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Pharmacokinetics and Tolerability of Prulifloxacin after Single Oral Administration

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Summary

The pharmacokinetic properties and tolerability of three different strengths of prulifloxacin (CAS 123447-62-1), a new antibacterial agent prodrug of AF3013 (CAS 112984-60-8), have been investigated in a randomized, cross-over study performed in 12 Caucasian male subjects (age range 19–34 years). Prulifloxacin was administered as a single oral dose at the dosages of 300, 450 and 600 mg. Plasma concentrations of the active metabolite AF3013 were determined in blood samples collected before the administration (pre-dose) and at 15, 30, 45 min, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 h after dosing. Urine samples were also collected. Determination in biological samples was performed using validated and specific HPLC methods. The following parameters were calculated: C_{max}, t_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$, V/F, Ae_{u}, CL_{ren} and fe. The analysis of variance performed on dose-normalized data after logarithmic transformation evidenced no statistically significant differences between the three doses concerning C_{max} and AUC. Friedman's test applied to t_{max} and $t_{1/2}$ did not show any statistically significant difference between doses. A significant linear relationship between doses and $AUC_{0-\infty}$ was detected ($p < 0.05$). Very high urinary concentrations and the relatively long terminal half-life (10–12 h) suggest that a once-daily application would show adequate clinical efficacy, especially in urinary infections. The safety profile of the three doses was very good.

Key words

- AF3012
- CAS 112984-60-8
- CAS 123447-62-1
- Fluoroquinolones
- NM441
- Prulifloxacin, clinical pharmacokinetics

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Zusammenfassung

Pharmakokinek und Verträglichkeit von Prulifloxacin nach einmaliger oraler Verabreichung

In einer randomisierten Crossover-Studie an 12 kaukasischen männlichen Probanden (Alter 19 bis 34 Jahre) wurden die pharmakokinetischen Eigenschaften und Verträglichkeit drei verschiedener Stärken von Prulifloxacin (CAS 123447-62-1, AF3012 bzw. NM441), einem neuen antibakteriell wirksamen Prodrug von AF3013 (CAS 112984-60-8), evaluiert. Prulifloxacin wurde als einmalige orale Dosis von 300, 450 bzw. 600 mg verabreicht. Die Plasmakonzentrationen des aktiven Metaboliten AF3013 wurden in Blutproben bestimmt, die vor bzw. 15, 30, 45 min, 1, 11/2, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 und 48 h nach Verabreichung der Substanz gesammelt wurden. Urinproben wurden ebenfalls gesammelt. Der Nachweis in den Proben erfolgte mit Hilfe validierter und spezifischer HPLC-Methoden. Es wurden folgende Parameter ermittelt: C_{max}, t_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$, V/F, Ae_{ur}, Cl_{ren} sowie fe. Die mit den nach logarithmischer Umwandlung dosisnormalisierten Daten durchgeführte Varianzanalyse ergab keine statistisch signifikanten Unterschiede zwischen den drei Dosierungen für C_{max} und AUC. Der Friedman-Test, mit dem tmax und $t_{1/2}$ evaluiert wurden, zeigte ebenfalls keine statistisch signifikanten Unterschiede zwischen den Dosierungen. Dagegen zeigte sich eine signifikante lineare Relation zwischen den Dosierungen und $AUC_{0-\infty}$ ($p < 0,05$). Außerordentlich hohe Harnkonzentrationen sowie die relativ lange terminale Halbwertzeit (10–12 h) lassen vermuten, daß eine einmal tägliche Verabreichung insbesondere bei Harnwegsinfektionen klinisch wirksam sein könnte. Das Sicherheitsprofil der drei Dosierungen war sehr gut.

1. Introduction

The role of fluoroquinolones is increasingly important due to the growing rate of resistance of common pathogens to traditional antimicrobial therapies, which is accomplished to a still limited incidence of resistance to fluoroquinolones. This is probably due to their interaction with dual bacterial targets, the related DNA gyrase and topoisomerase IV enzymes, and the avoidance of bacterial efflux-resistance mechanisms, which may contribute to the lower frequencies of selection of resistant mutants in the laboratory [1].

Clinical applications of fluoroquinolones, beyond genito-urinary tract infections, include upper and lower respiratory infections, gastrointestinal infections, sexually transmitted diseases, and some skin and soft tissue infections [2]. The new fluoroquinolones are recommended as first-line therapy for uncomplicated urinary tract infections in patients who live in areas with known resistance to trimethoprim-sulfamethoxazole, and are also considered as a valid therapeutic choice both in simple and complicated chronic bronchitis [3, 4].

The efficacy of antibiotic treatment is influenced by several variables, such as broad spectrum antibacterial activity, patient compliance, individual absorption rate and, overall, penetration of the drug into the target tissues. In this context, the pharmacokinetic profile of the drug represents a key factor in the success of treatment, including the possibility to use a once or twice daily administration that significantly enhances patient compliance, rather than 3 or more administrations [5].

Prulifloxacin (6-fluoro-1-methyl-7{4-[(5-methyl)-2-oxo- 1,3- dioxol- 4- yl) methyl]- 1- piperazinyl}- 4- oxo-1H,4H- [1,3] thiazeto [3,2a] quinolin-3-carboxylic acid; CAS 123447-62-1; AF3012 or NM441) is a new antibacterial agent, prodrug of AF3013 (or NM394, CAS 112984-60-8), characterized by a broad spectrum of antibacterial activity against Gram-negative and Gram-positive strains.

In recent European isolates, the in vitro activity of AF3013 against Gram-positive bacteria was greater than that of ofloxacin, comparable to those of ciprofloxacin and levofloxacin, but lower than those of grepafloxacin, moxifloxacin and trovafloxacin [6, 7]. Prulifloxacin, and in particular its derivative AF3013, belongs to the group of fluoroquinolones with major activity on Gram-negative bacteria, where it was found to be more active than ciprofloxacin and generally even more active than ofloxacin, levofloxacin, sparfloxacin, trovafloxacin and moxifloxacin, being the agent with the greatest activity against enterobacteria and some respiratory pathogens, such as *Haemophilus influenzae* and *Moraxella catarrhalis* [6, 7].

After oral administration, prulifloxacin is absorbed from the intestinal tract and is mainly transformed by a paraoxonase into AF3013, the active compound; no other known potentially active metabolites are formed [8, 9].

The aim of this study was to investigate the pharmacokinetics and tolerability of three different strengths of prulifloxacin, namely 300, 450 and 600 mg given as single oral doses.

2. Materials, subjects and methods

2.1. Subjects

Twelve Caucasian healthy male volunteers with an age range comprised within 18–40 years, body weights between 50 and 85 kg and within the limits of the ideal weight as described in the Reference Standard of the Metropolitan Life Insurance Company, and without any clinically significant laboratory abnormalities, were included after having given their written informed consent.

Prior to the starting of the study, the ethical approval of the protocol was obtained from the C.C.P.P.R.B. (Comité Consultatif

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de Protection des Personnes dans la Recherche Biomedicale) of Versailles (France). The clinical part of the study was performed at the Unité de Pharmacologie Clinique VERSUS (Aubervilliers, France) in accordance with the European Good Clinical Practice guidelines.

2.2. Study design

The trial was a randomized, cross-over study. Within 2 weeks prior to the start of the study, a screening visit including medical history, vital signs, physical examination, ECG and routine clinical laboratory tests was performed. No additional medications were allowed throughout the study, starting 1 week before administrations. Subjects were hospitalized for 24 h on 3 occasions, with a 6-day period of wash-out between hospitalizations. The consumption of tobacco and alcoholic beverages, or those containing xanthic bases, and fruit juices were forbidden during the 48 h preceding and for the whole duration of hospitalization. Subjects were fasting overnight from 22 h and remained fasted up to 4 h after administrations.

In each period, subjects randomly received one single tablet of 300 mg (batch number C14), 450 mg (batch number C13) or 600 mg (batch number C15) prulifloxacin[1)] with 150 ml of plain mineral water.

During the entire study periods, subjects were asked to report the occurrence of any adverse events. Safety evaluations, namely medical history, physical examination, vital signs, laboratory tests and ECG were repeated at the end of each period of the study.

2.3. Bioanalytics

Drug assay in biological fluids (plasma and urine) was performed by the CEPHAC Research Centre (Saint-Benoit, France) analytical laboratories. A validated high-performance liquid chromatographic (HPLC) method was used to determine the concentrations of AF3013. Sample preparation consisted on the isolation of AF3013 from biological fluids by means of solid phase extraction (C2 cartridge) followed by liquid/liquid extraction/derivatization with methylene chloride containing ethylchloroformate. The dry extract was re-dissolved and analyzed by reversed phase chromatography and UV detection.

The intra-batch and inter-batch precision and accuracy of the analytical method was verified by assaying calibration curves and quality control samples produced from blank plasma and urine spiked with different concentrations of AF 3013 (in the range 0.05 to 5 µg/ml in plasma including the limit of quantification, LOQ, and in the range 0.5 to 20 µg/ml in urine including the LOQ).

Specificity was tested in blank plasma and urine samples to check for the presence of endogenous interferences. The interference due to minor metabolites of prulifloxacin was also verified in blank samples spiked with original metabolites.

2.4. Pharmacokinetics

For plasma level concentrations measurement, 5 ml of venous blood samples were collected into heparinized tubes at predose and at 15, 30, 45 min, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 h after dosing. The blood was immediately centrifuged at 4 °C at 3000 rpm for 10 min. The supernatant plasma was transferred into two plastic tubes and stored at −20 °C.

Urine samples were collected before treatment and at the following time intervals: 0–2, 2–4, 4–8, 8–12, 12–24 and 24–48 h after dosing and stored at +4 °C. Two samples in plastic tubes of 10 ml from each urine collection were stored at −20 °C.

The following non-compartmental parameters were derived from individual plasma and urine concentrations: peak concentration (C_{max}), time to peak concentration (t_{max}), area under the plasma concentration-time curve from time 0 to the time of the last measurable concentration (AUC_{0-t}) by the linear trapezoidal rule, area under the curve extrapolated to infinity ($AUC_{0-\infty}$, = $AUC_{0-t} + C_{last}/k$, where C_{last} was the last quantifiable concentration), apparent elimination rate constant (k, slope of the last plasma concentrations, log-transformed, vs. time), apparent elimination half-life ($t_{1/2} = 0.693/k$), apparent volume of distribution (V/F), amount excreted through urine (Ae_{ur}), renal clearance (CL_R), and fraction of the dose excreted in the urine (fe).

2.5. Statistical analysis

Descriptive statistics on demographic data, vital signs (blood pressure and pulse rate), clinical laboratory parameters, results of ECG recordings and adverse events were performed. All clinical laboratory tests outside the normal range were identified.

Statistical analysis of pharmacokinetic data was performed using the SAS® package. Analysis of dose-normalized C_{max} and AUC was carried out by analysis of variance using PROC GLM on the logarithmically transformed data followed by the LS-means statement with adjustment for multiple comparisons according to Tukey's procedure. Analysis of t_{max} and $t_{1/2}$ was based on the non-parametric Friedman's test followed by the Wilcoxon test in case of significance. The linearity between log-transformed C_{max} and both AUC versus doses, and the correlation between AUC and Ae_{ur} were also evaluated.

The level for statistical significance was set at 5 %.

3. Results

3.1. Subjects

Twelve Caucasian male subjects aged 19 to 34 years were enrolled. Demographic and baseline characteristics are reported in Table 1. At the screening, no clinically significant alterations in laboratory tests, physical examination, ECG and vital signs were detected. No protocol deviations were observed and no concomitant medications were administered during the entire course of the study.

Table 1: Demographic and baseline characteristics.

	Mean	Standard deviation	Range
Age (years)	25.7	5.0	19–34
Weight (kg)	71.2	4.9	58.8–74.5
Height (cm)	177.5	6.1	167–187
Systolic blood pressure (mmHg)	126	5.5	115–133
Diastolic blood pressure (mmHg)	65	7.5	54– 80
Heart rate (bpm)	67.8	11.4	50– 87
Creatinine clearance (ml/min)	114.7	15.7	86–144

[1)] Manufacturer: A.C.R.A.F. S.p.A., Rome (Italy).

Table 2: Pharmacokinetic parameters.

	300 mg	450 mg	600 mg
t_{max} (h) median (range)	1 (0.5–2)	0.75 (0.25–2)	1 (0.5–2)
C_{max} (μg · ml^{-1}) mean (SD)	1.03 (0.53)	1.4 (0.7)	1.6 (0.9)
$t_{1/2}$ (h) mean (SD)	12.1 (4.8)	10.6 (3.1)	10.7 (2.6)
AUC_{0-t} (μg · ml^{-1} · h) mean (SD)	3.9 (1.7)	5.6 (2.4)	6.2 (2.9)
$AUC_{0-\infty}$ (μg · ml^{-1} · h) mean (SD)	5.0 (1.8)	6.6 (2.5)	7.3 (2.8)
V/F (l) mean (SD)	905 (495)	993 (648)	1231 (939)
Ae_{ur} (mg) mean (SD)	52.3 (28.3)	66.5 (25.1)	76.1 (34.5)
CL_R (ml/min) mean (SD)	171.6 (58.4)	173.1 (35.2)	170.1 (37.0)
fe (%) mean (SD)	23.0 (12.5)	19.5 (7.3)	16.7 (7.6)

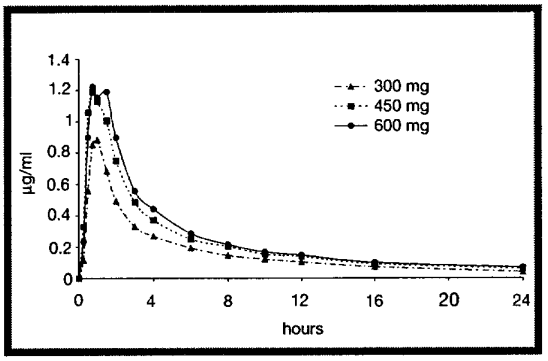

Fig. 1: Mean plasma concentration-time curves of AF3013.

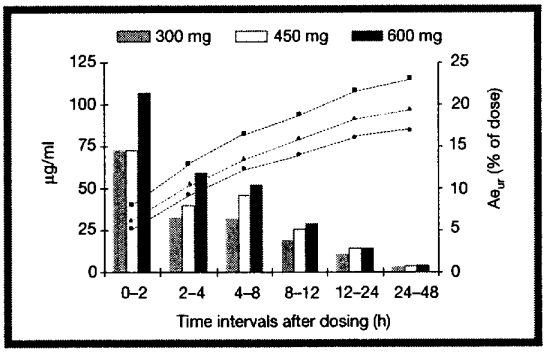

Fig. 2: Mean AF3013 urine concentrations (bars) and mean cumulative amounts of drug (as % of the dose) excreted in urine (■ 300 mg, ▲ 450 mg, ● 600 mg).

3.2. Bioanalytics

The bioanalytical method for measurement of AF3013 was linear from 0.05 to 5 μg/ml in plasma and from 0.5 to 20 μg/ml in urine. The LOQ was 0.05 in plasma and 0.5 μg/ml in urine. The correlation coefficient of calibration curves was always > 0.99. The precision (coefficient of variation, CV) of calibrators was in the range from 1.23 to 5.64 % in plasma and from 1.48 to 6.90 % in urine. The percentage of inaccuracy was in the range of –7.10 to 10.97 in plasma and –3.92 to 5.29 in urine. The overall precision (CV) of the quality control samples was in the range 4.15–9.13 % in plasma and 2.39–5.00 % in urine. The percentage of inaccuracy was in the range 2.57–4.60 % in plasma and –1.62–2.72 % in urine.

The specificity of the assay was confirmed in blank plasma and urine samples against endogenous interferences and also against the minor metabolites of prulifloxacin.

3.3. Pharmacokinetic results

The mean pharmacokinetic parameters after single oral administration of 300, 450 and 600 mg prulifloxacin tablets are reported in Table 2, and the corresponding plasma concentrations-time profiles are shown in Fig. 1.

The analysis of variance performed on dose-normalized data after logarithmic transformation evidenced no statistical differences between the three doses concerning C_{max} and AUC. Friedman's test applied to t_{max} and $t_{1/2}$ did not evidence any difference between doses. The assessment of linearity with doses showed a significant relationship with $AUC_{0-\infty}$ ($p < 0.05$), a trend with AUC_{0-t} ($p = 0.06$), and no relationship with C_{max}. A highly significant correlation between $AUC_{0-\infty}$ and Ae_{ur} ($r^2 = 0.86$, $p < 0.001$) was also found.

Mean urine concentrations of AF3013 and mean amount of excreted drug, expressed as the percentage of the administered dose, are reported in Fig. 2.

3.4. Tolerability

At the end of trial visit, one subject presented a minor, transitory and spontaneously regressive increase in AST (aspartate aminotransferase) levels (serum glutamic-oxalacetic transaminase, SGOT) equal to 1.3 times the upper normal range, without any other clinical or laboratory alteration: the event was judged as not being related to the drug administration, but probably due to physical efforts during the preceding few days. Another subject reported mild gastralgia after the administration of prulifloxacin 450 mg, but the complete and spontaneous regression of the event was observed. Relationship with the trial medication was judged as doubtful.

No other clinically significant changes with respect to baseline assessments were observed in physical examination, vital signs, laboratory tests and ECG.

4. Discussion and conclusions

In this study the basic pharmacokinetic properties of three prulifloxacin strengths were determined in Caucasian young healthy volunteers. The safety profile of the three dosages was also investigated and was found to be very good and comparable between doses.

Prulifloxacin was rapidly absorbed in 0.75 to 1 h (median) and the active metabolite AF3013 reached mean maximum plasma concentrations comprised between 1 and 1.6 μg/ml. $AUC_{0-\infty}$ values ranged from 5 to 7.3 μg/ml · h and increased linearly with the dose. The elimina-

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tion half-life ranged between 10.6 and 12.1 h, whatever the dose. These data are substantially consistent with a previous study carried out in healthy male Japanese volunteers, who received three strengths of prulifloxacin in a single oral dose (132, 264 and 528 mg), except for half-life values (range 7.7–8.9 h) that were lower than those observed in this study [9]. This difference is probably due to a more accurate definition of the elimination phase carried out in Caucasian subjects.

Urinary excretion was significantly correlated with $AUC_{0-\infty}$ indicating that renal elimination was not affected by the dose. This was confirmed by prulifloxacin renal clearance that was not dose-dependent (about 170 ml/min), and was higher than creatinine clearance (114.7 ml/min), suggesting the presence of active tubular secretion for AF3013 besides glomerular filtration.

The relatively long terminal half-life of prulifloxacin observed in this study, anticipated that a once-daily application will show sufficient clinical efficacy and patient compliance.

Prulifloxacin produced AF3013 plasma levels that appeared to be slightly lower than those observed with the newer quinolones [10]. However, it should be noted that AF3013 is active in vitro at low concentrations. In fact, $MIC_{90\%}$ (minimum concentration that inhibits the growth of 90 % of bacteria) values of prulifloxacin against Gram-positive bacteria were lower than those of ofloxacin, similar to or lower than those of ciprofloxacin, and generally lower than $MIC_{90\%}$ values of moxifloxacin, trovafloxacin, grepafloxacin, levofloxacin against Gram-negative nosocomial and community isolated bacteria [6, 7]. The comparative assessment of prulifloxacin plasma levels and $MIC_{90\%}$ values suggests an adequate efficacy profile. Moreover, the high mean apparent volumes of distribution during the terminal phase provide evidence of high concentrations in peripheral target organs/tissues, as already observed in animal studies [11].

Prulifloxacin shows major activity against Gram-negative bacteria, including enterobacteria, *Pseudomonas* spp. and respiratory pathogens, such as *Haemophilus influenzae* and *Moraxella catarrhalis*, although in vivo data emerging from a pivotal clinical trial in patients with acute exacerbation of chronic bronchitis evidenced an interesting activity of prulifloxacin against *Streptococcus pneumoniae* as well [7, 12]. Besides the level of exposure to the drug, the finding of in vivo positive results calls for more detailed information on distribution of the drug into extravascular spaces and, in particular, its concentrations on the target tissues of infection.

Urinary concentrations of prulifloxacin are very high up to 24 h from administration, often exceeding more then 10 times the MIC values of the most frequent uropathogens 48 h after dosing. At this time, urinary concentrations are consistently higher than 3 μg/ml (MIC_{90} for *Escherichia coli* ≤ 0.015 μg/ml, MIC_{90} for *Proteus mirabilis* ≤ 0.015 μg/ml, MIC_{90} for *Klebsiella pneumoniae* 0.12 μg/ml [7]).

These data suggest a favourable efficacy profile of prulifloxacin in urinary infections, such as in acute uncomplicated or complicated lower urinary infections using a once daily administration. However, appropriate clinical trials are needed to confirm if the pharmacokinetic and pharmacodynamic characteristics of prulifloxacin are adequate to ensure clinical efficacy.

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