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Continuous versus intermittent infusion of temocillin, a directed spectrum penicillin for intensive care patients with nosocomial pneumonia: stability, compatibility, population pharmacokinetic studies and breakpoint selection

Raf De Jongh[1], Ria Hens[1], Violetta Basma[2], Johan W. Mouton[3], Paul M. Tulkens[2]* and Stéphane Carryn[2]

[1]Dienst Voor Intensieve Zorgen, Ziekenhuis Oost-Limburg, B-3600 Genk, Belgium; [2]Unité de Pharmacologie Cellulaire et Moléculaire, Université Catholique de Louvain, B-1200 Bruxelles, Belgium; [3]Afdeling Medische Microbiologie en Infectieziekten, Canisius Whilhemina Ziekenhuis, NL-6500 GS Nijmegen, The Netherlands

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***Background and aims*: Temocillin, a 6α-methoxy-penicillin stable towards most β-lactamases (including extended-spectrum β-lactamase), is presented as an alternative to carbapenems for susceptible Enterobacteriaceae in microbiological surveys. We aimed at documenting its potential clinical usefulness in intensive care (IC) patients using pharmacokinetic/pharmacodynamic approaches applied to conventional (twice daily) and continuous infusion (CI) modes of administration.**

***Methods*: (i) *In vitro* evaluation of temocillin stability and compatibility with other drugs under conditions pertinent of CI in IC patients; (ii) pharmacokinetic study in patients treated by CI (4 g/day; $n = 6$) versus [twice daily (2 g every 12 h); $n = 6$]; (iii) population pharmacokinetic analysis of twice daily with Monte Carlo simulations to determine 95% probability of target attainment (PTA_{95}) versus MIC (based on time above MIC $\geq$40% for measured free drug).**

***Results*: Temocillin was stable at 37°C in 8.34% solutions for 24 h and compatible with flucloxacillin and aminoglycosides, but not with several other antibiotic and non-antibiotic drugs. With CI, stable total serum concentrations were 73.5 $\pm$ 3.0 mg/L (SEM) and free concentration 29.3 $\pm$ 2.8 mg/L. With twice daily, C_{max} (total drug) was 147 $\pm$ 12.3 mg/L (SEM; free drug: 50.3 $\pm$ 15.8 mg/L), lowest trough (total drug) 12.3 mg/L, and PTA_{95} (free drug) obtained for MIC $\leq$8 mg/L.**

***Conclusions*: Temocillin (4 g/day) by CI yields stable free serum concentrations above the current breakpoint (16 mg/L), although individual variations may suggest lowering the breakpoint to 8 mg/L (as for twice daily) unless the daily dose or the frequency of administration is increased.**

Keywords: HPLC, Monte Carlo simulation, target attainment

Introduction

Empirical therapy of nosocomial pneumonia requires careful optimization of antibiotic administration. For β-lactams, the fraction of the dosing interval during which the free serum concentration exceeds the MIC of the offending organism ($fT >$ MIC) is the main parameter determining the treatment outcome.[1] Administration of β-lactams by continuous infusion (CI) has, therefore, been advocated for difficult-to-treat situations[2] and tested in several indications including nosocomial pneumonia.[3–5]

Temocillin is a 6α-methoxy-penicillin active against Enterobacteriaceae and resisting to most β-lactamases[6,7] [including extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases[8,9] and carbapenemases[10]]. It is a potential alternative to carbapenems in infections when and where ESBL producers and other cephalosporin-resistant strains have become prevalent as long as *Pseudomonas aeruginosa* can be excluded,[8] but clinical data are still scarce. We have examined the potential of temocillin for administration via CI in nosocomial pneumonia in comparison with its conventional, twice daily mode of administration. On the basis of previous experience of CI with other

*Corresponding author. Tel: +32-2-764-7371; Fax: +32-2-764-7373; E-mail: tulkens@facm.ucl.ac.be

β-lactams,[11–13] we first examined the stability of temocillin and its compatibility with other common medications used in the intensive care (IC) patients. As the number of patients that could be recruited in a reasonable time frame was small, we performed a population pharmacokinetic analysis and a Monte-Carlo simulation[14] to calculate target attainment probabilities[15,16] and determine pharmacokinetic/pharmacodynamic (PK/PD) breakpoints.

Materials and methods

Stability and compatibility studies

Based on the temocillin registered daily dose (4 g) and on practical experience with the preparation of β-lactam solutions for CI in IC units[13] (daily dose prepared in a volume of 48 mL), stability was tested at a concentration of 83.4 g/L in water (Milli-Q Academic Ultrapure® Water System, Millipore Corp., Bedford, MA, USA) at temperatures ranging from 20 to 37°C for up to 24 h and under exposure to normal room light. To detect incompatibilities related to the infusion of temocillin with other drugs through a common infusion set,[11,13] temocillin and each other drug were mixed at those concentrations susceptible to be observed in a common infusion line (following conventional conditions of administration) and left at 25°C for 1 h before being examined for physical and chemical compatibilities as described previously.[11,13]

Assay of temocillin

Temocillin was assayed by HPLC with ticarcillin as internal standard and detection of both epimers of the two drugs[17] [see Supplementary data available at *JAC* Online (http://jac.oxfordjournals.org/) for details].

MIC determination

MICs were determined using standard CLSI procedure for Enterobacteriaceae with *Escherichia coli* ATCC 25922 as control organism.[18]

Overall design of the clinical study, patient selection and treatment modalities

The study was prospective and randomized but not blinded, and the protocol approved by the *ad hoc* Ethics Committee (Ziekenhuis Oost-Limburg, Genk, Belgium). Inclusion criteria were (i) a high probability of infection from nosocomial origin [body temperature >38 or <35.5°C not induced by external factors; leucocytosis or leucopenia; one or several suspected infection foci (based on X-ray pathognomonic image, purulent sputum, white blood cells in urine, or other accepted clinical sign)]; and (ii) no suspicion of an infection by *Pseudomonas* spp. or another temocillin-resistant bacteria. Exclusion criteria were (i) age <18 or >75 years; (ii) patient's weight <50 or >100 kg; (iii) renal insufficiency (estimated clearance <45 mL/min); (iv) haemodialysis; (v) estimated survival <5 days; (vi) documentation of temocillin-resistant organism; (vii) meningitis or other proven infections of the CNS; (viii) IgE-mediated allergy to penicillins; (ix) severe granulocytopenia (<500 polymorphonuclear leucocytes/mm^3); (x) pregnancy; (xi) patients having participated in another study <30 days before; and (xii) retrospectively, marked deterioration of the renal function during the study period. No patient was included more than once. All patients were categorized using APACHE II and SOFA scores. Patients received temocillin according to the following schemes: CI, loading dose (2 g) administered in 30 min in 50 mL of *pro injectione* water followed by infusion [4 g in 48 mL of *pro injectione* water infused at a rate of 2 mL/min (2.78 mg/min)]; twice daily, 2 g temocillin (in 50 mL of *pro injectione* water) every 12 h injected over a 30 min period. All patients also received flucloxacillin (six times 1 g/day).

Sample collection and preparation for analysis

Under CI, samples were withdrawn 1, 2, 3, 6, 12, 24, 48, 72, 96 and 120 h after loading dose; twice daily 1, 2, 3, 6 and 12 h after the first administration on day 0 and just before and after the ninth administration on day 4. All samples were taken from arterial catheter or from an infusion-free upper extremity. Serum (obtained by centrifugation after blood clotting) was frozen (−70°C) until analysis. Total antibiotic was extracted by a solid-phase method (OASIS® HLB Extraction Cartridge System, Waters Corp.; typical recovery, 95% to 97%). The free fraction of temocillin was measured on serum ultrafiltrates (Centrifree® devices, Millipore Corp.).

Pharmacokinetic analyses, population pharmacokinetics and calculation of probability target attainment rate

For patients treated twice daily, a one-phase exponential decay function was fitted to the data (GraphPad Prism version 4.03; GraphPad Software, San Diego, CA, USA), and individual AUC_{0-24} and $fT > MIC$[19] values were determined by graphical intrapolation. For data from patients treated by CI, AUC_{0-24} was calculated at steady state by intragraphical integration of values obtained >12 h after the initial loading dose. Population pharmacokinetic modelling was performed with WinNonMix® software (Pharsight Corp., Mountain View, CA, USA) using a one-compartment open model. Population pharmacokinetic parameters[20] were estimated from patients treated twice daily and used to derive the value of $fT > MIC$ of free drug as a function of the MIC.[21] Hereto, a Monte Carlo approach was applied using the full covariance matrix (MICLAB version 2.33 program, Medimatics, Maastricht, The Netherlands) and a log-normal distribution of parameters to simulate 10 000 subjects for each regimen, and the results were used to calculate the median and 95% confidence interval of the $fT > MIC$ over MIC (0.25–128 mg/L).[22]

Statistical analyses

Stability data were analysed by one-way analysis of variance with the Tukey–Kramer multiple comparisons test for individual differences within groups (GraphPad InStat version 3.01, GraphPad Software), patient data with two-tailed unpaired *t*-test (with Welsh correction) or with Student's *t*-test for parametric data (GraphPad Instat), and with Wilcoxon's test for non-parametric data (JMP 5.1, SAS Institute Inc., Cary, NC, USA).

Materials

Temocillin and ticarcillin were obtained as NEGABAN® (Eumedica s.a., Brussels, Belgium) and TIMENTIN® (GlaxoSmithKline Belgium, Rixensart, Belgium), respectively. All other drugs were procured as described previously.[11,13] Products for chromatography were of HPLC grade and obtained

from Sigma-Aldrich Corp. (St Louis, MO, USA) or E. Merck AG (Darmstadt, Germany).

Results

Laboratory studies

Stability studies. Temocillin solutions maintained at temperatures up to 37°C showed <2% loss in total drug content with only minor (13%) change in the *R* to *S* epimer ratio [see Supplementary data available at *JAC* Online (http://jac.oxfordjournals.org/)].

Compatibility studies. Table 1 shows the results of the compatibility studies. Among antibiotics, flucloxacillin, cefuroxime, aminoglycosides (gentamicin, tobramycin and amikacin), erythromycin and moxifloxacin were compatible. All other β-lactams tested caused a >10% loss of temocillin. Vancomycin, clarithromycin and clindamycin were physically incompatible, and iprofloxacin was chemically incompatible. Fluconazole was compatible. All sedatives, anticonvulsants and analgesics tested were compatible, except propofol, midazolam and piritramide. Among the other drugs tested, only nicardipine, milrinone, ranitidine and vitamin K were incompatible.

Clinical study

Demography and clinical outcomes. The twice daily and CI groups were similar in terms of demographic and disease- and treatment-related characteristics and drop-outs unrelated to the treatment (Table 2). The clinical outcome of all patients was favourable with no temocillin-related adverse effect (including drug incompatibility or neurotoxicity).

Pharmacokinetic and microbiological studies. The temocillin serum concentrations (free and total drug) from patients treated twice daily or by CI are shown in Figure 1, with MIC and key pharmacokinetic parameters presented in Table 2. For the twice daily group, these parameters were consistent with previously reported values for patients with normal renal function.[23] For CI, the total concentration levels stabilized after ~12 h (the first peak being due to the administration of a loading dose of 2 g) to a mean total drug value of 73.5 ± 3.0 mg/L [SEM; see Supplementary data available at *JAC* Online (http://jac.oxfordjournals.org/) for values of individual patients]. The percentage of free drug was 23.7 ± 6.15 (SD) for patients treated twice daily and 29.3 ± 2.8 (SD) for patients treated by CI ($P = 0.022$; 95% confidence interval of the difference: 0.96–10.28).

PK/PD modelling and calculation of probability of target attainment. For patients treated twice daily, a one-compartment model best fitted to the data (total drug), with estimates of 14.3 ± 0.87 L for the volume of distribution (V) and 0.172 ± 0.059 L/h for the elimination constant (k_{el}; corresponding to a mean half-life of 4.03 h), with good correlation between predicted and observed concentrations [see Supplementary data available at *JAC* Online (http://jac.oxfordjournals.org/)]. These parameter estimates and the actual data on free drug percentages were used to predict the free concentrations during CI (at steady state), which was 19.5 versus 21.5 mg/L measured (mean).

A Monte Carlo simulation for target attainment ($fT >$ MIC) for the twice daily group was then performed using 25% average free drug. Figure 2 shows that an $fT >$ MIC of 40% or more will be reached with 95% probability for an MIC slightly above 8 mg/L [to move this value to 4 mg/L (one dilution), the actual free fraction of temocillin should have been as low as 15%]. Simulating 2 g every 24 h (less than recommended) or 2 g every 8 h (off-label but often used by clinicians) gave an $fT >$ MIC >40% for isolates, with MICs of 4 and 16 mg/L, respectively, for the mean values of the population [see Supplementary data available at *JAC* Online (http://jac.oxfordjournals.org/)].

Discussion

The present study is a first approach to better delineate the potential usefulness of temocillin in IC patients based on a PK/PD approach and examining its use by both discontinuous infusion and CI. The data were also used to rationally define potential breakpoints for temocillin.

In discontinuous administration, the value of the $fT >$ MIC parameter ensuring *in vitro* bacteriostatic effect and animal survival was found to be between 29% and 34% for penicillins against a variety of target organisms.[1] In the absence of experimental data for temocillin, we used a cut-off value of 40% as a precaution (this value has also been shown to be sufficient for ceftazidime against *P. aeruginosa*,[24] a drug–bacteria combination requiring an optimal antibiotic administration at least as effective as the temocillin/Enterobacteriaceae combination). We also know that the value of $fT >$ MIC parameter is not influenced by the presence of resistance mechanisms such as ESBL (of importance for temocillin) as the MIC includes that information.[25] On this basis, the Monte-Carlo simulations for temocillin, given at 2 g every 12 h, suggest a clinical breakpoint at an MIC of 16 mg/L (originally proposed as epidemiological cut-off)[26] if using the median values, but at 8 mg/L to cover the 95% confidence interval. An ongoing survey of MIC distributions of temocillin towards Enterobacteriaceae based on data published over the last 25 years (data on file, available upon request) shows that about 90% of all isolates have MIC ≤8 mg/L (confirmed by two recent independent surveys of ESBL-producing clinical isolates[9,27]).

The interest of administering β-lactams by CI has been repeatedly advocated,[1] but still needs support from both laboratory and clinical studies, which are presented here for temocillin.

We first show that the stability of temocillin largely exceeds the requirements of the European Pharmacopeia[28] even if stored at 37°C, which is in contrast to ceftazidime (>10% degradation in 8 h with the release of pyridine if stored above 25°C),[11,12] cefepime (>10% degradation in 8 h at temperatures >25°C, with appearance of so far unidentified coloured products if using commercial preparations),[13] or imipenem and meropenem (>10% loss in 3–5 h).[12] The change in *R*/*S* epimer ratio, although significant, is probably unimportant as it remains in a range within which activity is not affected.[29] We, however, document several incompatibilities with drugs often required for patients hospitalized in IC units. As drug incompatibilities are difficult to predict (and may vary among β-lactams),[11,13] clinicians need to seek specific information on all drugs they wish to use in combination with temocillin (beyond what is recorded

Table 1. Temocillin compatibility with other drugs under conditions mimicking their co-administration through the same line of infusion[a]

Drug	Dose (mg)[b]	Volume per administration (mL)	Time of infusion (h)	Drug/temocillin weight ratio	Result
Anti-infectives					
flucloxacillin	1000	4	0.33	18	c
cefuroxime	1500	50	0.5	18	c
amoxicillin/clavulanate	2000	100	0.5	24	i (chem)
piperacillin/tazobactam	4000	20	0.25	96	i (chem)
ceftazidime	2000	100	0.25	48	i (chem)
cefepime	2000	10	0.25	48	i (chem)
imipenem	1000	200	0.5	12	i (chem)
meropenem	2000	40	0.25	48	i (chem)
vancomycin	1500	50	1	9	i (phys)
erythromycin	100	20	0.33	1.8	c
clarithromycin	500	10	0.33	3	i (phys)
gentamicin	600	100	0.25	14.3	c
tobramycin	600	100	0.25	14.3	c
amikacin	1500	100	0.25	36	c
ciprofloxacin	400	200	0.5	4.8	i (chem)
moxifloxacin	400	250	0.5	4.8	c
clindamycin	1200	100	0.67	10.8	i (phys)
fluconazole	200	100	0.5	2.4	c
Sedatives/anticonvulsants/analgesics					
ketamine	480	48	24	0.12	c
propofol	300	300	24	0.07	i (phys)
thiopental	1	200	0.167	0.036	c
midazolam	600	120	24	0.15	i (phys)
valproic acid	1200	12	24	0.3	c
phenytoin	750	15	0.25	18	c
morphine	5	5	1	0.03	c
sufentanil	0.12	24	24	3×10^{-5}	c
piritramide	10	5	1	0.06	i (phys)
tramadol	100	2	0.25	2.4	c
paracetamol	1000	100	0.25	3	c
Bronchodilators					
theophylline	200	1	0.33	3.6	c
Antihypertensive drugs/vasodilators/other drugs acting on the sympathetic system					
nicardipine	120	120	24	0.03	i (phys)
nimodipine	200	1000	24	0.05	c
milrinone	75.6	378	24	0.018	i (chem)
uradipil	2400	480	24	0.6	c
isosorbide dinitrate	6	30	1	0.036	c
furosemide	960	96	24	0.24	c
dopamine	0.4	1	0.016	0.143	c
dobutamine	0.84	0.84	0.016	0.302	c
epinephrine	0.5	10	0.33	9×10^{-3}	c
Antacids					
ranitidine	50	20	2	0.15	i (chem)
omeprazole	40	100	0.33	0.72	c
Hormones					
insulin	60 IU	0.6	3	0.12 IU/mg	c
methylprednisolone	500	10	0.5	6	c
Anticoagulants					
enoxaparine	60	0.6	0.016	21.5	c

Continued

Table 1. *Continued*

Drug	Dose (mg)[b]	Volume per administration (mL)	Time of infusion (h)	Drug/temocillin weight ratio	Result
Antihaemorrhagics					
vitamin K	2	20	0.016	0.72	i (chem)
Miscellaneous					
N-acetylcystein	10 000	100	24	6.94	c
amino acid solution	18 000	1000	24	4.5	c

c, chemically and physically compatible; i, incompatible [phys, physically incompatible (precipitate and presence of particles as shown by particle analyser); chem, chemically incompatible (<90% recovery and >10% loss of antibiotic compared with nominal content)].
[a]Temocillin was assumed to be administered at a daily dose of 4 g and to be infused at a rate of 2.78 mg/min.
[b]Calculated (when appropriate) for a 70 kg male subject.

Table 2. Characteristics of patients and microbiological, pharmacokinetic and PD/PK parameters

	Twice daily	CI
Recruitment[a]		
no. of patients enrolled	10	7
no. of evaluable clinical records	6	7
no. of patients with evaluable laboratory samples	6	6
Patient biometric parameters[b]		
age (years)	56 ± 9	58 ± 8
sex (M/F)	4/2	5/1
Clinical scores at initiation of therapy[b]		
APACHE II	13 ± 2	12 ± 2
SOFA	7 ± 2	8 ± 1
Creatinine clearance at initiation of therapy (mL/min)[b]	94 ± 11	102 ± 18
Type of infection[b]		
pneumonia (ventilated at initiation of therapy), *n*	6 (3)	6 (4)
urinary tract infection, *n*	1 (co-infection)	0
Duration of therapy (days)[b], mean (range)	8.8 (6–13)	8.5 (6–11)
Clinical outcome[b]		
clinical outcome considered successful, *n*	6	6
survival at 28 days, *n*	6	6
Microbiological and PK/PD parameters[b]		
MIC (mg/L), median (range)	7 (2–16)	6 (2–16)
AUC_{24} (mg·h/L)[c,d]	1856 ± 282 (1018–2752)	1759 ± 188 (1395–2639)
half-life (h)[c]	4.3 ± 0.3 (3.8–5.3)	NA
clearance (mL/min)[c]	40.7 ± 6.5 (24.2–65.4)	39.7 ± 3.4 (25.2–47.8)
C_{max} (mg/L)[c]	147 ± 12 (85–223)	NA
C_{min} (mg/L)[c]	28.2 ± 4.5 (12.3–63.0)	NA
C_{ss} (mg/L)[c,d]	NA	73 ± 3 (40–142)
free C_{min}/highest MIC	1.09 ± 0.33 (0.51–2.6)	NA
free C_{ss}/highest MIC	NA	1.91 ± 0.39 (1.18–3.80)
percentage of dosing interval (mean) during which the free drug concentration remains above the highest MIC[d]	51	100

Unless otherwise stated, values are given as means ± SEM (with ranges of mean values when appropriate).
CI, continuous infusion; NA, not applicable.
[a]Reasons for drop-outs: twice daily, progressive haemodynamic instability with multiple organ failure due to dramatic neurological deterioration (1), temocillin administration fault (1), antibiotic change due to the presence of non-invasive *P. aeruginosa* (2); and CI retrospectively excluded because of an undetermined substance interfering with the assay of temocillin in all plasma samples (1).
[b]Limited to the patients with evaluable laboratory samples (twice daily, $n = 6$ and CI, $n = 6$).
[c]Total drug.
[d]Using equilibrium values only (all data points >12 h post-initiation of the infusion).

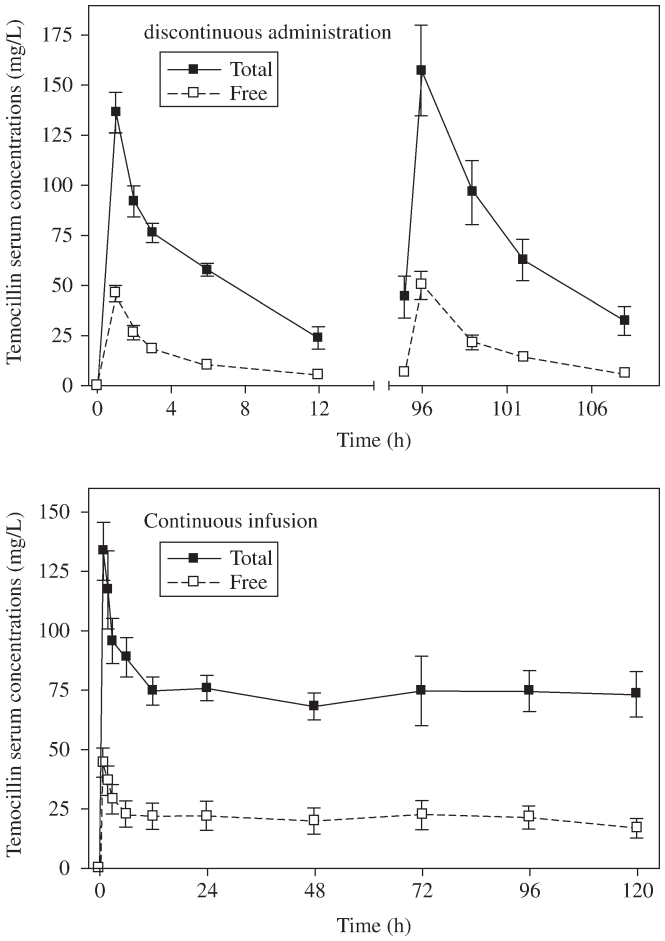

Figure 1. Serum concentrations (total and free) of temocillin in patients receiving temocillin. Upper panel: patients received 2 g every 12 h (discontinuous administration) with samples taken after the first administration (0–12 h) and 1 h before and after the ninth administration (95–108 h). Lower panel: patients received a loading dose of 2 g followed by a CI at a rate of 4 g per 24 h (CI). All values are means ± SD ($n = 6$ for each treatment group).

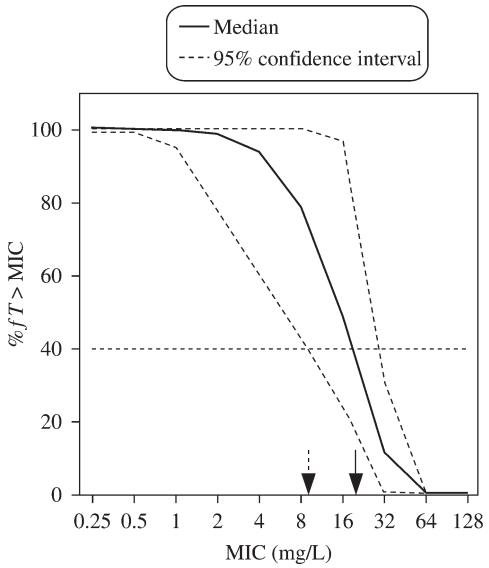

Figure 2. Probabilities of target attainment of temocillin (as obtained with the Monte Carlo simulation: solid line, median value; dotted lines, 95% confidence interval) for the currently registered treatment (2 g every 12 h), using the pharmacokinetic data of the six patients treated according to this dosage and schedule in this study (twice daily group). The abscissa shows the MIC range used for the simulations and the ordinate the fraction of time (as a percentage) during which free serum levels remain above the corresponding MIC. The horizontal dotted line indicates the 40% $fT >$ MIC limit achieving a bacteriostatic effect and survival for penicillins in animal models with Gram-negative bacteria.[1] The highest MIC at which this target will be obtained is shown by the vertical arrows (arrow with solid line, median; arrow with dotted line, 95% probability).

here), if administration through a common line is envisaged. Secondly, we show that stable levels can be obtained and used to assess the potential efficacy of temocillin. Although *in vitro* studies suggest that the free serum concentration at steady state (C_{ss}) must be greater than or equal to four times the MIC for maximal bactericidal effect,[30,31] a static effect (obtained by definition at one times the MIC) may be sufficient *in vivo* if the patients are not immunocompromised. This would set a susceptibility breakpoint for temocillin (4 g/day) slightly above 16 mg/L. Because of inter-individual variabilities in serum levels, however, a more conservative limit of 8 mg/L seems appropriate. These variabilities are, actually, quite puzzling, as the observed mean value at equilibrium (73 mg/L) was close to the predicted one [64 mg/L; based on the temocillin average clearance (~40 mL/min)[20,23] and the rate of infusion used]. Similar inter-individual variabilities in serum levels have been observed for ceftazidime[32,33] and are not specific to CI (as variations in C_{max} in twice daily patients are quite as large). Thus, serum monitoring of β-lactams may be more necessary than originally thought, especially with isolates having MICs close to PK/PD breakpoints. CI makes it easy to perform because sampling time is not critical (if disregarding the post-loading dose period).

Our study could not be powered to provide direct information about the clinical outcomes of the administration of temocillin by CI versus twice daily treatment. With Temocillin being a niche product, conventional, large-scale clinical studies are indeed ethically and economically difficult to perform in a reasonable time frame. The Monte Carlo simulation approach, however, allows one to draw a general conclusion out of a limited patient sample and to predict target attainment rate values that integrate interpatient variability in drug exposure, drug potency and *in vitro* susceptibility data.[34] This study may, therefore, provide guidance for a prudent use of temocillin by CI based on its PK/PD properties if the susceptibility profile of the target organisms is carefully assessed.

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Transparency declarations

S. C. is working under contract with Eumedica s.a., Brussels, Belgium, and R. D. J. and P. M. T. are unpaid advisors to Eumedica s.a., Brussels, Belgium. The rest of the authors do not have any conflicts to declare.

Supplementary data

Supplementary data are available at *JAC* Online (http://jac.oxfordjournals.org/).

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Supplementary data

Table S1. Stability of temocillin in concentrated aqueous solution (8.34% w/v; corresponding to a daily dose of 4 g in a 48 mL infusion syringe) at increasing temperatures maintained for 24 h.

Temperature (°C)	Total (% of original amount)	R/S epimer ratio
20	102.8±1.1^{A}	1.908±0.015^{A}
25	101.5±0.7^{A}	1.792±0.011^{B}
30	101.5±2.6^{A}	1.729±0.024^{C}
37	98.1±0.3^{B}	1.660±0.002^{D}

Samples were analysed by HPLC with differential detection of the *R* and *S* epimers (see Figure S1).
Data are means±SD (*n*=3).
Note that a drug loss upon storage ≤10% fulfills the requirements of the European Pharmacopeia [see Note for guidance on Manufacture of the Finished Dosage Form (CPMP/QWP/486/95), pp 1-6. The European Agency for the Evaluation of Medicinal Products (EMEA), London, UK].

Statistical analysis [one-way analysis of variance (ANOVA)]:
Analysis by column: for total (% of original amount), P=0.02; for R/S epimer ratio, P<0.0001.
Analysis for individual differences within columns (Tukey-Kramer Multiple Comparisons Test): figures with different letters are significantly different from each other (at P<0.05 or less).

Table S2. Fraction of time (%) during which the free serum concentration (median values) remains above a given MIC (%*f*T>MIC) after administration of 2 g of temocillin every 24 h (q24 h; once daily], every 12 h (q12 h; BID) or every 8 h (q8 h; TID); results obtained by Monte Carlo simulation starting from the pharmacokinetic data of the patients treated BID in the present study

	*f*T>MIC (in %) of free temocillin (2 g) given		
MIC (mg/L)	q24 h	q12 h	q8 h
0.50	100.00	100.00	100.00
1.00	87.50	100.00	100.00
2.00	70.60	100.00	100.00
4.00	53.70	100.00	100.00
8.00	36.70	79.70	100.00
16.00	19.40	45.10	80.30
32.00	1.60	9.50	26.90
64.00	0.00	0.00	0.00

Figure S1. Assay of temocillin. Chromatographic profile of temocillin as typically observed in serum samples from untreated patients and spiked with temocillin. TMO, temocillin; TIC, ticarcillin (added as an internal standard: *R*, *R* epimer; *S*, *S* epimer). Inset: correlation between the ratio of the combined areas of the temocillin (both epimers) to the ticarcillin (both epimers) peaks and the actual concentration of temocillin in blank serum samples spiked with increasing concentrations of temocillin. Both epimers were used for concentration calculations. Specific conditions for chromatography: LiChrospher 100 RP-18 5 µm column (Merck AG); elution buffer 100 mM Na acetate buffer pH 7/acetonitrile (95:5, v/v); flow rate 1 mL/min; Waters 2690 Alliance System (Waters Corp., Milford, MA, USA), with quantification at 235 nm.

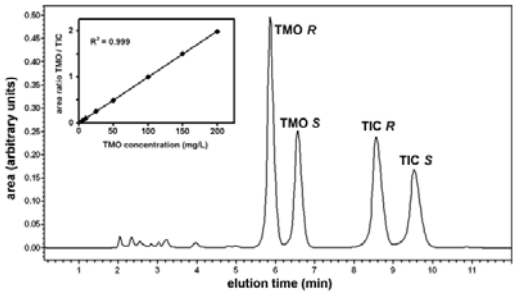

Figure S2. Total serum concentration of temocillin in individual patients treated by continuous infusion and measured 12, 24, 48, 72, 96 and 120 h after the initiation of the infusion. The horizontal bars show the means of the corresponding individual values.

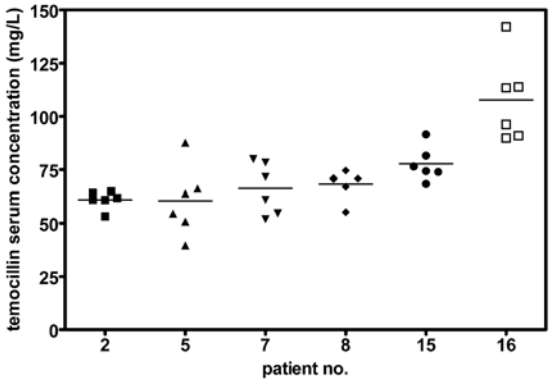

Figure S3. Correlation between the observed and predicted total serum concentrations in patients treated with the BID mode of administration. The prediction was based on a one-compartment model (V, 14.3±0.87 L; k_{el}, 0.172±0.059 L/h).