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Determination of the Predictive PK/PD Parameter for the Efficacy of Friulimicin B in a Murine Staphylococcal Thigh Muscle Infection

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Revised Abstract

Background: Friulimicin B (FRI) is a novel lipopeptide antibiotic and is intended for the treatment of severe infections caused by Gram-positive pathogens. To determine the predictive PK/PD parameters for efficacy, FRI was evaluated in a murine thigh muscle infection caused by *Staphylococcus aureus*.

Methods: Mice were rendered neutropenic by injection of cyclophosphamide. Mice were inoculated into the right hind leg with $5x10^5$ *S. aureus* DSM 11823/mouse. Treatment with FRI was initiated 0.5 h post infection. Mice (n=3) were treated subcutaneously (s.c.) for 24 h with various divided doses (1, 2, 4 or 8). 24 h after start of therapy thigh muscles were removed aseptically and CFUs were determined by plating. Plasma concentrations were also determined after s.c. administration of various doses. For each dose and time point (n=3), the FRI concentration was determined using LC/MSMS. The PK/PD parameters were determined according to AAC 50: 243, 2006. The correlation between efficacy and each of the three PK/PD parameters (AUC/MIC, Cmax/MIC, T>MIC) was determined.

Results: After s.c. administration to mice, FRI showed dose proportional pharmacokinetics. In the thigh muscle infection, FRI demonstrated concentration-dependent killing of *S. aureus* DSM 11823 with a static dose of 9 mg/kg and a maximum reduction in CFU of 4 logs. FRI showed good correlation between CFU-reduction per thigh muscle and AUC/MIC (R-squared value 0.881) as well as Cmax/MIC (R-squared value 0.817) and no correlation to T> MIC. The best predictor for efficacy was AUC/MIC.

Conclusions: The best predictive PK/PD-parameter for efficacy was AUC/MIC while with Cmax/MIC, a slightly less strong relationship with efficacy was seen and no correlation was found to T>MIC.

Introduction

Friulimicin B (FRI) is a novel lipopeptide antibacterial agent that exhibits potent activity against a variety of Gram-positive bacteria[1-4]. It is structurally similar to daptomycin but has a distinct mode of action[5-6]. Its chemical structure is shown in Figure 1.

The aim of the present study was to determine the pharmacokinetic and pharmacodynamic parameters and to identify the predictive PK/PD-parameters for efficacy using a murine thigh muscle infection model.

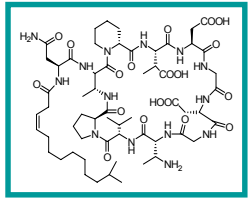

Fig. 1 Friulimicin B

Methods

Bacterial strain: A clinical isolate of *S. aureus,* strain DSM 11823, from the culture collection of Bayer HealthCare AG, Germany was used. The MIC of FRI determined by broth microdilution was 0.425 mg/L.

Antibiotic: FRI provided by Combinature Biopharm AG was used and dissolved in 0.9% NaCl. The specified dosages were related to the free friulimicin acid.

Animals and neutropenia: Female CFW-1 mice (18-20 g body weight) were used (Harlan-Winckelmann, Germany). The animals were kept under conventional housing conditions and rendered neutropenic by injecting intraperitoneally 150 mg/kg and 100 mg/kg of cyclophosphamide on days -4 and -1 before infection or administration of FRI.

Pharmacokinetic studies: FRI was administered subcutaneously (0.1 ml per mice) by single and divided doses (1, 2, 4 h). Samples were collected from 3 mice/time point by cardiac puncture and the concentration of FRI determined by HPLC coupled to a tandem mass spectrometer (LC/MSMS). The assay was linear over the range 100 – 100,000 µg/ml using a volume of 1 µl plasma.

Infection model: Neutropenic mice were infected with 100 µl of a *S. aureus* suspension in physiological saline ($5x10^5$ CFU/mouse) into the thigh muscle of the right hind leg (i.m.). Treatment started 0.5 h post infection (p.i.). Viable bacterial counts in the homogenized thigh muscles were determined at 24 h p.i. by plating serial tenfold dilutions onto Columbia blood agar plates. Bacterial colony forming units (CFU) were counted after overnight incubation of the plates at 37°C.

PK/PD parameter determinations: The PK/PD parameters were determined according to Andes & Craig[7] using 3 mice/time point. Mice were treated for 24 h with 29 varying dosing regimens with total doses of between 1.33 to 170.04 mg/kg in 1, 2, 4, or 8 doses.

For the analysis, the sigmoid dose-effect model was used. The correlation between efficacy (CFU reduction at 24h) and each of the three PK/PD parameters (AUC/MIC, Cmax/MIC, T>MIC) was determined.

Results and Discussion

Pharmacokinetic studies:

Pharmacokinetics of FRI administered subcutaneously to mice by single or multiple dosing were dose proportional between 1.594 and 102.02 mg/kg. There were no differences in the pharmacokinetic profiles after single or multiple administration and a good fit between measured and predicted concentrations using a one compartment model could be demonstrated (Figure 2). Therefore, the pharmacokinetic parameters could be calculated with high precision (Table 1). The predicted PK parameters were then used for the simulation of all concentration vs. time profiles after administration of the different doses and using the different dose regimens. Furthermore, AUC/MIC, Cmax/MIC and T>MIC were calculated from the simulated concentration vs. time profiles.

Infection model - Dose finding study:

Even a low dose of 1.06 mg/kg FRI resulted in a significant reduction of the bacterial load in the thigh muscle infection. Bactericidal killing, a 3 log CFU reduction, was achieved with 5.31 mg/kg s.c. (data not shown).

PK/PD determinations:

In the thigh muscle infection, FRI resulted in a dose dependent reduction of bacterial load (data not shown). A maximum CFU reduction was reached with doses equal or higher than 21.25 mg/kg/24h. Over the whole dose range tested, the efficacy was independent of the dosing frequency (Figure 3).

Results and Discussion

There was a good correlation between Δ-log10 CFU per thigh muscle and AUC/MIC (Fig. 4) as well as between Δ-log10 CFU per thigh muscle and Cmax/MIC (Fig. 5) and no correlation between Δ-log10 CFU per thigh muscle and T> MIC.

The best predictor for efficacy in the thigh muscle is AUC/MIC.

Calculation of kill rate: The AUC/MIC ratios that are necessary to produce units of killing as related to CFU at 24h in untreated controls were calculated according to the formula:

$$AUC / MIC = \sqrt[S]{\frac{ED_{50}^{\ S} \times E}{E_{max} - E}}$$

The following results were obtained:

log units killing	AUC/MIC ratio	Dose [mg/kg]
1	227	~ 3.4
2	408	~ 6.2
3.65*	1520	~ 23

*corresponds to 1 log unit killing as related to the initial inoculum

Maximum efficacy amounts to ~4 log units reduction of CFU after 24 h. Half maximum efficacy was reached at an AUC/MIC ratio of about 401 corresponding to a dose of ~ 6.1 mg/kg in mice.

The bacteriostatic activity (equivalent to a 2.65 log unit reduction of CFU), related to the initial infection inoculum, was achieved at an AUC/MIC ratio of about 586 which corresponds to a dose of 8.8 mg/kg.

		Estimate	CV[%]
AUC	[hr*mg/L]	716	4.72
AUC_{norm}	[kg·h/L]	28.1	4.72
C_{max}	[mg/L]	146	6.02
$C_{max,norm}$	[kg/L]	5.74	6.02
Tmax	[hr]	0.641	13.1
$t_{1/2}$	[hr]	2.91	9.76
V_F	[L/kg]	0.150	8.35
CL_F	[L/hr/kg]	0.0356	4.72

Table 1 Predicted pharmacokinetic parameters (using a one compartment model) after multiple (every 6h) subcutaneous administration of 25.5 mg/kg FRI to female CFW 1 mice

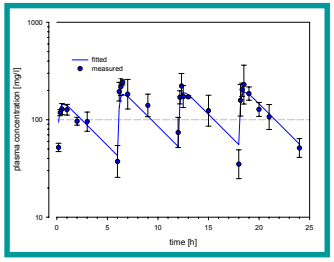

Fig. 2 Measured and fitted concentrations in plasma after multiple (every 6h) subcutaneous administration of 25.5 mg/kg FRI to female CFW 1 mice.

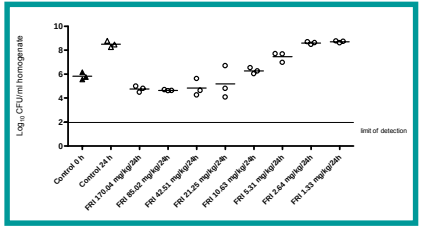

Fig. 3 Effect of eight doses of FRI on numbers of staphylococci in mouse thigh tissue (N=3). Inoculum 9.4 X 10^5

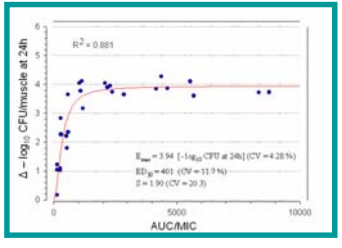

Fig. 4 Relationship between Δ-log10 CFU per thigh muscle and AUC/MIC.

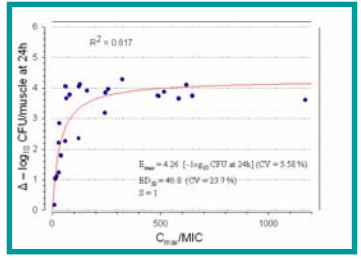

Fig. 5 Relationship between Δ-log10 CFU per thigh muscle and Cmax/MIC

Conclusions

- In a mouse thigh muscle staphylococcal infection, the best predictor for efficacy of friulimicin B was the AUC/MIC. With Cmax/MIC, a slightly less strong relationship to antibacterial efficacy was seen. No correlation was found with T>MIC
- The AUC/MIC ratios that are necessary to produce 1 or 2 units killing or the maximum kill rate were calculated and the corresponding doses were deduced

Literature

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