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Original Article

DOSE OPTIMIZATION OF CEFTRIAXONE-SULBACTAM COMBINATION IN ADULTS USING *IN VITRO* SYSTEMS, PK/PD MODELING AND STOCHASTIC SIMULATIONS APPROACHES

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ABSTRACT

Objective: To optimize the dosage regimen of fixed-dose combination (FDC) of ceftriaxone/sulbactam (2/1 w/w) using *in vitro* system, pharmacokinetic/pharmacodynamic (PK/PD) modeling and Monte-Carlo simulations (MCS).

Methods: One compartment *in vitro* system was used for identification of PK/PD driver that best correlates with therapeutic potential of FDC against ESBL positive *E. coli* infection. Using *in vitro* approach, the best exposure from dose escalation study was fractionated twice-a-day (BID) and thrice-a-day (TID) to determine a best dosage regimen of the FDC. In second approach i.e. *in silico* PK/PD modeling, dose response curve was constructed to estimate curve parameters (EC_{50}, γ, and E_{max}), which were then used to develop PK/PD model for the FDC. In the third approach, MCS were employed to evaluate the impact of different dosage regimen against mild-to-severe infections. Lastly, the recommendation of dose adjustments for patients with renal impairment was also presented.

Results: Based on all three approaches, the best antibacterial effect was obtained from the exposure of 20 x MIC_{comb}, which when fractioned to twice-daily dosing showed a maximum reduction in bacterial densities for severe infections. Dose reduction was recommended for patients with several renal impairments.

Conclusion: FDC dosage regimen of 1.5g BD or 3g OD was recommended for mild to moderate infections; whereas 3g BD was required for severely infected patients.

Keywords: PK/PD modeling, Monte-carlo simulations, Ceftriaxone, Sulbactam, Dose optimization

INTRODUCTION

Extended-spectrum beta-lactamases (ESBLs) and metallo-β-lactamases (MBL) represent major worldwide threat among resistant bacterial isolates, with its alarming prevalence in Europe and South-East Asian countries [1, 2]. Mono-therapy with a carbapenem is a currently available treatment against which increased in cases of resistance have been reported [3]. The limited therapeutic options coupled with dried pipeline of drug development guided us to look for alternatives. However, the scarcity of alternative options for monotherapy lead us to see beyond the effects of β-lactams and thus other non-antibiotic compounds like adjuvants have been studied for their activity along with antibiotics against ESBL-/MBL-producing isolates.

The current fixed dose combination (FDC) of ceftriaxone/ sulbactam/Na_2EDTA (2/1/0.074 w/w/w) was developed on same lines; where ceftriaxone is a base beta-lactam antibiotic and sulbactam is a beta-lactamase inhibitor; providing protection against ESBL and extending the antibacterial spectrum to cover *Acinetobacter baumanni* [4]. Additionally, the FDC has a non-antibiotic adjuvant ethylenediamine tetraacetate (EDTA), which further extends its anti-bacterial effect against MBLs. Mechanistically; ceftriaxone inhibits bacterial cell wall synthesis following attachment to penicillin binding proteins (PBPs) and exerts *in vitro* activity against a wide range of gram-negative and gram-positive microorganisms [5]. Sulbactam prevents β-lactamases induced inactivation of ceftriaxone through irreversible binding to the enzymes and prevents its further interaction with ceftriaxone. Therefore, sulbactam addition broadens the antibacterial spectrum of ceftriaxone. Also, third component i.e. EDTA [non-antibiotic adjuvant] synergise FDC action by chelating divalent ions produced by MBLs, reducing efflux transporter expression, inhibiting the conjugal transfer of resistant gene, eradicating bacterial biofilm, and inhibition of curli formation [6].

After carefully selecting pharmacodynamics options for a FDC, the next step is dose optimization by identifying PK/PD relationships. The dosing regimens of antibiotics are based on MIC estimates (a surrogate PD marker for antibacterial response characterization) and the quantification of exposure (changes in the concentration of an individual component of FDC)-response (the reduction of bacterial count) relationship. The MIC estimates mainly explains antibiotic-infection relationship; whereas the exposure-response (E-R) relationships identifies the PK/PD drivers which directly affect the therapeutic potential of anti-bacterial agents in clinical conditions. Maximum literature for these kinds of PK/PD studies is available for single antibacterial agents [7-9]; the effect of combination therapy on the PK and PD, and subsequently on PK/PD indices is very limited [10].

The objective of the present study was to predict the therapeutic efficacy of combination therapy and optimize the dosage regimen of the FDC formulation (fig. 1). Thus, *in vitro* studies were integrated with fractional inhibitory concentrations (FIC) approach to identify the optimum exposure and PK/PD driver of FDC under the specified conditions. The information was then utilized for dose optimization using *in vitro* method, PK/PD modeling and Monte-Carlo simulations (MCS). All three methods use same pieces of information as their input but vary in the specificity of output. The MCS, being least specific, have also employed population-PK parameters of ceftriaxone and sulbactam to carry out stochastic simulations, and for dose recommendation. Dose adjustment in case of renal impairment was also presented in the last section of the study.

MATERIALS AND METHODS

Compound, microorganism, and media

The FDC of ceftriaxone/sulbactam/Na_2EDTA (2/1/0.074) vials (Elores®) was obtained from Venus Pharma GmbH, Germany. Genetically characterized ESBL isolates (NCTC 13353; CTX-M-15) with reduced susceptibility to ceftriaxone were taken from isolate bacterial bank of Venus Medicine Research Centre, Baddi, India. Media Mueller-Hinton broth (Becton Dickinson, Sparks, MD) was

used to perform all *in vitro* studies involving MIC determination, bacterial kinetics, dose ranging, fractionation and response studies.

Bacterial density, minimum inhibitory concentration, and fractional inhibitory concentration index determinations

Bacterial density (colony forming units [CFU]), AST and MIC determinations were conducted according to Clinical Laboratory Standard Institute (CLSI) guidelines [11]. FIC index was determined using checkerboard method.

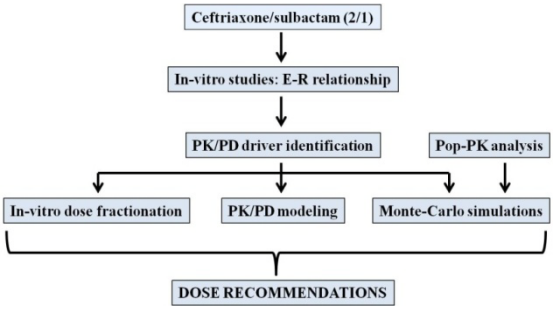


Fig. 1: Schematics of the approach used in the study. *In vitro* studies were performed to study Exposure-Response (E-R) relationships and PK/PD driver identification. Using the information of *in vitro* studies, three approaches i.e. *in vitro* studies, PK/PD modeling, and Monte-Carlo simulations were employed for dose recommendations

***In vitro* system**

One-compartment dilution system

The *in vitro* modeling of FDC was done using a single-compartment chemostat infection system [12]. Briefly, the chemostat system assembly consisted of a 500 ml glass central reservoir chamber with ports for the addition and removal of media via silicone tubes connected to peristaltic pumps, injection of drug (antibiotic combination) solution, and removal of medium samples. Prior to each experiment, ESBL colonies were grown overnight to obtain a starting inoculum of 10^8 CFU/ml in 500 ml central reservoir flask containing media. An aqueous solution of FDC was prepared in accordance with dilution advised by the manufacturer. The "in" and "out" flow rates from the central reservoir were adjusted as per the half-lives of active components. Samples from the central reservoir were collected at different time points from 0 to 24 h post-FDC addition. These samples were analyzed for individual component concentrations and CFU determination for bacterial densities. The bacteria growth control experiments were also performed using the same experimental set-up without adding any drug. Net PD effects of all doses of FDC were then reported after accounting the bacterial dynamics of the growth control experiment. Pre-and post-FDC exposure, MICs were determined for evaluating any changes in MICs due to FDC exposure or changed bacterial dynamics.

Hollow fiber system

The selected drug exposures were also evaluated in hollow fiber system [13]. Initial inocula of 10^8 CFU/ml was achieved by injecting 10 ml of ESBL producing *E. coli* into extra-capillary space, which was separated from the central reservoir by semi-permeable hollow fibers. After 2 h of incubation in media, FDC was injected into the central reservoir. The drug can freely cross back and forth between the extra-capillary space and central reservoir so that ESBL *E. coli* were exposed to same drug concentration as those in the central compartment. Central compartment was connected to inlet and outlet reservoir through a pump whose flow rates were adjusted as per the half-lives of active components. Samples were collected from extra-capillary space for three days and were analyzed for drug concentration and bacterial densities.

Dose escalation, fractionation and dose response curve studies

The *in vitro* chemostat model was used to perform dose escalation studies. The drug concentrations were varied from 1-200 folds MICs to determine the most efficacious exposure based on their PK/PD parameters. The best exposure from dose escalation study was then fractionated twice-a-day (BID) and thrice-a-day (TID) to determine a dosage regimen of antibiotic which can enhance bacterial killing at a lower concentration. The promising dosage regimen was then employed to construct dose-response curve (DRC). The dosage regimen at 80%, 50%, and 33% of original concentration, and the corresponding change in bacterial density in 24 h was measured. The DRC was plotted between the dose percentage and log reduction in bacterial density to calculate EC_{50} (concentration required to produce half of the PD response).

Pharmacokinetic analysis

The concentrations-time profiles were fitted to one compartment model and PK parameters of ceftriaxone and sulbactam were calculated. The parameters included the area under the plasma concentration-time curve (AUC), highest concentration reached (the peak; Cmax), elimination rate constant (K_e), half-life ($t_{1/2}$), the volume of distribution (V_D), and clearance (CL). These parameters were calculated for all dose escalations and dose fractionation studies. The parameters obtained from concentration profile data were compared with reported PK parameters in the literature (Table 1).

Pharmacokinetic/pharmacodynamic analysis

The FIC curves for ceftriaxone and sulbactam combinations were generated. Briefly, the concentrations of each antibiotic at every time-point were divided by their respective MIC contributions towards MIC_{comb} to obtain $FIC_{ceftriaxone}$ and $FIC_{sulbactam}$ values. These values were added (FIC_{comb}) for each time points and plotted against time. The resultant FIC-time profile was fitted to one compartment model to obtain PK/PD parameters i.e. AUC_{comb}, $C_{max\text{-}comb}$, $\%T>MIC_{comb}$ and $\%T>FIC_{comb}$.

Pharmacokinetic/pharmacodynamic modeling

In one compartment chemostat infection model, bacterial infection is dynamically exposed to an antibiotic formulation. During this time course, drug act on bacterial infection, and decrease the bacterial density. Since, the additional filter was not placed in the outlet of the *in vitro* chemostat system to check the bacterial elimination; the resultant bacterial loss was compensated in the model. A schematic illustration of PK/PD model is shown in fig. 2.

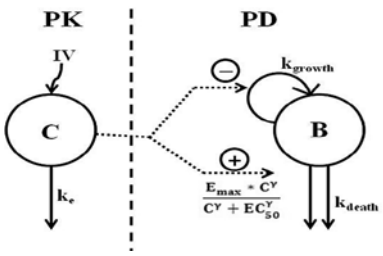


Fig. 2: Schematics of the pharmacokinetics (PK)/pharmacodynamic (PD) model. The drug administered intravenously (IV) into one compartment *in vitro* system and follow first order elimination kinetics. Drug was in direct contact with bacterial infection (B), where it reduces the bacterial density either by inhibiting the growth rate (K_{growth}) and/or increase death rate (K_{growth}) of bacteria following sigmoidal-E_{max} model

Semi-mechanistic pharmacokinetic/pharmacodynamic modeling and validation

a. Bacterial modeling

The bacterial kinetics determination involves modeling of a single bacterial compartment (B) with first-order rate constants for bacterial multiplication (k_{growth}) and bacterial death (k_{death}) [14]. The equation 1 explains the observed exponential growth of bacteria

until it reaches a stationary bacterial level without the addition of antibiotics (control experiments); where B and B_{max} are initial bacterial density and maximum bacterial density, and k_{net} is (k_{growth} - k_{death}).

$$\frac{dB}{dT} = k_{net} * (1 - \frac{B}{Bmax}) * B \dots\dots\dots\dots(1)$$

b. Pharmacokinetic/pharmacodynamic modeling

The effect (E: bacterial load: PD endpoint) was evaluated at 8, 9.5, 12 and 24 h. The relationship between the effect and the corresponding PK/PD indices was evaluated according to a sigmoidal E_{max} type function as described in equation 2 [14]:

$$E = E_0 - \frac{PD_{max} * X^{hill}}{X^{hill} + EX_{50}{}^{hill}} \dots\dots\dots\dots (2)$$

Where, E is the PD endpoint i.e. bacterial density calculated as change in $\log_{10}$CFU/ml after 8, 9.5, 12 or 24 h of treatment, E_0 is the baseline effect i.e. PD endpoint without antibiotic treatment; X is PK/PD index; PD_{max} is a maximum effect; EX_{50} is magnitude of X that is needed to achieve 50% of the PD_{max}; Hill is the sigmoidicity factor, reflecting the steepness of the relationship.

All data from the different dosing regimens was fitted to model mentioned above (equation 2). Curve fitting was performed in GraphPad Prism (version 4.01, GraphPad Software, San Diego, CA) using the non-linear regression analysis. The coefficients of determination (R^2), sigmoidicity factor, and the visual inspection of observed versus predicted values graphs were used to select the best PK/PD index and the best-predicted endpoint of antibacterial effect.

c. Anti-bacterial-PK/PD modeling

The basic assumption for the dependence of antibacterial effect is generally based on a non-linear relationship with concentration data of antibiotics. Higher the sigmoidicity factor, lesser is the predictability of PD effect with respect to PK/PD index. The concentration-effect relationships were incorporated in the bacterial model (equation 1) to predict the bacterial count from PK/PD model (equation 3). In the constructed model, the antibacterial effect of FDC of ceftriaxone/sulbactam is hypothesized as a combination of bacterial growth inhibition (k_{growth}) and bacterial killing enhancement (k_{death}) [14].

$$\frac{dB}{dT} = k_{net} * B - (\frac{E_{max} * C^{\gamma}}{C^{\gamma} + EC_{50}{}^{\gamma}}) * B \dots\dots\dots\dots (3)$$

Model validation

All data from the different drug exposure and dosage regimen was fitted to model mentioned above (equation 3) for the time period of 8 and 24 h respectively using SCIENTIST (MicroMath, version 3.0, Saint Louis, Missouri, USA). The 95% confidence intervals were drawn for all the predicted values and compared with the observed values (obtained from *in vitro* system). Additionally, coefficients of determination (R^2) between predicted and observed data values were determined and evaluated for the validation of the antibacterial-PK/PD model.

Monte-Carlo simulations

Monte-Carlo simulations for 1000 adult subjects were performed to determine how likely the FDC dose of 0.75, 1.5, 3, 6, and 9 g would achieve 70% $T>MIC_{comb}$ at different values of MIC_{comb} i.e. 1, 4, 8, 16, 32, 64 µg/ml. The population-PK parameters (CL and K_e) were obtained from population-PK model of the FDC and were utilized in simulations. The concentration-profiles of once-a-day and twice-a-day dosing regimens were generated using CL and K_e of each simulated subjects and $\%T>MIC_{comb}$ was calculated for all simulated subjects for different exposures at all MIC values. The PTA (target PTA minimum 90%) was then defined as the percentage of simulated subjects showing $\%T>MIC_{comb}$ of more than 70.

Data analysis

Descriptive statistics were used for reporting all PK variables and summary tables were prepared using mean, standard deviation (SD), median, and range (whichever appropriate). Log transformed data was used wherever applicable. The statistical analysis was done using GraphPad Prism (version 4.01, GraphPad Software, San Diego, CA).

RESULTS AND DISCUSSION

Dose optimization and its recommendation mainly depend on upon its pharmacological (including PK and PD) properties of a drug. The complexity increases with the addition of another active drug in the formulation, similar to the one presented in the study. The current formulation is a FDC of ceftriaxone and sulbactam in 2:1 (w/w), mainly used for ESBL infections. The synergism of antibacterial effect of both drugs results in a reduction of MIC from 256 to 8 µg/ml against bacterial strain NCTC 13353 (ESBL bacterial strain employed in this study). Also, FIC index of the FDC was less than 0.5 which confirms the pharmacodynamic synergism offered by FDC.

MIC selection for *in vitro* studies using microbiological studies

The ESBL producing *E. coli* isolates were used as a prototype of ESBL infections. Dose optimization of the FDC was performed using *in vitro* studies, PK/PD modeling and Monte-Carlo simulations. For *in vitro* studies, target MIC needs to be selected against which therapeutic effect of drug combination could be evaluated. The same information was also needed for other two approaches i.e. PK/PD modeling (for dose individualization based on bacterial density) and Monte-Carlo simulations (for dose recommendation based on MIC). Thus, the MIC selection was carried out using microbiological studies of ESBL isolates.

A total of 515 strains of ESBL producing *E. coli* were collected from different Microbial Collection Centres across the country. Clinical breakpoints were determined using CLSI guidelines [11] and MIC distribution of bacterial isolates of *E. coli* were evaluated (fig. 3). As shown in fig. 3, more than 90% ESBL bacterial isolates exhibiting MIC of less or equal to 8 µg/ml were susceptible to FDC treatment. Also, MIC 8 µg/ml was at the borderline of clinical breakpoints of susceptible and intermediate bacterial strains, which means that the exposure-response relationship and resistance emergence (if any) against the FDC could be better identified at the given MIC. Therefore, MIC of 8 µg/ml was selected to carry out *in vitro* studies for dose optimization of FDC.

Identification of optimum exposure against a given MIC

In vitro studies for dose, optimization requires PK and PD inputs to evaluate the PK/PD of a given exposure of drug against an infection. For PK input, PK parameters reported in the literature [15-16] for ceftriaxone and sulbactam (table 1) were utilized. The PD target i.e. MIC of 8 µg/ml was selected using microbiological studies, as explained in above section. One compartment *in vitro* chemostat system with first order elimination was designed for dose optimization of FDC. The PD effect was defined as a logarithmic reduction in ESBL bacteria densities over 24 h period.

The ESBL infection was treated with six different exposures (1-200 folds of MIC 8 µg/ml) of FDC for 24 h to find PK/PD driver that can predict the PD effect. Another objective was to find the lowest exposure that can give maximal PD effect against the infection. The drug concentration-bacterial CFU-time profiles were obtained from *in vitro* chemostat model. Focusing on PK aspects, the PK parameters calculated in *in vitro* chemostat infection model for individual drugs were in concordance with reported literature values (table 1), suggesting successful reproduction of clinical conditions using *in vitro* system.

In terms of PD effect, bacterial killing increase with an increase in drug exposure till the saturation is achieved (fig. 4a). After saturation (>20 fold MIC for 9.5 h; >100 fold MIC for 24 h), there was no substantial increase in antibacterial effect. Dose of 100 x MIC was quite high, which can potentially affect therapeutic window of the drug combination. However, the dose of 20 x MIC (106.67 µg/ml of ceftriaxone and 53.33 µg/ml of sulbactam) was the minimum exposure for which maximum PD effect (~5 fold reduction) was observed after 9.5 h of drug administration; and moderate (~2 fold reduction) bacterial killing was seen post-24 h of drug treatment. Therefore, the exposure (20 x MIC) was selected for further dose optimization studies.

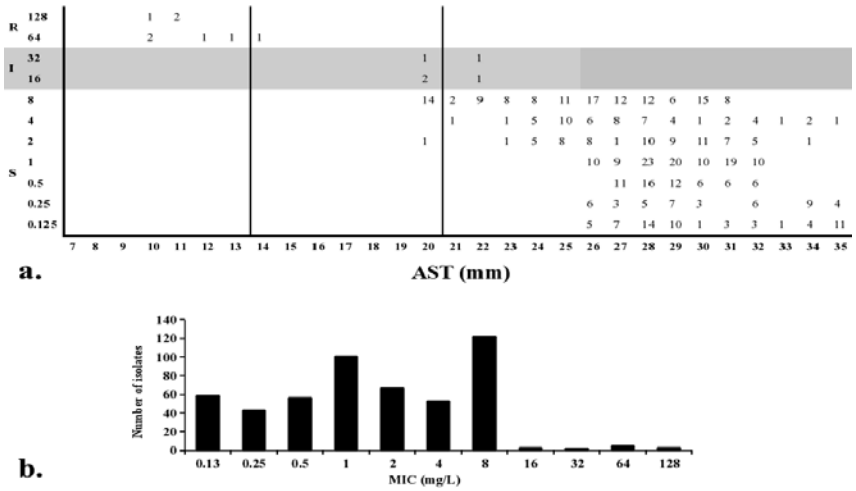


Fig. 3: MIC selection for *in vitro* studies using microbiological studies. (a) The plot of MIC values of various strains against the zone diameter for E. coli and segregated based on the MIC breakpoints of 8 and 32 mg/l; (b) The distribution of the bacterial isolates of E. coli based on their MICs. R: Resistant, I: Intermediate, S: susceptible

Table 1: Pharmacokinetic parameters of ceftriaxone and sulbactam employed in the study [15-16] and their comparison with the values estimated from *in vitro* one compartment dilution system

Parameters	As reported in literature		Estimated from *in vitro* system	
	Ceftriaxone	Sulbactam	Ceftriaxone	Sulbactam
Clearance (L/h)	0.88	11.90	1.13	11.10
Volume of distribution (L)	10.1	22.82	12.3	20.87
Half life (h)	5.9	0.97	7.42	1.5

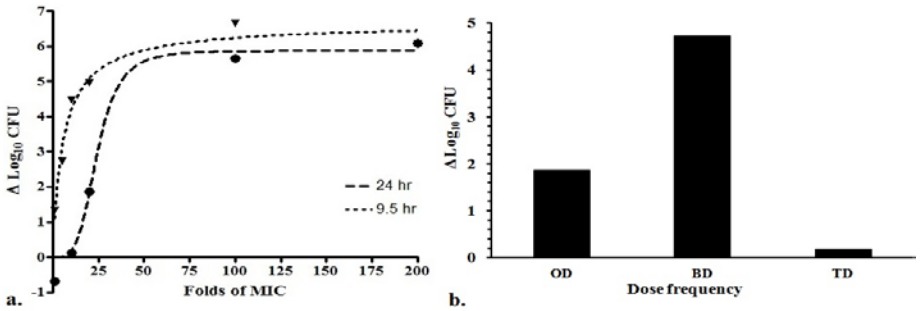


Fig. 4: Selection of dosage regimen using dose escalation (a) and dose fractionation (b). As shown above in dose escalation (a), exposure of 20x MIC was optimum and thus selected for further dose fractionation (b) in which the dose was divided into twice-a-day (BD) and thrice-a-day (TD) regimen and corresponding change in bacterial density (Δ $\log_{10}$ CFU) was observed

Identification of PK/PD driver for therapeutic outcome

Further dose optimization of FDC requires an understanding of PK/PD driver responsible for the therapeutic potential of the FDC. For instance, if the FDC's PD effect is exposure (AUC)-dependent, it is recommended to increase both dose (increase C_{max}) and dose frequency (increase %T>MIC) to increase the anti-bacterial response of FDC. Thus, identification of PK/PD driver of the FDC was then carried out using sigmoidal-E_{max} model [14]. Since, the present formulation is a combination of drugs, fractional inhibitory concentrations (FIC) approach was used for PK/PD analysis in order to combine the contribution of both drugs as per their concentration and MIC contribution in the FDC [10]. Briefly, FIC-time profiles were generated from concentration-time profile of both drugs for all doses as described above (See 'PK/PD analyses' in 'Materials and Method' section). The PK/PD indices i.e. AUC_{comb}, $\%T>MIC_{comb}$ and %T>FIC were calculated from the FIC-time curves. All PK/PD indices were plotted against the logarithmic reduction in bacterial densities of *E. coli* ESBL strain for 8, 9.5, 12 and 24 h. The time periods of 8, 12 and 24 h were chosen considering TID, BD and OD regimen of the FDC. The time point of 9.5 h was included because the second inoculum was added at this time point which might affect the net reduction in bacterial density. The PD effect was found to be better correlated with 9.5 h and 24 h time point as compared to 8 and 12 h, thus shown here in fig. 5.

The objective was to find the PK/PD index that can predict the antibacterial effect of the FDC as a single unit. From FIC-t profiles, AUC_{comb}, $\%T>MIC_{comb}$, and %T>FIC were calculated and plotted against bacterial killing post 9.5 h and 24 h drug exposure (fig. 5). The data was fitted with a sigmoidal-E_{max} type function (see 'materials and methods', equation 2) and selection of best PK/PD driver was done based on coefficient of correlation, and sigmoidal factor. Sigmoidal factor is the steepness of the sigmoidal curve and represents the predictability of the model. Higher the steepness, lesser is the predictability of the model. It must be noted that the initial concentration or $C_{max\text{-}comb}$ is pre-decided when the drug was injected in *in vitro* system. Higher coefficients of correlation of $\log C_{max\text{-}comb}$ vs. log ΔCFUs were obvious and, thus not considered to identify the PK/PD driver of the current FDC.

Focusing on results, higher correlation coefficient were obtained after 24 h drug treatment as compared to ones after 9.5 h [compare correlation coefficients after 9.5 h (fig. 5a) vs 24 h (fig. 5b), i.e. 0.91

vs 0.98 for log AUC_{comb}; and 0.87 vs 0.98 of %T>MIC_{comb}]. The %T>FIC was poorly correlated with bacterial reduction. Focusing on 24 h drug exposure, the R^2 is highest for %T>MIC_{comb} i.e. 0.9880 with high predictability (γ = 3.38). The good R^2 of 0.9873 was also obtained from the plot of logAUC_{comb} vs log ΔCFU, but steepness was quite high, which restricts the predictability of the model (γ =14.25; average predictability) (fig. 5b). The high value of sigmoidicity factor suggests all-or-none function of the system and thus decreases the predictability of model. Therefore, % T>MIC_{comb} was considered the best PK/PD driver followed by AUC_{comb}, to predict the PD effect of FDC in the study.

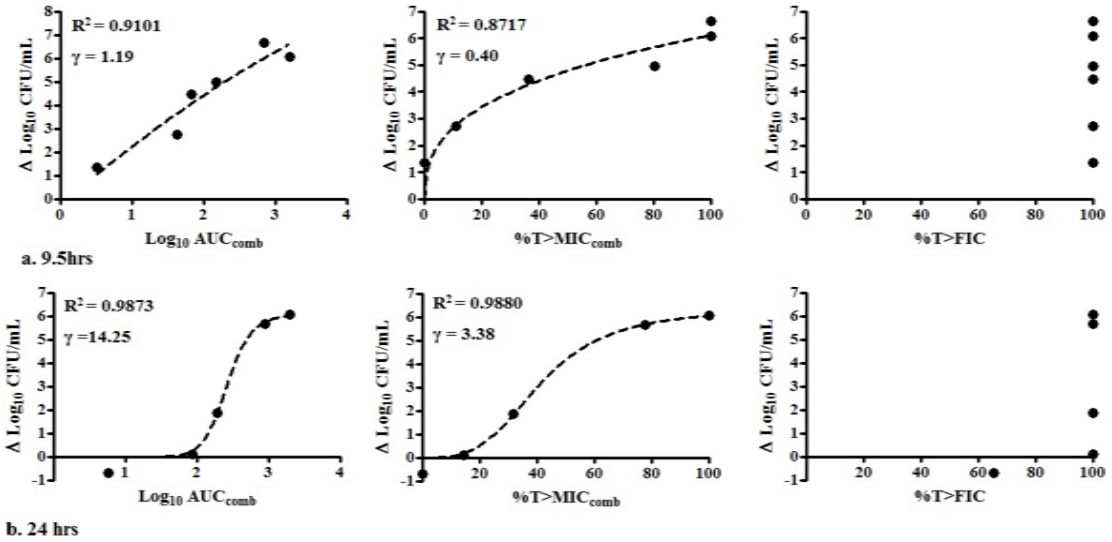


Fig. 5: Selection of PK/PD drivers of the FDC using fractional inhibitory concentration (FIC) approach. Three PK/PD drivers i.e. $Log_{10}AUC_{comb}$, %T>MIC_{comb}, and %T>FIC were plotted against corresponding pharmacodynamic effect after 9.5 h (a) and 24 h (b) [measured as Δ log_{10} CFU]. The sigmoidal E_{max} model was employed to find the best fit for the observed data to identify PK/PD driver of the FDC

After selection of PK/PD driver (i.e. %T>MIC), the next objective was to identify a desired value of %T>MIC that one should target to assure good therapeutic effect of the FDC. Thus, plot of %T>MIC vs. Δ log_{10}CFU was evaluated to estimate desired %T>MIC value (fig. 5 b-ii). It was clearly observed that PD effect of the FDC become saturated after 70 %T>MIC, prompting us to select 70 as a target value of PK/PD i.e. %T>MIC for dose optimization. In other words, one can assure good therapeutic effect of the FDC, if the drug concentration in body remains above MIC for more than 70% of the time of drug exposure.

The identification of PK/PD driver of FDC and its desired value was further used for dose optimization and recommendation. Three approaches were used i.e. *in vitro* approach, PK/PD modeling and Monte-Carlo simulations. All three approaches use same piece of information and give useful information for dose optimization and vary from each other in terms of specificity of information. The first approach (*in vitro* approach) mainly tells about the therapeutic potential of a certain drug under a specific MIC. The PK/PD model is generally employed for dose individualization; whereas, Monte-Carlo simulations broadly recommends dose regimen based on MIC of the target infection. All three approaches were utilized and explained below.

Dose optimization using *in vitro* system

a. One-compartment dilution system

The time-dependence of FDC to exhibit its PD effect, prompted us to fractionate the dose, with an objective to increase the duration of exposure (above MIC) and decrease the overall concentration (to reduce side effects). As explained above, the dose exposure of 106.67/53.33 µg/ml (ceftriaxone/sulbactam) was selected for dose fractionation. Considering the patient compliance, antibiotic half-life, and MIC of drug against *E. coli* ESBL strains, dose was fractionated to mimic a dosage regimen of once-a-day (OD), twice-a-day (BID), and thrice-a-day (TID). Similar to dose escalation study, the *in vitro* dilution system was used for these dosage regimens and results are shown in fig. 4b. The antibacterial effect of the FDC was increased from once-a day dosing to twice-a-day dosage regimen in 24 h time period (~2 fold to ~5 fold reduction in bacterial density). Further fractionation of dose (i.e. TID) decreases the drug concentration below MIC for longer time period and thus reduces the time of exposure during which the drug concentration remains above the MIC (only 0.2 fold reductions in bacterial density). The selected dosage regimen roughly corresponds to 3.0 g FDC of ceftriaxone (2 g) and sulbactam (1 g), after correcting for protein binding of 80% and 38%; and volume of distribution of 10.1 L and 18-25 L of both components i.e. ceftriaxone and sulbactam respectively (Table 1). The MIC values remained same during 24 h drug-bacterial incubation period for all drug exposures (data not shown), thus ruling out the possibility of antimicrobial resistance development in *E. coli* ESBL isolates during *in vitro* studies.

b. Hollow fiber system

In vitro dilution system is considered good for evaluating exposure-response relationship of a drug. However, it is sub-optimal in mimicking clinical conditions where bacterial suspension is concentrated in a small volume with no bacterial loss. Therefore, hollow fiber system was employed to confirm the results of 3 g BD dose of FDC, obtained from *in vitro* dilution system for. As expected, similar results were obtained from hollow fiber system, where the bacterial killing of 3 log reductions was observed for 3 g twice-a-day regimen. The relatively lower bacterial reduction in case of hollow fiber system as compared to *in vitro* dilution system was a result of more rigorous and closer-to-reality set-up of hollow fiber system; where the bacterial infection was kept in extracellular matrix secluded from the central compartment through a semi-permeable membrane [13]. The hollow fiber set-up elevates the severity of bacterial infection, manifested as lower CFU reduction, by preventing bacterial loss and providing a favourable condition for resistance development. The results of both *in vitro* systems have shown similar antibacterial efficacy for 3.0 g BD dosage regimens in adults. To summarize, a dose of 3 g BD is to be fractionated into twice-a-day dosage regimen to maintain the same therapeutic efficacy while keeping the possibilities of adverse events low.

Dose optimization using PK/PD modeling

a. PK/PD model development

The PK/PD model was developed using equation 3 of sub-section

section 'antibacterial-PK/PD modeling' under 'Materials and Method' section, which was a differential equation with one independent (T) and two dependent variables (B and C). The remaining variables were K_{net}, E_{max}, EC_{50} and γ. The bacterial net growth (K_{net}) was determined from growth kinetics of bacteria without adding drug. For the determination of E_{max}, EC_{50} and γ, dose-response curves (DRC) were constructed and analyzed using *in vitro* dilution system.

The dose-response relationship is an important aspect of any drug as it directly relates the dose of the drug with its pharmacological action. Depending upon the therapeutic action needed, the dose can be adjusted to enhance therapeutic effects using the dose-response relationship. ESBL infections were exposed to 33%, 50%, 80% and 100% of a dosage regimen in *in vitro* chemostat system and the corresponding logarithmic change in bacterial densities of-2.3, 1.0, 3.8 and 4.7 were observed respectively. The DRC was plotted (fig. 6) and the data was fitted with sigmoidal function; and EC_{50} of 49 % (defined as a percentage of dose required to attain the 50 percent of PD effect), γ of 5.06 (steepness of DRC curve) and E_{max} of 5.7 (maximum therapeutic effect displayed by FDC) was calculated. All these parameters were employed to develop a semi-mechanistic antibacterial bacterial-PK/PD model.

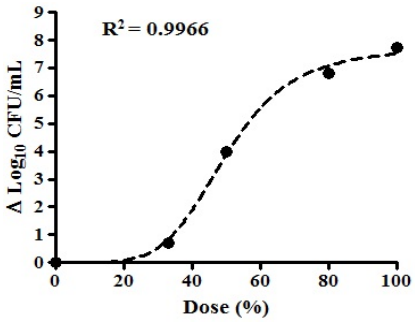


Fig. 6: Dose-response curve (DRC) of the FDC was plotted between different fractions [0, 33, 50, 80 and 100%] of FDC 3.0 g BD and the corresponding changes in bacterial densities to calculate EC_{50} and gamma (γ) value. The net therapeutic effect of all dose fractions was increased by 2 units to convert all negatives CFU values [corresponding to lower drug concentrations] into positive values

b. PK/PD model validation

The final PK/PD equation was validated to check its suitability in dose recommendation using SCIENTIST. The experimental data of dose escalation studies was fitted using equation 3. The goodness of fit and 95% confidence interval of simulated data were evaluated for first 8 h (before the addition of the second inoculum). Simulations after 8 h of drug exposure were not shown in the case of dose-escalation study in order to focus mainly on initial exposure of drug, where most of the bacterial killing occurred. The semi-mechanistic-PK/PD model has moderately predicted the drug exposure. For instance, R^2 values for the fitted values of dose exposures of 10x, 20x and 100x MIC were 0.7508, 0.7154 and 0.8914 respectively (fig. 7-I) suggesting good predictability of model under given exposures. Additionally, most of the experimental data points were under 95% confidence interval of simulated data, which further supports the validity of PK/PD model.

In the case of dose fractionation, dose frequency was the main factor; thus model predictions were extended to 24 h. The observed values of experiments and predicted values of the model were plotted for all three dosage regimens i.e. once-a-day, twice-a-day and thrice-a-day (fig. 7-II). The addition of the second inoculum at 9.5 h had raised the CFU counts, as shown by a downward peak in all three cases. Also, the model had weaker predictability at lower concentrations of the drug, as observed in PD prediction during 20–24 h for all three dose frequencies of FDC. Therefore, the weaker correlation was observed in all three cases as compared to dose escalation studies. The correlation coefficient was increased from 0.4506 of once-a-day dosing to 0.6361 of twice-a-dosing of the FDC of antibiotics. Further increase in dose fractionation drastically reduced the correlation coefficient to 0.2703, which suggests the model weakness in the prediction of lower drug concentrations. However, the model was appropriate for the twice-a-day dose frequency, which was also the best dosage regimen for FDC according to *in vitro* results. The same conclusions were derived from 95% confidence interval of simulated data. Most of the data of twice-a-day dosing were in the upper and lower limits of the confidence interval, followed by once-a-day dosing (fig. 7-II). As expected in the case of thrice-a-day dose frequency, predicted values were poorly correlated with the experimental values.

Dose optimization using monte-carlo simulations

The *in vitro* studies were done using the fixed values of primary PK parameters and MIC; which did not account for variability in the clinical population. Therefore, Monte-Carlo simulations were performed in order to account for the randomness in human PKs and MICs. The probability of target attainment (PTA) was defined using *in vitro* results, which is a percentage of simulated subjects showing $\%T>MIC_{comb}$ of more than 70 (fig. 8). The objective was to identify the antibiotic exposure that can give maximum bacterial killing with minimal side effects.

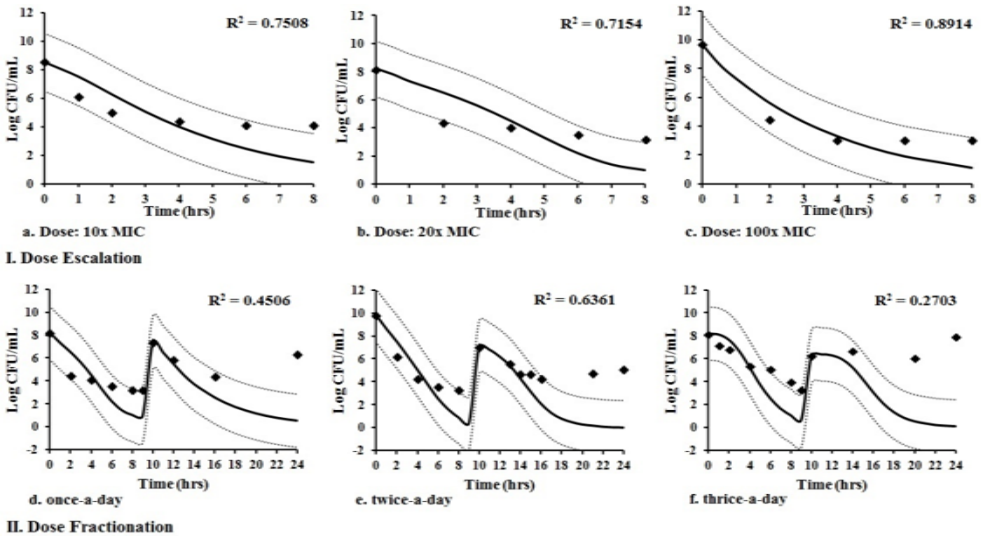


Fig. 7: Validation of PK/PD model for the data of dose-escalation (I) and dose fractionation (II) studies using SCIENTIST. Weaker predictions were observed at a lower concentration of FDC. Dotted lines represent 95% confidence interval of the predicted values, whereas the solid lines represent prediction by the final PK/PD model. Solid squares represent the observed values obtained from *in vitro* studies

Monte-Carlo simulations of 1000 concentration-time profiles were performed using population PK parameters estimated from both populations (healthy and infected) combined. The population PK parameters utilized in simulations were CL of 0.75±0.2 L/h and 16.52±4.63 L/h for ceftriaxone and sulbactam respectively [17]. The K_e was set to 0.09 and 0.51 for ceftriaxone and sulbactam respectively. The FDC in a dose range of 0.75 to 9 g as OD and BD regimen were used to calculate PTA over a range of MIC_{comb} (1-64 µg/ml) (fig. 7). The FDC was noted to be very effective with PTA of ~100% at dose strength≥ 1.5 g OD and ≥0.75g BD for the strains corresponding to MIC of 1 and 4 µg/ml. For MIC 8 µg/ml, doses less than 3g were not effective. Even 3 g OD achieved PTA~80%, which can be improved to 95% when the same exposure was divided into two doses (1.5 g BD). Further increasing the dose to 3 g BD maximized the PTA to 100%. For MIC 16 µg/ml, OD regimen was ineffective as the maximum PTA achieved at highest 9 g OD dose was only 35%. The FDC was ineffective at all drug exposures (PTA~0-5%) for the MIC of 32 and 64 µg/ml.

In summary, a high PTA (≥90%) for a target 70%T>MIC with MIC ≤8 µg/ml was observed with 1.5 g BD dose; and with same total exposure in OD dose (3 g), ~80% PTA was attained. The dosage regimen of 3g BD showing PTA of 100% indicates the improvement in response if required, in cases of severe infections. This claim was further supported from the clinical data of cUTI patients, where >90% cure was achieved with 3 g BD FDC dose [18]. The results were also in conformance with the dose optimization study of the FDC in paediatrics [19].

Recently, Sharma et al. have reported cost effectiveness of the FDC at the same dosage regimen of 3 g BD over meropenem treatment [20].

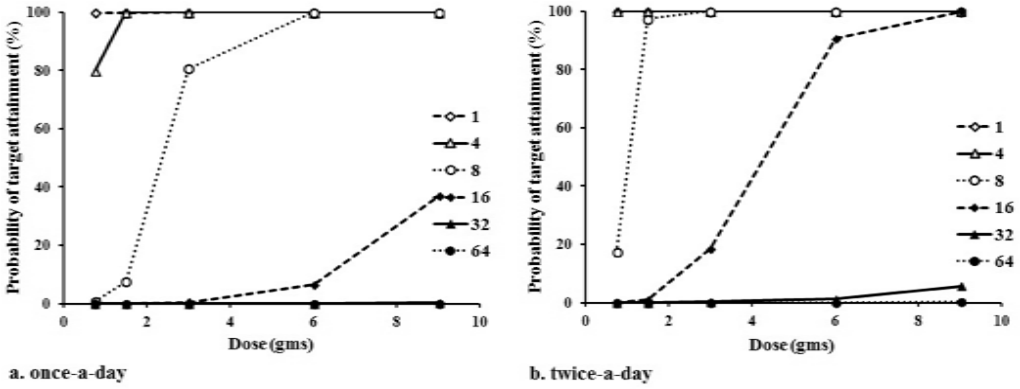
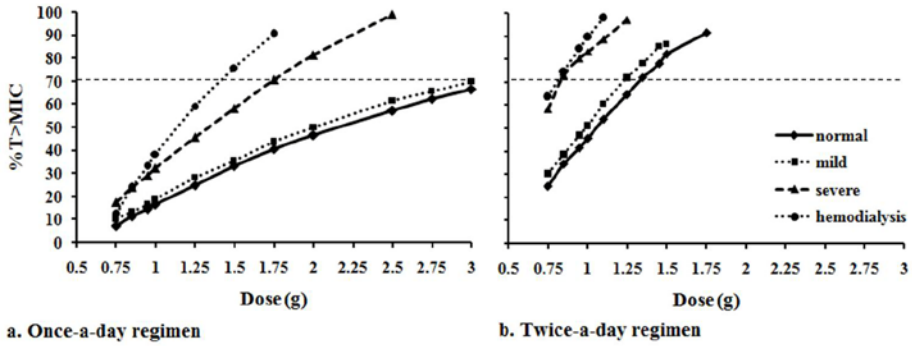


Fig. 8: Monte-Carlo simulations of 1000 subjects were performed and PTA (70 %T>MIC) was calculated for 0.75-9.0 g once-a-day (a) and twice-a-day (b) dosage regimen of FDC against MIC of 1-64 µg/ml

Dose optimization for renal impairment

Subjects with renal impairment have reduced renal clearance as per the severity of the disease. Reduction in renal clearance of drug would increase the net drug exposure and thus necessitate dose reduction to avoid adverse side effects without compromising therapeutic effect. Ceftriaxone undergoes 50-60% renal clearance; whereas sulbactam has the renal clearance of 70-80% [15, 16]. Dose adjustments were done based on achieving PK/PD driver i.e. 70% T>MIC using the PK parameters of mild, moderate, severe and hemodialysed patients (fig. 9). The assumption was made that body would not initiate any compensatory mechanism in case of reduced renal clearance. It was observed that only 4-7% dose reduction in both OD and BD regimen was required for mild renal impairment. However, in principle, this minor dose reduction can easily be compensated by the body. Additionally, ceftriaxone was mainly responsible for the therapeutic action of the FDC and had another major route of elimination (biliary excretion).

Therefore, dose reduction was not required for mild renal impairment. However, the same conclusion was not valid for patients with moderate to severe renal impairment. For moderate-to-severe infection, 37-38% dose reduction in OD and BD regimen was recommended. In case of anephric patients, a significant reduction in renal clearance occurs and thus dose should be reduced by 40-50% for the same therapeutic effect. To summarize, there was no need to adjust the dose of the FDC in patients with mild renal diseases; whereas dose reduction would be required for severe renal impairment cases.



Renal impairment	GFR (mL/min)	Once-a-day regimen (% of regular dose)	Twice-a-day regimen (% of regular dose)
Mild	31-60	96	93
Moderate-to-severe	5-30	62	63
Hemodialysis	<5	49	60

Fig. 9: Dose adjustment in renal impairment for once-a-day (a) and twice-a-day (b) regimen

CONCLUSION

Dose optimization of fixed dose combination of ceftriaxone/ sulbactam/Na_2EDTA (2/1/0.074) was performed using three approaches i.e. *in vitro* systems, semi-mechanistic PK/PD modeling and Monte-Carlo simulations. In the first approach (*in vitro* system), the best antibacterial effect was obtained from the exposure of 20x MIC_{comb} which when fractioned to twice-daily dosing showed a maximum reduction in bacterial densities. The second approach (semi-mechanistic PK/PD model incorporating bacterial kinetics and drug's PK/PD relationship) have showed good predictability at therapeutic exposures. Using the third approach (stochastic simulations with PTA of 70%T>MIC), further dose recommendations were made, wherein, 1.5 g BD or 3 g OD can combat pathogens up to MIC of 8 µg/ml; while for combating pathogens with MIC 16-32 µg/ml, 3 g BD would provide benefit. Thus, the study corroborates the validity of antibacterial effect of FDC ceftriaxone/sulbactam/Na_2EDTA (2/1/0.074 w/w/w) which can be extrapolated to "real" clinical population with the unique advantage of giving both components together in similar dosing frequencies.

CONFLICT OF INTERESTS

Declare none

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