Influence of Rifampin on Fleroxacin Pharmacokinetics

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Staphylococcus aureus infections have been successfully treated in animal models with the combination of fleroxacin and rifampin. We studied the influence of rifampin, a potent cytochrome P-450 inducer, on the pharmacokinetics and biotransformation of fleroxacin in 14 healthy young male volunteers. Subjects were given 400 mg of fleroxacin orally once a day for 3 days to reach steady state. After a wash-out period of 2 days, the same subjects received 600 mg of rifampin orally once daily for 7 days. On days 5 to 7 of rifampin treatment, 400 mg of fleroxacin was again administered once daily. Concentrations of fleroxacin as well as its two major urinary metabolites, N-demethyl- and N-oxide-fleroxacin, in plasma and urine were determined by reverse-phase high-performance liquid chromatography. The extent of hepatic enzyme induction by rifampin was confirmed by a significant increase of 6-β-hydroxycortisol urinary output from 160.8 ± 41.4 to 544.8 ± 120.7 μg/4 h. There were no significant changes in the peak fleroxacin concentration in plasma (6.3 ± 1.2 versus 6.2 ± 1.9 μg/ml, time to maximum concentration of fleroxacin in plasma (1.1 ± 0.9 versus 1.3 ± 1.1 h), or renal clearance (53.5 ± 16.4 versus 61.9 ± 13.4 ml/min). The area under the curve AUC (71.4 ± 15.8 versus 62.2 ± 13.7 μg · h/liter) and the terminal half-life of fleroxacin (11.4 ± 2.2 versus 9.2 ± 1.1 h) decreased (P < 0.05), while the total plasma clearance increased from 97.7 ± 21.6 to 112.3 ± 25.8 ml/min (P < 0.01). Despite being statistically significant, this 15% increase in total plasma clearance does not appear to be clinically relevant. Metabolic clearance by N demethylation was increased (6.9 ± 2.4 versus 12.5 ± 3.2 ml/min; P < 0.01), whereas clearance by N oxidation did not change (5.8 ± 1.1 versus 5.8 ± 1.5 ml/min). Fleroxacin elimination was slightly increased (about 15%) through induction of metabolic Clearance to N-demethyl-fleroxacin. Since fleroxacin levels remained above the MIC for 90% of the tested isolates of methicillin-susceptible S. aureus for at least 24 h, dose adjustment does not appear necessary, at least for short-term treatments.

Fluoroquinolones, including fleroxacin, have recently emerged as interesting antibacterial agents in the therapy of deep-seated infections. This is in part attributable to their antibacterial spectra, the extent of their oral absorption, and their high rate of penetration into biological fluids and tissues (45).

Rifampin has long been used as an adjunctive treatment of staphylococcal infections because of its antibacterial effects on staphylococci and its extensive distribution into tissues. However, rifampin monotherapy almost invariably leads to failure due to development of resistance. The combined use of both families of antibacterial agents, fluoroquinolones and rifampin, has been advocated for the treatment of serious staphylococcal infections to avoid the resistance of resistant bacteria, especially in long-term treatments (3, 14–16, 22, 23, 25, 26, 28, 35, 38, 44, 48).

The combination of fleroxacin plus rifampin has been used successfully in animal models, in particular to cure chronic staphylococcal infections (8, 28). Fleroxacin is virtually completely absorbed; its absolute bioavailability after oral administration amounts to 100%. It is eliminated primarily by renal clearance (CLR), about 60 to 70% of a dose being recovered unchanged in the urine within 96 h. The terminal half-life (t1/2b) reaches approximately 13 h. Two major metabolites in urine have been identified, N-oxide-fleroxacin and N-demethyl-fleroxacin. They amount to approximately 5 to 10% and 7%, respectively, in healthy subjects (47).

Rifampin is metabolized by the hepatic microsomal enzyme system (1, 43) and is known to induce many enzymatic catabolic pathways (20). By acting on the hepatic mixed-function oxidase system in humans (including the cytochrome P-450 III A CYP3A isoenzymes) (34), it enhances the elimination of a large number of drugs (2). Because the two metabolites of fleroxacin are formed by oxidative processes in the liver, an influence of rifampin on fleroxacin kinetics must be considered. Therefore, it was important to assess the influence of rifampin on steady-state fleroxacin pharmacokinetics and to determine its effect under controlled conditions. In order to estimate the efficiency of enzymatic induction by rifampin, the urinary output of 6-β-hydroxycortisol was measured. This physiologic nonconjugated metabolite of cortisol is synthesized mainly in the endoplasmic reticulum of the liver and poorly in the adrenal cortex and placenta. It is produced by an isoenzyme of the cytochrome P-450 superfamily (probably CYP3A4) and is therefore an accurate marker to control environmental induction (17, 46). The results of the present study will be useful to assess the dosages necessary for clinical studies. applying this drug combination in the therapy of staphylococcal infections. (This work was presented in part at the 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Anaheim, Calif., 11 to 14 October 1992 [39].)

MATERIALS AND METHODS

Volunteers. Thirteen healthy male volunteers (age range, 22 to 36 years) participated in this investigation. Subjects were admitted to the University Hospital of Geneva, Geneva, Switzerland, after ethics committee approval and informed written consent were obtained. The age, weight, serum creatinine levels, and creatinine clearance (9) of these

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subjects were (mean ± standard deviation) 27.4 ± 4.0 years, 75.9 ± 8.5 kg; 96.6 ± 12.5 μmol/liter, and 130 ± 31.2 ml/min, respectively.

Exclusion criteria were known or suspected hypersensitivity to nalidixic acid, quinolones, or rifampin; clinically relevant deviation from normal in the physical examination or in the routine laboratory tests; and any use of drugs within 2 weeks before the study.

Antacids as well as other drugs were strictly avoided. Alcohol consumption and smoking (more than 10 cigarettes per day) were prohibited. Only one volunteer was a moderate smoker (1 to 3 cigarettes per day), and he refrained from smoking throughout the study. Medical histories, physical examinations, and results to a panel of laboratory tests (renal and liver chemistries, complete blood cell and platelet counts, and urinalysis) were obtained before the study and on days 3, 10, and 12 of the study. Whenever volunteers came for blood sampling, they were systematically questioned about the occurrence of symptoms such as nausea, vomiting, abdominal pain, dizziness, sleep disorders, and pruritus. The symptoms were graded as discrete, moderate, or severe, and, if any were reported, their reversal was actively checked.

Study design. This was an open-label, nonrandomized, within-subject comparative study. Two 3-day active treatment periods were programmed (floxacin alone and fleroxacin plus rifampin) to achieve steady-state conditions. Each was followed by a 2-day wash-out period. For hepatic enzyme induction, a 7-day run-in period with rifampin was allowed to (32, 36, 43) overlap with the second 3-day fleroxacin treatment period. The overall study design diagram (Fig. 1) demonstrates the 14-day time schedule of this trial.

In the course of period A (floxacin alone), every volunteer received, after an overnight fast, one oral dose of 400 mg of fleroxacin daily for 3 days (days 1 to 3). After a 2-day wash-out period (days 4 to 5), a daily morning 600-mg dose of rifampin was given for 7 days (days 6 to 12). Rifampin was taken alone for 4 days; during the last 3 days (days 10 to 12), rifampin was taken 3 h after the dose of fleroxacin, 400 mg orally, with the volunteer still fasting to avoid any interference during the absorption. Overnight fasting and a minimum of 150 ml of water for drug intake were required. Volunteers were allowed to stop fasting 3 h after fleroxacin intake.

Blood and urine samples for assays were taken at steady state (days 3 and 12). Sampling schedules were identical for both periods. Blood was collected (5-ml Vacutainer tube [F. Hoffmann-La Roche, Basel, Switzerland] containing sodium fluoride and potassium oxalate) before intake and at 0.5, 1, 1.5, 2, 3, 4, 8, 12, 24, 48, and 72 h after fleroxacin intake. After centrifugation, plasma was transferred into polyethylene-stoppered glass tubes covered with aluminum foil to prevent photodegradation and then stored at −20°C until assay.

Urine samples were collected over the following 6 periods after the third fleroxacin dose: 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, and 48 to 72 h. At the end of each time period, the portions were carefully mixed. pH and total volume were recorded, and an aliquot (10 ml) was transferred to a tube covered with aluminum foil and frozen at −20°C until assay. During the first urine collection period of days 3 and 12, a further aliquot (10 ml) was kept for a 6β-hydroxy cortisol assay as a control measure of hepatic enzyme induction.

Floxacin assay. Concentrations of fleroxacin and its two main metabolites, N-oxide-fleroxacin and N-demethylfleroxacin, were determined by means of a reverse-phase high-performance liquid chromatography (HPLC) method (11). The mobile phase was 5 mM tetrabutylammonium hydrogen sulfate-methanol (18:7; vol/vol), and separation was performed on a Toyo Soda ODS-120 T 5 μm column. Concentrations of fleroxacin in plasma and urine were detected fluorimetrically (excitation at 290 nm, emission at 450 nm). In plasma samples, the limit of quantification was 20 μg/liter (RSD, 2.5%), with a linearity of >0.99 over a range of 0.02 to 10 mg/liter, and 12.5 to 4.8% over that concentration range. The sensitivity for urinary fleroxacin was 1 mg/liter (RSD, 0.98%), and the linearity precision was 0.98 to 4.5% over a concentration range from 1 to 200 mg/liter (linearity, >0.99 over that range). The limit of quantification for fleroxacin urinary metabolites was 0.5 mg/liter (RSD, 5%), with an assay precision of 3.2 to 4.2% over a concentration range from 1 to 40 mg/liter (linearity, >0.99 over that range).

6β-Hydroxy cortisol assay. 6β-Hydroxy cortisol urinary output is highly individual (17) and has a circadian rhythm (50). Therefore, each volunteer was his own control, and sampling schedules were identical for both periods. Urine samples were collected following an initial pretreatment period, from 7 a.m. to 11 a.m. The volume was determined, and, after mixing, an aliquot was frozen at −20°C until analyses could be performed. We used an immunoenzymatic reaction of a competitive type with 6β-hydroxy cortisol peroxidase as a conjugate and orthophenylenediamine as a chromogenic substrate (51). Urine samples and calibrating solutions were measured by enzyme-linked immunosorbent assay (ELISA) (Stabilgen, Nancy, France). The limit of quantification was 50 pg/ml, with a linearity superior to 0.99 over the range of 50 to 1,000 pg/ml. The intra-assay coefficient of variation throughout the range of quantifiable values was no greater than 10%.

Pharmacokinetic analysis. Visual inspection of the log linearized data showed monophasic fleroxacin concentration decline in all subjects. Monophasic elimination with first-order absorption was therefore assumed, and a biexponential model was fitted to the untransformed data with weighting proportional to the inverse of the predicted values. The maximum concentration in plasma, the time to reach maximum plasma concentration, and the minimum concentration in plasma (24 h after the drug intake) were determined at steady state; the area under the concentration-time curve
RESULTS

Tolerance. One volunteer was excluded because of a moderate allergic reaction (diffuse urticarial rash and nausea) 30 min after ingestion of the first dose of feroxacin. This adverse effect disappeared spontaneously within 1 h. Otherwise, the tolerance of the drug combination was good; in particular, no effects on vital signs were observed. Minor manifestations (mostly gastrointestinal complaints such as nausea [5], epigastric discomfort [2], vomiting [1], and diarrhea [1]) in nine volunteers were reported. Two volunteers complained of increasing photosensitivity, with an unusually rapid suntan, and two noted dizziness on the first day of the study. This fairly high frequency of minor adverse events may be explained, partly at least, by the systematic questioning of the volunteers as required by the protocol. Complete blood cell count, urea, serum creatinine, alanine aminotransferase, aspartate aminotransferase, and bilirubin were within normal ranges for all volunteers. Marginal elevation of alkaline phosphatase levels during the last control may have been related to the use of rifampin.

Hepatic enzyme induction. Urinary excretion of 6-β-hydroxycortisol increased in all the volunteers by a mean of approximately 350%, as shown in Fig. 2, demonstrating successful induction of the cytochrome P-450 hepatic microsomal enzyme system by rifampin.

Pharmacokinetic parameters. The concentration-time curves obtained either before or after rifampin treatment are presented in Fig. 3 and 4. The pharmacokinetic parameters for feroxacin at the end of periods A and B are presented in Table 1. After rifampin pretreatment, feroxacin t1/2 and AUC from 0 to 24 h decreased significantly \((P < 0.05)\), by 19 and 13%, respectively. This was associated with a 15% increase in CL. Pretreatment with rifampin also had a significant influence on the minimum concentration in plasma, resulting in a 30% decrease (1.4 to 1.0 mg/liter). However, no significant influence on the maximum concentration in plasma or the time to maximum concentration in plasma was observed.

CLR was not significantly altered, whereas metabolic clearance increased by 28% (39.4 to 50.4 ml/min) in subjects who were pretreated with rifampin. This higher metabolic clearance was also reflected by a significant increase in urinary recovery of N-demethyl-feroxacin, from 6.9 to 12.5 ml/min, following rifampin pretreatment. The urinary excretion of unchanged drug and N-oxide metabolite decreased by 8 and 15%, respectively, but without reaching a statistically significant difference when expressed as clearance.

The cumulative urinary excretion (0 to 24 h) of the native drug and its two main metabolites accounted for 70% and remained unchanged after rifampin pretreatment.

DISCUSSION

Rifampin administered alone is one of the best available antistaphylococcal agents. It has an extremely low MIC against S. aureus and is extensively distributed into tissues. Moreover, rifampin is known to concentrate within neutrophils and kill intracellular organisms (29). This mechanism may be important in the treatment of purulent infections (5, 29). However, this agent is not used as monotherapy because resistance develops rapidly (30, 48, 49). Quinolones have been used in several clinical studies as single agents in the therapy of staphylococcal infections (12, 13, 18, 19, 27). There is also considerable concern about the increasing development of S. aureus resistance to quinolones (6, 10, 40, 42), both during therapy and on the epidemiological level. Therefore, quinolone-rifampin combinations have been of interest to clinicians. Theoretically, combined therapy could improve the rate of therapeutic success and limit the development of resistance by staphylococci to either agent.

Many experimental studies have been conducted with such combinations for the therapy of staphylococcal infections. Successful results were observed with different animal models. A rat model was used to compare the efficacies of various agents, alone and in combination, for the therapy of methicillin-resistant S. aureus chronic osteomyelitis (15, 23). The efficacy of a 3-week combination of a quinolone (either ciprofloxacin [15, 23] or pefloxacin [15]) and rifampin was shown to be equivalent (15) or statistically superior (23) to that of a vancomycin-rifampin combination. In any case, the efficiencies of these combination regimes were equivalent to that of rifampin alone but superior to that of any other single agent.

Kaatz et al. reported that ciprofloxacin, alone or in combination with rifampin, was not better than vancomycin alone in the treatment of a rabbit model of S. aureus...
endocarditis (26). However, they suggested that the addition of rifampin to ciprofloxacin may decrease the frequency at which high-level resistance to ciprofloxacin emerges.

Rabbits with intraperitoneal foreign bodies were infected with *S. aureus* (3). The efficacy of ciprofloxacin alone (8 days) was not different from that in the control group, whereas the addition of rifampin showed significantly better results.

Finally, a rat model for subcutaneous foreign-body abscesses was developed. A 6-day therapy of fleroxacin plus rifampin was shown to be equivalent to rifampin alone or in combination with vancomycin but clearly better in terms of resistance to rifampin (28). A prolonged therapy (21 days) demonstrated a clear advantage after the combination of fleroxacin and rifampin versus monotherapies, including vancomycin (8).

There is controversy about the possible pharmacokinetic interactions between quinolones and rifampin, since ri-

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**FIG. 3.** Mean fleroxacin concentrations in plasma after the last (third) 400-mg fleroxacin dose: influence of rifampin. Period A, fleroxacin alone; period B, fleroxacin after rifampin induction.

**FIG. 4.** Time course of fleroxacin concentrations in plasma within a dosage interval (24 h). The levels were assessed under conditions similar to those for Fig. 3. Period A, fleroxacin alone; period B, fleroxacin after rifampin induction. *P < 0.05 pertains to the two rightmost values in the figure.
TABLE 1. Fleroxacin and its main metabolites: summary of pharmacokinetic parameters obtained for 13 volunteers*  

<table>
<thead>
<tr>
<th>Parameters (unit)</th>
<th>Period A</th>
<th>Period B</th>
<th>% Difference</th>
<th>Statistics</th>
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<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>C_{max} (mg/liter)</td>
<td>6.3</td>
<td>1.2</td>
<td>6.2</td>
<td>1.9</td>
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<td>C_{min} (mg/liter)</td>
<td>1.4</td>
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<td>1.0</td>
<td>0.4</td>
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<td>( t_{1/2} ) (h)</td>
<td>1.1</td>
<td>0.9</td>
<td>1.3</td>
<td>1.1</td>
</tr>
<tr>
<td>( f_{125} ) (h)</td>
<td>11.4</td>
<td>2.2</td>
<td>9.2</td>
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<tr>
<td>AUC_{0-24} (mg·h/liter)</td>
<td>71.4</td>
<td>15.8</td>
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<td>AUC_{0-24} (mg·h/liter)</td>
<td>78.5</td>
<td>18.2</td>
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<td>CL (ml/min)</td>
<td>97.7</td>
<td>21.6</td>
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<td>CL_{R} (ml/min)</td>
<td>58.3</td>
<td>16.4</td>
<td>61.9</td>
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<tr>
<td>CL_{N-d} (ml/min)</td>
<td>39.4</td>
<td>10.4</td>
<td>50.4</td>
<td>9.0</td>
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<td>CL_{N-d} (ml/min)</td>
<td>6.9</td>
<td>2.4</td>
<td>12.5</td>
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<td>CL_{N-O} (ml/min)</td>
<td>5.8</td>
<td>1.1</td>
<td>5.8</td>
<td>1.5</td>
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<tr>
<td>Fe Fleroxacin (%)</td>
<td>59.2</td>
<td>8.5</td>
<td>54.3</td>
<td>6.1</td>
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<tr>
<td>Fe N-Demethyl (%)</td>
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<td>1.9</td>
<td>11.3</td>
<td>2.5</td>
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<tr>
<td>Fe N-Oxide (%)</td>
<td>6.0</td>
<td>1.0</td>
<td>5.2</td>
<td>1.0</td>
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<tr>
<td>Cumulative urinary excretion (%)</td>
<td>72.5</td>
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<tr>
<td>Cumulative urinary excretion (%)</td>
<td>81</td>
<td></td>
<td>81</td>
<td></td>
</tr>
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</table>

* Period A, fleroxacin alone; period B, fleroxacin plus rifampin; difference, ratio between periods B and A — 1; \( C_{max} \), maximum concentration in plasma; \( C_{min} \), minimum concentration in plasma; \( t_{1/2} \), time to reach \( C_{max} \); \( f_{125} \), terminal half-life; AUC_{0-24}, area under the concentration-time curve from 0 to 24 h; AUC_{0-24}, AUC from 0 to infinity; CL, total plasma clearance; CL_{R}, renal clearance; CL_{N-d}, nonrenal clearance; CL_{N-O}, formation clearance; Fe, fraction of dose excreted as a urine metabolite from 0 to 24 h.

a P < 0.05 for period B versus period A.

Fleroxacin is known to induce enzymes responsible for increased hepatic drug catabolism. For ciprofloxacin, a rabbit model demonstrated an increased catabolism requiring a quinolone dose adaptation (4). However, no clinically significant change in ciprofloxacin pharmacokinetics in elderly volunteers (48) or in elderly patients (7) was observed. In one of these trials, Weinstein et al. (48) excluded a significant rifampin effect, but the experimental protocol included a very short rifampin induction time (2 days). Different results were obtained with a pefloxacin-rifampin combination. In eight healthy volunteers a significant interaction was observed, but dosage modification was not required (24).

In a recent clinical study, Dworkin et al. (16) successfully treated right-sided S. aureus endocarditis in parenteral drug users with a combined regimen of ciprofloxacin and rifampin. One disadvantage of ciprofloxacin is its rapid elimination (\( t_{1/2} \approx 4 \) h). Fleroxacin has a more prolonged half-life and its levels may be maintained continuously above the MIC for methicillin-susceptible staphylococcal strains (1 \( \mu g/ml \)) (37) during therapy with a single 400-mg daily dose. It was therefore necessary to assess whether coadministration of rifampin would alter the hepatic clearance of fleroxacin and thus its pharmacokinetic properties.

Our study was not designed as a crossover because the time required for reversion of the increased hepatic enzyme activity is dependent on each individual and therefore no standard duration for the restoration of baseline status can be predicted.

Pharmacokinetic parameters of fleroxacin in the initial period were similar to those previously reported. For rifampin, the increased urinary output of 6-β-hydroxy cortisol confirms that each healthy young volunteer underwent a significant hepatic enzyme induction. The results indicate a significant pharmacokinetic influence of rifampin on fleroxacin. This interaction may be considered to be moderate, with an increase in CL of 15%. As CL_{R} was not affected, the mechanism of interaction seems to be mainly an induction of metabolic pathways leading to N-demethyl-fleroxacin. Whereas N oxidation is dependent on flavoproteins (31), N-demethylation is predominantly mediated by cytochrome P-450 (41). Without any hepatic enzyme induction, cumulative urinary excretion of the major fleroxacin metabolites remains in a narrow range. Estimates of N-oxide-fleroxacin vary in the literature from 5.7% ± 0.8% of the total dose for 72 h (\( n = 6 \)) (21) to 6.3% ± 1.7% (\( n = 12 \)) (41); the N-demethyl derivative represents from 6.6% ± 1.3% (\( n = 30 \)) (33) to 6.9% ± 2.0% (\( n = 6 \)) (21). Our results before rifampin therapy were similar to those reported previously. Hepatic enzyme induction by rifampin led to a significant increase in N-demethyl metabolite production, whereas N-oxide-fleroxacin clearance remained unchanged. There are presently no available data concerning the influence of other hepatic metabolism inducers (either smoking or other drugs) on fleroxacin pharmacokinetics. It should be emphasized that despite a statistically significant effect of rifampin on the pharmacokinetics of fleroxacin, the extent of that interaction has little clinical importance. It should be noted that the mean minimum concentration in plasma (24 h after a 400-mg fleroxacin dose and upon 600-mg daily rifampin treatment) is around 1.0 mg/liter, which corresponds to the minimal concentration inhibiting 90% of strains of methicillin-susceptible S. aureus and Staphylococcus epidermidis. The present results now allow the concomitant administration of fleroxacin and rifampin in clinical trials, without any a priori dose modification of fleroxacin.

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