estimate the maximal possible contamination of ejaculate and prostatic and seminal fluid by urine. GTX was well tolerated. The means (standard deviations) for the following parameters were as indicated: time to maximum concentration of drug in serum, 1.66 (0.91) h; maximum concentration of drug in serum, 2.90 (0.39) μg/ml; area under the concentration-time curve from 0 to 24 h, 25.65 μg · h/ml; and half life, 7.2 (0.90) h. Within 12 h about 50% of the drug was excreted unchanged into the urine. The mean renal clearance was 169 ml/min. The gatifloxacin concentrations in ejaculate, seminal fluid, and prostatic fluid were in the range of the corresponding plasma concentrations which were 1.92 (0.27) μg/ml at approximately the same time point (4 h after drug intake). The concentrations in sperm cells (0.195, 0.076, and 0.011 μg/ml) could be determined in three subjects. The good penetration into prostatic and seminal fluid, the good tolerance, and the previously reported broad antibacterial spectrum suggest that GTX may be a good alternative for the treatment of chronic bacterial prostatitis. Clinical studies should be performed to confirm this assumption.

Fluoroquinolones have already been used successfully in the treatment of chronic bacterial prostatitis (CBP) and are recommended as first-line treatment for this indication (1, 6). This recommendation is based on their antibacterial activity; on their ability to penetrate into prostatic tissue, prostatic fluid, seminal fluid, and ejaculate; and on clinical studies (6). Although in about 60% of patients with symptoms of chronic prostatitis significant prostatic inflammation can be demonstrated (4), an etiologically recognized pathogen, such as Escherichia coli, Klebsiella spp., Proteus spp., Enterococcus faecalis, or Pseudomonas aeruginosa, is only isolated in up to 10% of these patients (14). In the vast majority of patients, bacterial evaluation either fails to identify a pathogen (nonbacterial prostatitis), or identifies so-called atypical bacteria, like Mycoplasma spp., Ureaplasma spp., and Chlamydia spp. These atypical pathogens are, however, not well covered by the antibacterial activity of the classical fluoroquinolones, e.g., ciprofloxacin or ofloxacin. Thus, the treatment of CBP remains a challenging issue, and new fluoroquinolones with improved antibacterial activity also against gram-positive pathogens and Mycoplasma and Chlamydia species, as well as against anaerobes, may be considered for the treatment of CBP.

Gatifloxacin, a new fluoroquinolone antibiotic, has a broad spectrum of activity encompassing both gram-positive and gram-negative organisms, as well as anaerobes (2). It also has activity against Mycoplasma and Chlamydia spp. (5). Since the antibacterial spectrum and the concentrations in the target tissues are crucial for the treatment of CBP, it was of interest to investigate its penetration into prostatic and seminal fluid. The results of this study could serve as a basis for a clinical study protocol (dosage selection and estimate of clinical and bacteriological efficacy) to test gatifloxacin in the treatment of CBP and vesiculitis.

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MATERIALS AND METHODS

Study design. This was a single-dose, one-way, open-labeled, noncontrolled, single-center, phase 1 study. The study was approved by the institutional and local ethics committees, and written informed consent was obtained from each volunteer.

Study subjects. Ten male Caucasian volunteers, 18 to 33 years old (mean age, 23 years) with a body weight ranging from 63 to 97 kg (mean, 77 kg) and a body height ranging from 172 to 190 cm (mean, 180 cm) were included. The subjects were considered healthy according to history, physical examination, electrocardiogram, and standard laboratory tests, including hepatitis virus and human immunodeficiency virus screen. Prior to administration of the study drug, and 24 h after dosing, routine hematology, urine, biochemistry, and electrocardiogram analyses were repeated. Vital signs (blood pressure, pulse rate, and oral

* Corresponding author. Mailing address: Department of Urology, Hospital St. Elisabeth, St.-Elisabeth-Str. 23, 94315 Straubing, Germany. Phone: 49-9421-710-1700. Fax: 49-9421-710-270. E-mail: NaberK@klinikum-staebling.de.

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over the whole concentration range. The intraday precision of the assay for gatifloxacin in ejaculate, cell-free seminal fluid, prostatic fluid, and cells was ≤7.2%, with an intraday accuracy of ≤7.3%.

**Assay of iohexol.** Iohexol in urine, prostatic fluid, ejaculate, and cell-free seminal fluid was analyzed by a HPLC technique using UV detection. The method used has been implemented at that laboratory. Urine, prostatic fluid, ejaculate, and cell-free seminal fluid samples (50 μl) were deproteinized by addition of 25 μl of acetonitrile-perchloric acid, mixed, and centrifuged for 30 min at 11,000 rpm. Ten microliters of each sample were analyzed by HPLC with UV detection (254 nm). For human urine three iohexol standards were prepared (12.5, 25, and 50 mg/ml) in human urine. Using the theoretical concentrations of these standards and measured peak heights, concentrations were determined and clinical samples were analyzed against these standards. Concentrations of iohexol in clinical urine samples ranged from 0.48 to 2.0 mg/ml.

For validation and calibration, ejaculate and cell-free seminal fluid calibration curves were used for gatifloxacin. For validation and calibration, ejaculate and cell-free seminal fluid calibration curves were used for validation and calibration. Calibration was performed by weighted (reciprocal of standard concentration) linear regression. The resulting coefficients from linear regressions were at least 0.999. The limit of quantification of iohexol was set to 1.95 μg/ml for human ejaculate and 1.89 μg/ml for both human cell-free seminal fluid and prostatic fluid. For validation of the iohexol concentration in ejaculate and cell-free seminal fluid, three QC samples for each fluid were prepared.

The gatifloxacin assay was cross-checked for gatifloxacin, and no interference was found. This result was expected, since iohexol is a polar compound unlikely to be extracted by the solvent used to extract gatifloxacin (methylene dichloride at pH 2.8). In addition, the mobile phase used for the assay of gatifloxacin is more polar than that used for iohexol, suggesting that iohexol would elute much earlier than gatifloxacin.

**Analytical data management.** For plasma and urine measurements of gatifloxacin, the detector of the analytical instrument was digitized by means of an analog-to-digital converter connected to a personal computer. The signals were identified and processed for peak height calculation with the Turbochrome 4 software. For calibrations, the nominal concentrations associated with these measurements were computed through weighted linear regression (weighting factor: reciprocal of standard concentration squares). Concentrations in the subject’s samples and the QC samples were calculated using the slope and the intercept obtained from the calibration lines. Concentrations were expressed as micrograms of anhydrogatifloxacin per milliliter of fluid.

The evaluation of the calibration standards of gatifloxacin and iohexol in prostatic fluid, ejaculate, cell-free seminal fluid, and sperm cells was performed by a weighted linear regression (reciprocal of standard concentration) with theoretical concentrations of calibration standards and measured peak height ratios. The gatifloxacin concentrations were calculated by using the following: maximum concentration in plasma (Cmax) at time at which Cmax occurs (Tmax, area under the curve from 0 to 24 h (AUC(t→24)), plasma elimination half-life (t1/2), and renal clearance (CLr), calculated as U (amount excreted unchanged in urine, in milligrams)/AUC (in milligrams·hour/milliliter).

**Assay of gatifloxacin in prostatic fluid, ejaculate, cell-free seminal fluid, and sperm cells.** Gatifloxacin in prostatic fluid, ejaculate, cell-free seminal fluid and sperm cells was analyzed by a validated HPLC method with fluorescence detection at IBMP. Prostatic fluid, ejaculate, and seminal fluid (50 μl) were deproteinized by precipitation of drug insoluble material with cooling in a mixture of acetone and ethanol at 11,000 rpm. The sperm cell samples (50 μl) were diluted with distilled water. Fifteen microliters of samples were in this way analyzed by HPLC with fluorescence detection. The Turbochrom 3 software (1991 release, version 3.2; PE Nelson, Cupertino, Calif.) was used for evaluation of chromatograms.

For validation and calibration, ejaculate, seminal fluid, and sperm cell calibration curves using nine standards (including a blank sample) and sets of spiked QC samples were prepared. The resulting coefficients from linear regressions of the standard curve were at least 0.999. For prostatic fluid and for sperm cells no calibration curve could be prepared due to the low volume of drug-free prostatic fluid or sperm cells available. Therefore, prostatic fluid and sperm cell samples were analyzed against the seminal fluid calibration curve.

The QC samples were measured and treated in the same manner as subjects' samples. The interday precision of QC samples of gatifloxacin in ejaculate and that in cell-free seminal fluid were ≤9.3%, with an interday accuracy of ≤7.9% over the whole concentration range. The intra- and interday precision of the assay for gatifloxacin in ejaculate, cell-free seminal fluid, prostatic fluid, and cells was ≤7.2%, with an intraday accuracy of ≤7.3%.

**Pharmacokinetic analysis methods.** Pharmacokinetic parameters, derived from concentrations in plasma and urine measured in samples collected immediately before and within 24 h after dosing, included the following: maximum concentration in plasma (Cmax), time at which Cmax occurs (Tmax, area under the curve from 0 to 24 h (AUC(t→24)), plasma elimination half-life (t1/2), and renal clearance (CLr), calculated as U (amount excreted unchanged in urine, in milligrams)/AUC (in milligrams·hour/milliliter).
Urinary contamination of prostatic fluid, ejaculate, and seminal fluid. The theoretical (maximum) urinary contamination of prostatic fluid, ejaculate, and seminal fluid was estimated assuming that the total iohexol concentration of these fluids could have been derived from the corresponding iohexol urinary concentration according to the following formula: urinary contamination of fluid (%), urinary contamination of fluid (micrograms per milliliters) × 100/urinary iohexol (micrograms per milliliter).

Theoretical maximum percent urinary contaminations of ejaculate, seminal fluid, and prostatic fluid were calculated from the iohexol concentrations in urine and the corresponding fluids. The theoretical maximum percent urinary concentration of the ejaculate ranged between 0.01 and 0.04%, that of the seminal fluid ranged between 0.01 and 0.04%, and that of prostatic fluid ranged between 0.01 and 0.16%. Thus, the highest possible urinary contamination of 0.16% in prostatic fluid could have contributed up to 0.64 µg/ml to the prostatic fluid concentration of gatifloxacin (patient 6), considering the urinary gatifloxacin concentration of 399 µg/ml. The measured gatifloxacin concentration of that prostatic fluid was 1.91 µg/ml. If urinary contamination is assumed as mentioned before, the real concentration in prostatic fluid would have been 1.27 µg/ml, with a corresponding concentration in plasma of 1.75 µg/ml, resulting in a true prostatic fluid-to-plasma ratio of 0.73.

If all fluid-to-plasma ratios are corrected by this means, the median (range) true fluid-to-plasma ratios of gatifloxacin can be calculated as 1.10 (0.73 to 1.61) for prostatic fluid, 0.99 (0.77 to 1.17) for seminal fluid, and 0.99 (0.83 to 1.25) for ejaculate. Concentrations in sperm cells could only be determined in three subjects and were 0.195, 0.076, and 0.011 µg/ml.

Gatifloxacin was well tolerated. A total of two treatment-emergent AEs (headaches) were reported by 2 of the 10 (20%) subjects. Both were classified to be of moderate intensity and were judged to have no relationship to study medication but rather to study conditions, e.g., no caffeine intake.

**TABLE 1. Pharmacokinetic parameters of gatifloxacin following a single oral dose of 400 mg administered to healthy, male volunteers (n = 10).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>1.66</td>
<td>0.91</td>
<td>1.50</td>
<td>1.00–4.00</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/ml)</td>
<td>2.90</td>
<td>0.39</td>
<td>2.93</td>
<td>2.34–3.89</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>7.20</td>
<td>0.90</td>
<td>7.10</td>
<td>5.86–8.83</td>
</tr>
<tr>
<td>AUC$_{0-24}$ (µg · h/ml)</td>
<td>25.65</td>
<td>2.53</td>
<td>25.36</td>
<td>21.32–29.90</td>
</tr>
</tbody>
</table>

**DISCUSSION**

This study of 10 healthy volunteers shows that a single oral dose of 400 mg of gatifloxacin is well tolerated and is rapidly and well absorbed with a mean (SD) $T_{\text{max}}$ of 1.66 (0.91) h, an estimated $C_{\text{max}}$ of 2.90 (0.39) µg/ml, and a mean $t_{1/2}$ of 7.20 (0.90) h. The AUC$_{0-24}$ was 25.65 (2.53) µg · h/ml. Thus, the $C_{\text{max}}$ and the $t_{1/2}$ of gatifloxacin are higher than those observed for comparable doses of enoxacin, norfloxacin, and ciprofloxacin in a similar setting (15). In this study we investigated urinary excretion only up to 12 h after oral administration, and within this time about 50% of the substance was excreted.
unchanged into urine. Earlier investigations showed that approximately 65 to 90% of gatifloxacin is excreted unchanged into the urine over 24 to 72 h (12, 17; H. Stahlberg, K. Goehler, M. Guillaume, and A. Mignot, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., p. 8–8, 1998). The concentrations in plasma and urinary excretion of gatifloxacin found in this study are somewhat lower than those found by other investigators (3, 12; Stahlberg et al., 38th ICAAC). Since the CLR was practically identical with that found by other authors (3, 12, 17), we assume that lower absorption in our volunteers was the reason for the lower concentrations in plasma and urinary excretion.

The determination of the concentrations of antibiotics in prostatic and seminal fluid may be biased by urinary contamination (16). To estimate potential contaminations of prostatic and seminal fluid by urine, we used a model which has been published earlier (7–11). It has been proposed that by using this model it is possible to estimate the maximum possible amount of urinary contamination. In brief, the renal contrast agent iohexol, which is excreted almost exclusively via glomerular filtration by the kidneys, was administered intravenously to the subjects at the same time the study drug was given. Since iohexol concentrations in urine are several hundred-fold greater than those in plasma or prostatic fluid, possible urinary contamination (13) the concentration of such a substance can be expected to be higher in prostatic fluid as well as in seminal fluid than that achieved with weak acids such as, e.g., β-lactam antibiotics (9, 10).

We used gatifloxacin concentrations in prostatic and seminal fluid, corrected by maximal urinary contamination, to estimate the true fluid/plasma ratios. These median (range) ratios were 1.10 (0.73 to 1.61) for prostatic fluid, 0.99 (0.77 to 1.17) for seminal fluid, and 0.99 (0.83 to 1.25) for ejaculate, respectively. Thus, gatifloxacin concentrations in ejaculate, seminal fluid, and prostatic fluid after a 400-mg oral dose are in the range of the corresponding concentrations in plasma. The median seminal fluid/plasma ratio is comparable to that of ofloxacin (1.0 to 1.3) but is somewhat lower than that of other fluoroquinolones such as fleroxacin (1.3 to 1.7), enoxacin (2.2), ciprofloxacin (5.8 to 7.1) (6), or ofloxacin (2.6 to 4.0) (7, 8, 11). In contrast, the median prostatic fluid/plasma ratio was at least twofold higher than those reported from similar studies with norfloxacin, ciprofloxacin, ofloxacin, enoxacin, or ofloxacin (6–8, 11), which were found to be in the range of 0.12 to 0.48.

Conclusion. The relatively high concentrations of gatifloxacin in prostatic and seminal fluid as compared to those of other fluoroquinolones, along with the extended antibacterial spectrum, indicate that gatifloxacin may be an appropriate therapeutic agent for CBP, which should be investigated in clinical trials. According to its pharmacokinetic properties and its good penetration into prostatic and seminal fluid, an oral dosage of 400 mg once daily appears to be suitable for investigating the efficacy of gatifloxacin in CBP.

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REFERENCES


