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 (EC50, γ, and Emax), which were then used to develop PK/PD model for the FDC. In the third approach, MCS was employed to evaluate the impact of different dosage regimens 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 MICcomb, which when fractionated 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-beta-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 beta-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/Na2EDTA (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 baumannii [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 beta-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] synergize FDC action by chelating divalent ions produced by MBLs; reducing efflux transporter expression, inhibiting the conjugal transfer of resistant gene, eradicate bacterial biofilm, and inhibition of curl 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 explain antibiotic-infection relationships; 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/Na2EDTA (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.

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**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 1 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 (Ko), half-life (1/2), the volume of distribution (Vd), 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 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.

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**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 is 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 ($K_{death}$) 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\text{max} are initial bacterial density and maximum bacterial density, and k_{\text{knet}} is (k_{\text{growth}}-k_{\text{death}}).

\[ \frac{dB}{dt} = \frac{dB_{\text{knet}}}{t} = B \cdot (1 - \frac{B}{B_{\text{max}}}) - B \cdot \ldots \ldots \ldots (1) \]

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 type function as described in equation 2 [14]:

\[ E = E_0 - \frac{E_{\text{PD max}} - E_{\text{PD min}}}{\left(\frac{X}{X_{\text{h/2}}}\right)^{*}X_{\text{h/2}}} \ldots \ldots (2) \]

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, E0 is the baseline effect i.e. PK/PD index without antibiotic treatment; X is PK/PD index; PD_{\text{max}} is a maximum effect; EX_{\text{h/2}} is magnitude of X that is needed to achieve 50% of the PD_{\text{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_{\text{growth}}) and bacterial killing enhancement (k_{\text{knet}}) [14].

\[ \frac{dB}{dt} = \frac{dB_{\text{knet}}}{t} = (k_{\text{growth}}) \cdot B - (k_{\text{knet}}) \cdot B \cdot \ldots \ldots \ldots (3) \]

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_{\text{comb}} at different values of MIC_{\text{comb}} i.e. 1, 4, 8, 16, 32, 64 µg/ml. The population-PK parameters (CL and K_{\text{c}}) 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_{\text{c}} of each simulated subjects and %T>MIC_{\text{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_{\text{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-Carl 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 PK 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 CPS-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 5.333 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.
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 logistic 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-comb is pre-decided when the drug was injected in in vitro system. Higher coefficients of correlation of logC_max-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 coefficients 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. 0.95].
period (~2 fold to ~5 fold reduction in bacterial density). Further fractionation of dose (i.e. TID) decreases the drug concentration dilution system was used for these dosage regimens and results are from once-a-day dosing to twice-a-day dosage regimen in 24 h time shown in fig. 4b. The antibacterial effect of the FDC was increased thrice-a-day (TID). Similar to dose escalation study, the antibacterial 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 talks 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 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
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 3.3%, 50%, 80% and 100% of a dosage regimen in vivo to evaluate the corresponding logarithmic change in bacterial densities of -2.3, 1.0, 3.8 and 4.7 were observed respectively. The DRC was plotted and the data was fitted with sigmoidal function; and EC50 of 49% (maximum therapeutic effect displayed by FDC) was calculated. All these parameters were employed to develop a semi-mechanistic antibacterial PK/PD model.

The dose-response curves (DRC) were constructed and analyzed using in vitro dilution system.

Dose optimization using monte-carlo simulations

The in vivo 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 vivo results, which is a percentage of simulated subjects showing %T>MICcomb of more than 70 (fig. 8). The objective was to identify the antibiotic exposure that can give maximum bacterial killing with minimal side effects.
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 corresponding to MIC of 1-64 \( \mu g/ml \) (fig. 7). The FDC was noted to be very effective with PTA of ~100% at dose strength 1.5 g OD and 0.75 g BD for the strains corresponding to MIC of 1 and 4 \( \mu g/ml \). For MIC 8 \( \mu g/ml \), 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 \( \mu g/ml \), 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 \( \mu g/ml \).

In summary, a high PTA (≥90%) for a target 70%T>MIC with MIC ≤8 \( \mu g/ml \) 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].

**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.

**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 \( \mu g/ml \)

**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 combination (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, which when fractionated 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%>MIC), further dose recommendations were made, wherein, 1.5 g BD or 3 g OD can combat pathogens up to MIC of 6 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/NaEDTA (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

REFERENCES