Supplementary Text 1.

Modeling analysis

In order to identify independent features of DAV131A dosing schedule associated to the reduction of fecal free moxifloxacin concentration and to link the DAV131A dosing regimen administered to the mortality rate, we performed a modeling analysis of the data.

Methods

Handling of data from outlier animals

We excluded from modeling the hamster from the group DAV131A 200 mg/kg/day which exhibited an outlier concentration of 463.4 µg/g, being 8 times higher than the median concentration in the control group.

The hamster for which fecal free moxifloxacin concentration was below the limit of quantification was excluded from the modeling analysis.

Mathematical model for the link between DAV131A daily dose and fecal free moxifloxacin concentration

The link between DAV131A daily dose and fecal free moxifloxacin concentration was analyzed using nonlinear regression. First, a basic model without covariate was fitted to observed concentrations. The predicted fecal free concentration of moxifloxacin $c_{pred,i}$ in the hamster i after administration of a DAV131A daily dose $Dose_i$ was described by a full sigmoid I_{max} model:

$$c_{pred,i} = C_0 \times (1 - \frac{Dose_i^{\gamma}}{{D_{50}}^{\gamma} + Dose_i^{\gamma}})$$

where the 3 parameters to be estimated are (*i*) C_0, the mean fecal free concentration of moxifloxacin in the absence of treatment by DAV131A, (*ii*) D_{50}, the daily dose of DAV131A that allow for 50% of the maximal effect of DAV131A and (*ii*) γ, the sigmoïdicity coefficient. In this full I_{max} model, we supposed that DAV131A would adsorb 100% of fecal free moxifloxacin residuals for a very large dose. This

hypothesis is indeed realistic as DAV131A is based on an activated charcoal. The observed fecal concentration of free moxifloxacin $c_{obs,i}$ in the hamster i can be written as:

$$c_{obs,i} = c_{pred,i} + \varepsilon_i$$

where the residual error ε_i is supposed to have a normal distribution with a mean of 0 and a variance of $(b.c_{pred,i})^2$ in the case of a proportional error model, or $(a + b \times c_{pred,i})^2$ in the case of a combined error model. Parameters estimates were obtained by maximum likelihood using generalized least squares (R function *gnls*). We compared the models with and without sigmoïdicity coefficient (γ=1) using the Bayesian Information Criterion (BIC, the lower the better). Proportional and combined error models were compared using BIC.

We then tested the effect of several covariates. The effect of each covariate j is introduced in the model above by transforming D_{50} as $D_{50} \times \left(\sum_k(\theta_{j,k} \times C_{j,k}) + 1\right)$, where $\theta_{j,k}$ is the effect on D_{50} of the k-th modality of the covariate j and $C_{j,k}$ is a dummy variable for the covariate j. The effect of the following covariates was studied: DAV131A intake 10 hours before the first administration of moxifloxacin (no/yes, ref=no), number of daily DAV131A intake (BID/TID, ref=BID) and DAV131A timing schedule (before/ together / after moxifloxacin administration, ref=before). Interaction between DAV131A administration 10 hours before the first administration of moxifloxacin (no/yes) and the number of daily DAV131A administrations (BID/TID) was also explored using the following composite covariate: no DAV131A treatment 10 hours before the first administration of moxifloxacin / DAV131A treatment 10 hours before the first administration of moxifloxacin and BID intake / DAV131A treatment 10 hours before the first administration of moxifloxacin and TID intake. Reference value of this composite covariate was no DAV131A treatment 10 hours before the first administration of moxifloxacin. In the univariate analyses, we performed likelihood ratio test (LRT) for each covariate. The final model was chosen from the covariates with a p-value < 0.2 in the univariate analyses, using backward selection based on the LRT with a significant threshold of 0.05.

Mathematical model for the link between fecal free moxifloxacin concentration and death

For the analysis of the probability of death at D_{12}, we performed a binary logistic regression of the probability of death according to the observed fecal free moxifloxacin concentrations. The probability of death p_i for the subject i was linked to the fecal concentration $c_{obs,i}$ using the model:

$$\text{logit}(p_i) = \alpha + \beta \times (c_{obs,i})$$

where α is the logit of the probability of death when fecal free moxifloxacin concentration equals 0, and β is the slope of the logit – concentration relationship.

Parameters were estimated by maximum likelihood using iteratively reweighted least squares (R function *glm*).

Joint model and predictions

We then developed the joint model using the fecal concentration $c_{pred,i}$ predicted by the final full sigmoid I_{max} model with covariates. In the joint model, statistical significance of the covariates was tested using the likelihood ratio test (LRT). Parameters of the joint model were estimated by maximum likelihood, using a simulated annealing algorithm (R function *optim*). The R code is available upon request to the first author (charles.burdet@inserm.fr). Initial estimates used were those estimated in the separate modeling. Standard errors of estimates were obtained from the inverse of the observed Fisher information matrix obtained from second derivatives of the log-likelihood. The 95% prediction intervals of the basic and final models were computed using the delta method.

The final model was used in order to predict the fecal free moxifloxacin concentration and the DAV131A daily dose needed to obtain various rates of mortality: 50%, 10%, 5% and 1%. Their 95% confidence intervals were computed using the delta method.

Model evaluation

The dose – concentration model was evaluated using standard goodness-of-fit plots: observed *vs* predicted values, standardized residuals *vs* predicted concentrations and standardized residuals *vs* observed DAV131A daily doses.

The logistic model was evaluated using the probability of death observed for the predicted concentration values in hamsters and their 95% confidence interval (CI) computed using the binomial distribution. The calibration of the model was evaluated using the Hosmer-Lemeshow test. Discrimination was evaluated using the area under the ROC curve and its 95% confidence interval computed using 1000 paired-bootstrap replicates (R functions *roc* and *ci.auc*). The discriminative abilities of the logistic model adjusted with observed and predicted concentrations were evaluated using the area under the ROC curve. They were compared using 1000 paired-bootstrap replicates [1].

Results

Data from 210 hamsters were available for modeling.

Model for the link between DAV131A daily dose and fecal free moxifloxacin concentration

The evolution of fecal free moxifloxacin concentration according to DAV131A daily dose was well described by a full sigmoid I_{max} model. The best residual error was a combined model. In the basic model without covariate, C_0 was estimated to 60.0 µg/g (relative standard error RSE=7.8%), and the D_{50} of DAV131A was estimated to 407.9 mg/kg/day (RSE=11.6%).

The administration of an additional initial dose of DAV131A at D_1H_{-10} decreased the D_{50} by 30% ($p<10^{-10}$), and DAV131A TID administration lead to a 20% decrease of the D_{50} when compared to BID administration (p=0.0009). The effect was still significant when studying the interaction between DAV131A intake at D_1H_{-10} and the number of DAV131A intakes combined in a composite covariate. However, after backward selection, the only covariate that was significantly associated with D_{50} was the administration of an additional initial dose of DAV131A at D_1H_{-10}. This covariate was thus kept for the joint model.

Model for the link between fecal free moxifloxacin concentration and death

The logistic model adequately described the relationship between fecal free moxifloxacin concentration and mortality. Intercept was estimated to -5.5 (RSE=14.6%), while the slope was estimated to 0.2g/µg (RSE=16.0%). The model calibration was satisfactory (p=0.9 for the Hosmer-Lemeshow test), as well as its discrimination (AUC of the ROC curve 0.97, 95%CI=0.95-0.99).

Joint model

Results of the joint model are presented in the Table 1 and Figure 1 below. In the joint model, the association between DAV131A administration at D_1H_{-10} and the D_{50} parameter remained significant ($p<10^{-8}$). The additive part or the residual error model was however poorly estimated, and a proportional model for the residual error was used in the joint model. Goodness-of-fit plots are presented in Figure 2 below. In hamsters not treated or treated by DAV131A at D_1H_{-10}, D_{50} was estimated to 405.3 mg/kg/day (95%CI=318.2-492.5) and 239.7 mg/kg/day (95%CI=179.1-300.3) respectively. The discrimination of the logistic model of the fecal free moxifloxacin concentration and the mortality was better when using the predicted concentration instead of the observed concentration (areas under the ROC curves, 0.99, 95%CI=0.98-0.99 *vs* 0.97, 95%CI=0.95-0.99, p=0.02).

Table 1. Parameter estimates and their relative standard errors (RSE, %) and 95% confidence interval in the final joint model of the link between DAV131A daily dose, fecal free moxifloxacin concentration and the probability of death in the 210 hamsters included in the modeling analysis. C_0 is the mean fecal free moxifloxacin concentration in the absence of DAV131A treatment, D_{50} is the DAV131A daily dose reducing the mortality to 50%, θ_{D1H-10} is the effect on D_{50} of the administration of one dose of DAV131A 10 hours before the beginning of moxifloxacin treatment, γ is the sigmoïdicity coefficient and b is the proportional residual error for the full I_{max} model. α (intercept) and β (slope) are the parameters of the logistic model.

Parameter	Parameter estimate	RSE (%)	95% confidence interval	p-value (LRT)
DAV131A daily dose – fecal free moxifloxacin concentration model				
C_0 (µg/g)	58.0	7.2	49.8 – 66.3	-
D_{50} (mg/kg/day)	405.3	11.0	318.2 – 492.5	-
θ_{D1H-10}	-0.4	7.8	-0.5 – -0.3	$p<10^{-8}$
γ	1.6	6.6	1.4 – 1.8	-
b	0.62	6.8	-	-
Fecal free moxifloxacin concentration – probability of death model				
α	-6.9	18.5	-9.4 – -4.4	-
β (g/µg)	0.3	23.8	0.2 – 0.4	-

Figure 1. Predicted relationships between DAV131A daily dose and fecal free moxifloxacin concentration (top, left), and fecal free moxifloxacin concentration and the mortality rate (top, right). The evolution of the mortality rate and DAV131A daily dose derived from these 2 models

is presented in the bottom panel. Models and data are represented according to treatment by DAV131A 10 hours before the first administration of moxifloxacin (green) or not (orange). In the top left panel, triangles, dots and squares represent the observed concentrations in studies 1, 2 and 3, respectively. In the top right panel, points and vertical colored lines represent the mortality rate observed for the predicted fecal free concentration of moxifloxacin and their 95% confidence interval. In the bottom panel, points and vertical colored lines represent the mortality rate observed for corresponding DAV131A daily dose and their 95% confidence interval. Full lines represent the model prediction, and shaded areas the 95% prediction intervals.

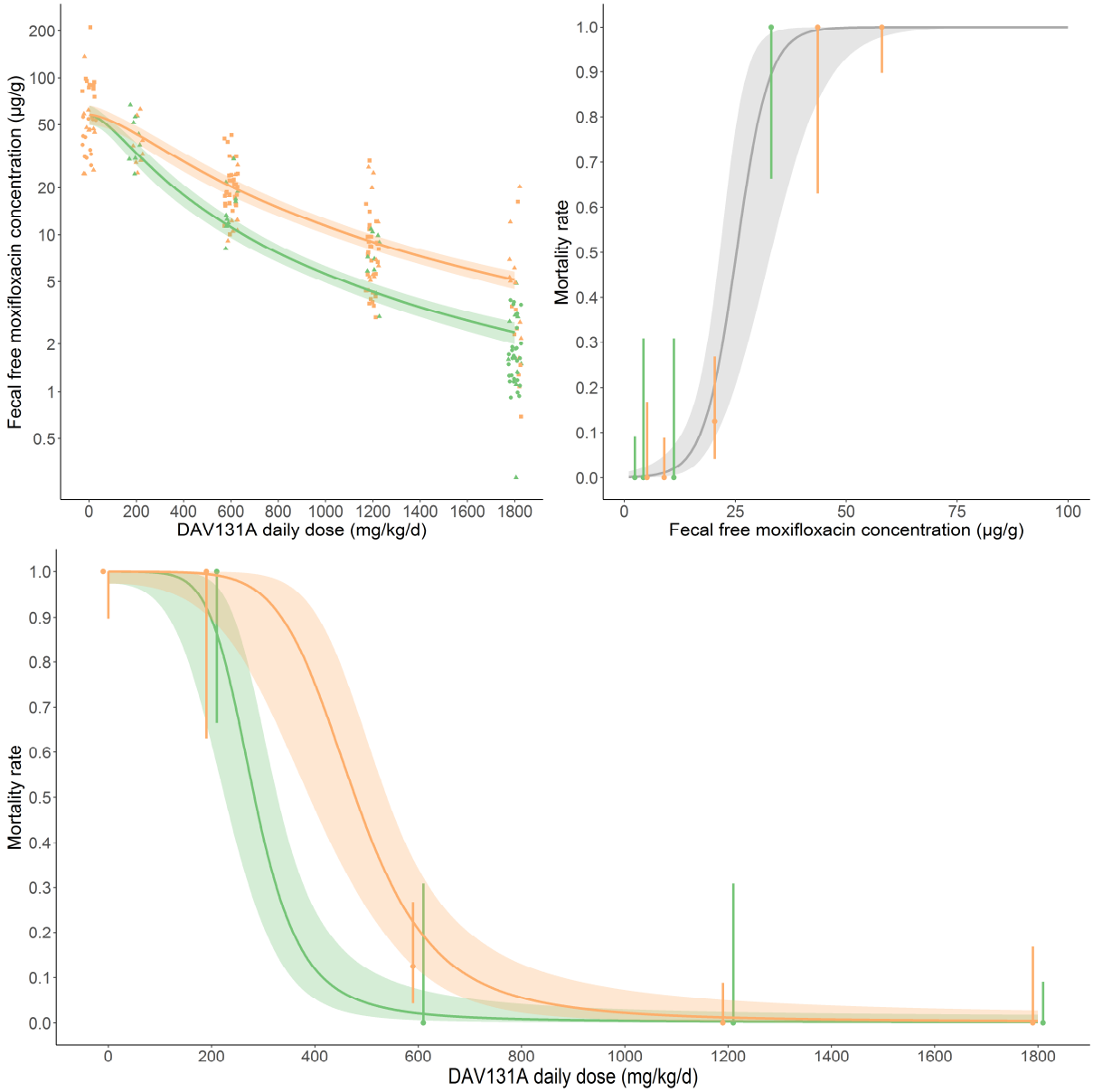
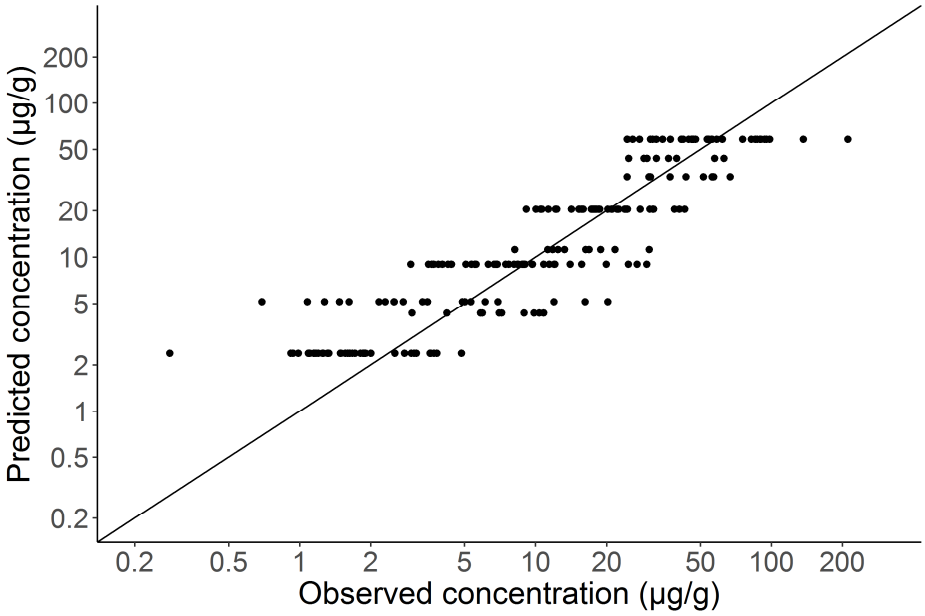
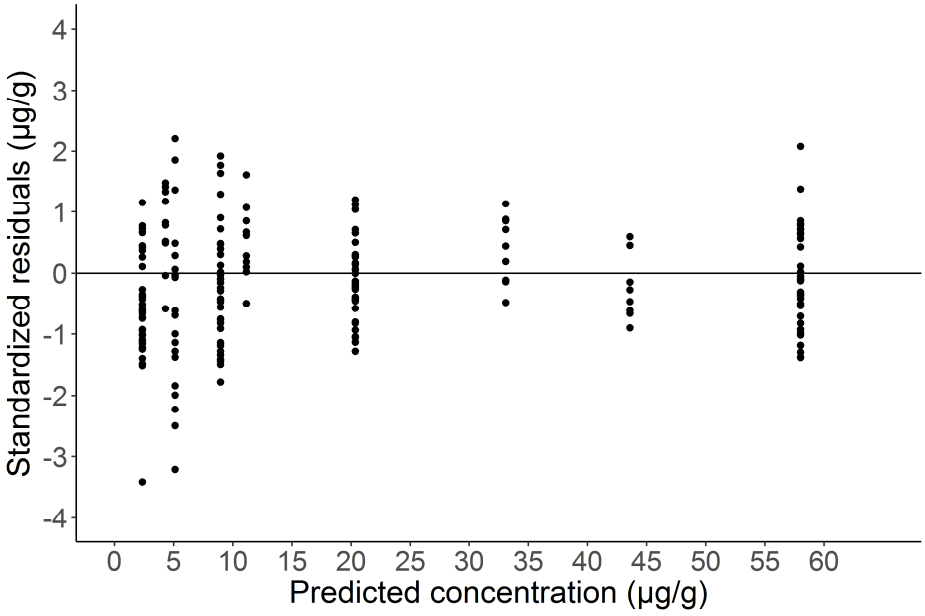
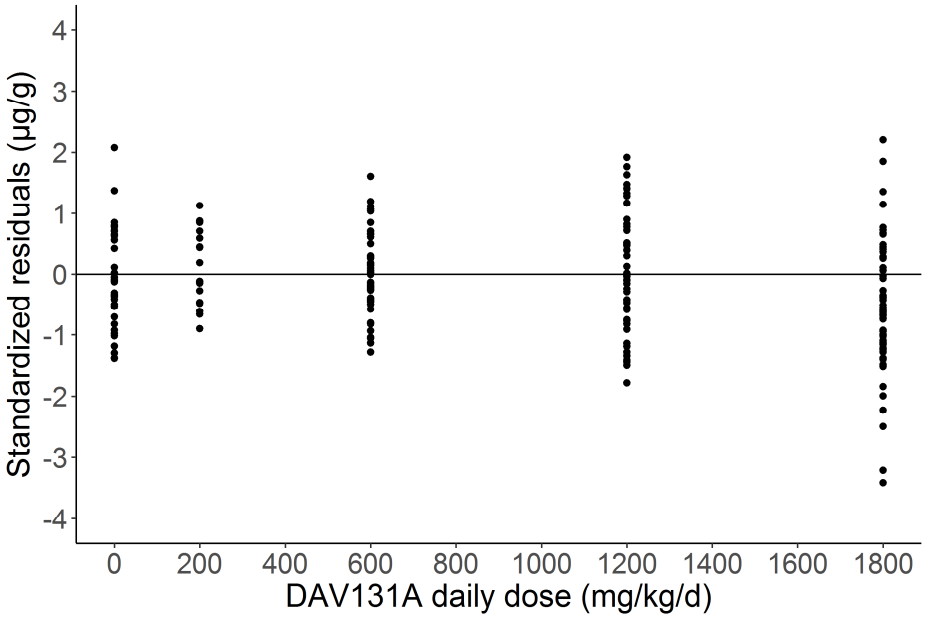


Figure 2. Diagnostic plots of the DAV131A daily dose – fecal free moxifloxacin concentration model drawn from the final model with covariate: Predicted *versus* observed fecal concentration of free moxifloxacin (top),

standardized residuals *versus* predicted fecal concentration of free moxifloxacin (middle), standardized residuals *versus* DAV131A daily dose (bottom).


Predicted concentration (µg/g)
200
100
50
20
10
5
2
1
0.5
0.2
0.2
0.5
1
2
5
10
20
50
100
200
Observed concentration (µg/g)


Standardized residuals (µg/g)
4
3
2
1
0
-1
-2
-3
-4
0
5
10
15
20
25
30
35
40
45
50
55
60
Predicted concentration (µg/g)


Standardized residuals (µg/g)
4
3
2
1
0
-1
-2
-3
-4
0
200
400
600
800
1000
1200
1400
1600
1800
DAV131A daily dose (mg/kg/d)

Our modeling approach enabled to forecast that reducing fecal free moxifloxacin concentration to 17.2 µg/g (13.8-20.7) was sufficient to reduce the mortality rate to 10%. The estimated DAV131A dose needed to achieve this mortality rate was 702.6 mg/kg/day (596.4-808.8). Results for other mortality rates are presented in Table 2 below.

Table 2. Fecal free moxifloxacin concentration and DAV131A daily doses (and their 95% confidence intervals) needed to decrease the mortality rate to 50%, 10%, 5% and 1% in the hamster model of moxifloxacin-induced *Clostridium difficile* infection.

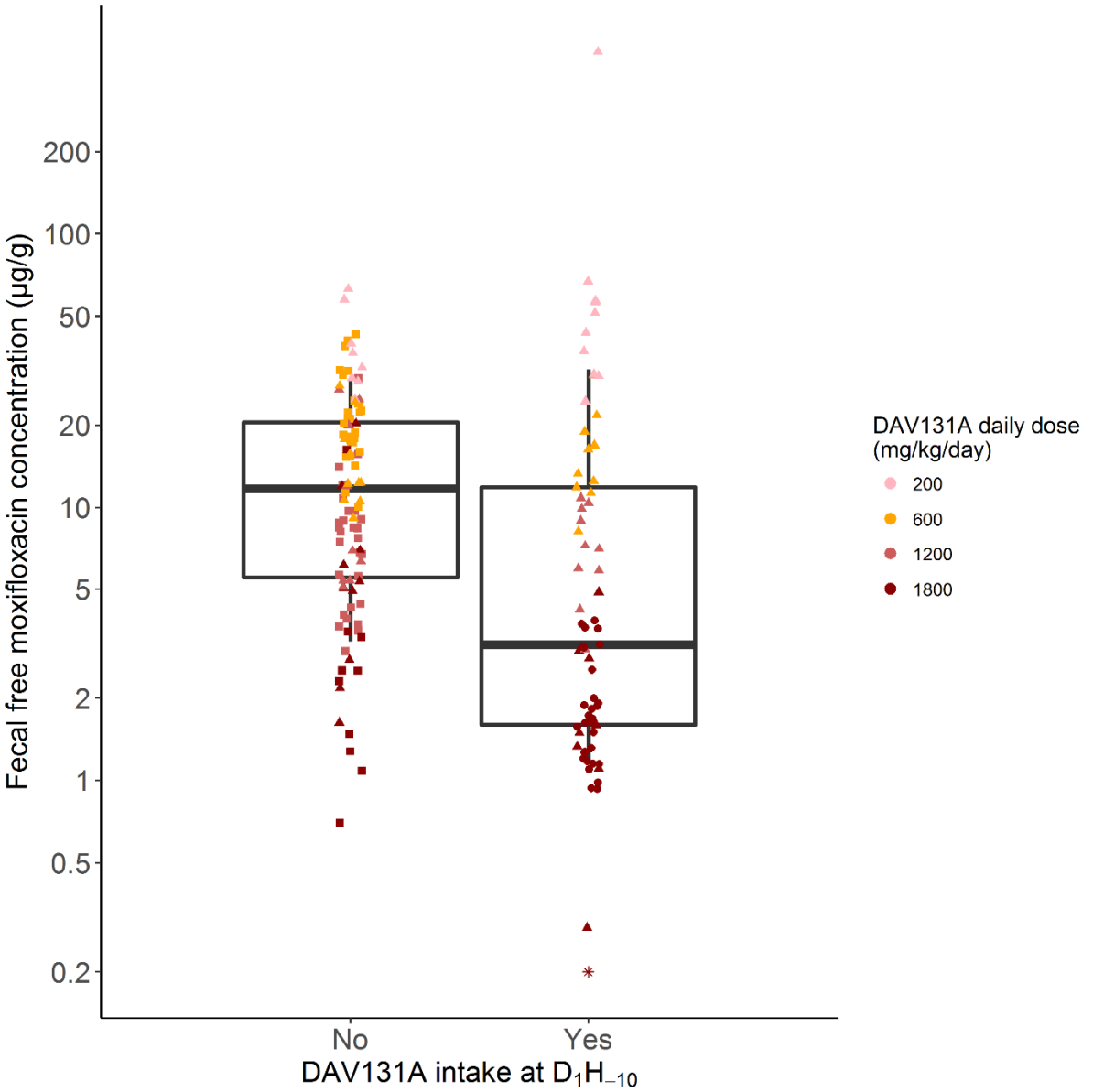
Mortality rate	**Fecal free moxifloxacin concentration**	**DAV131A doses needed**
50%	25.3 µg/g (20.5-30.1)	478.5 mg/kg/day (410.5-546.5)
10%	17.2 µg/g (13.8-20.7)	702.6 mg/kg/day (596.4-808.8)
5%	14.5 µg/g (10.7-18.4)	817.5 mg/kg/day (646.5-988.5)
1%	8.5 µg/g (2.8-14.2)	1251.4 mg/kg/day (628.2-1874.6)

Bibliography

1. Robin X, Turck N, Hainard A, et al. pROC: An Open-Source Package for R and S+ to Analyze and Compare Roc Curves. BMC Bioinformatics **2011**; 12:77.

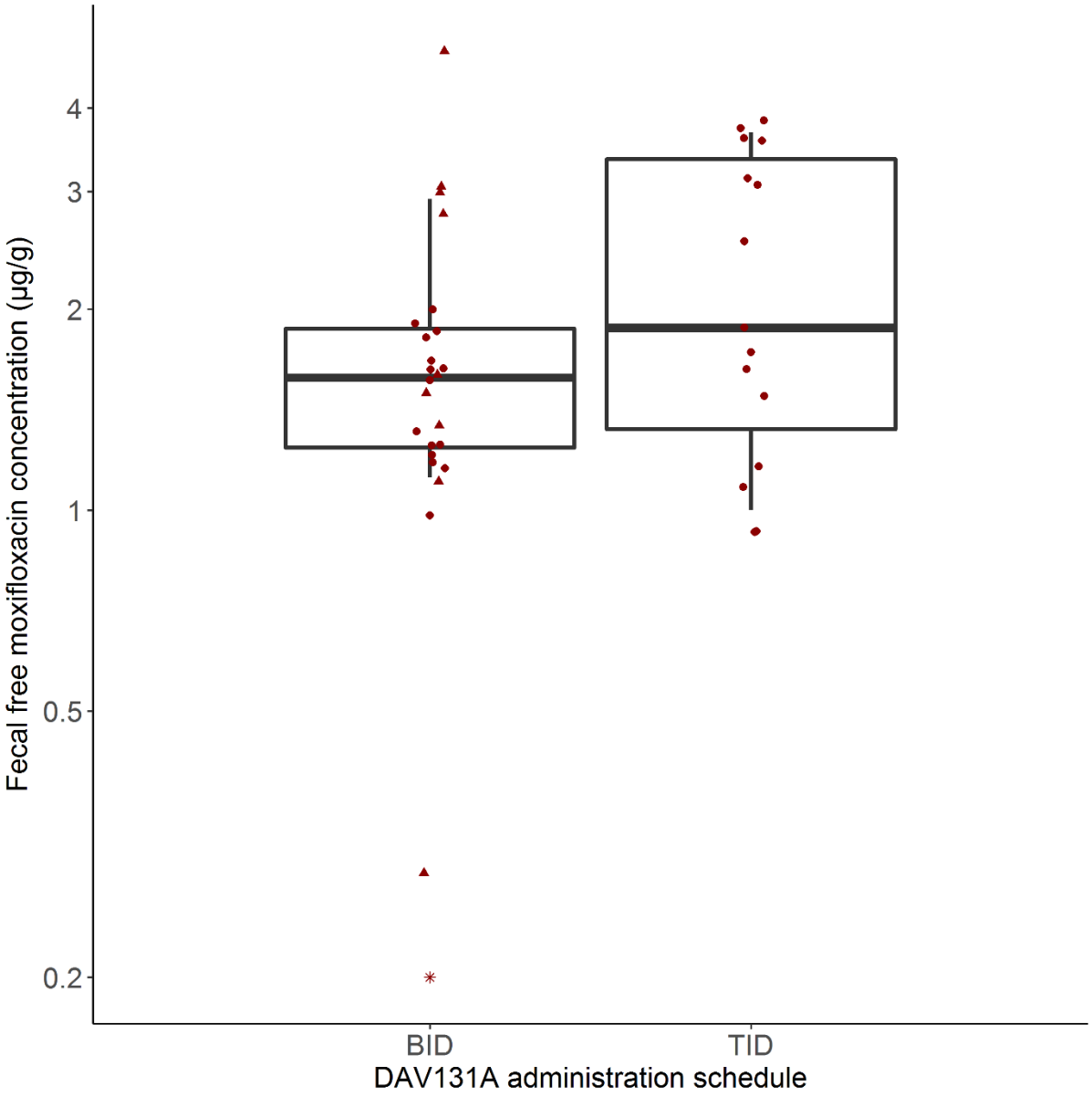
Supplementary Figure S1.

Boxplots of the fecal free moxifloxacin concentrations measured at D_3 in the 178 hamsters treated with 200-1800 mg/kg/day DAV131A that had received or not an additional initial dose of DAV131A 10 hours before the first administration of moxifloxacin. Triangles, dots and squares represent the observed concentrations in studies 1, 2 and 3, respectively. Whiskers represent 10^{th} and 90^{th} percentiles. Red symbol represents data below the limit of quantification.



Supplementary Figure S2.

Boxplots of the fecal free moxifloxacin concentrations measured at D_3 according to the number of DAV131A daily administrations, in the 40 hamsters treated with DAV131A 1800 mg/kg/day who received an additional initial dose of DAV131A 10 hours before the first administration of moxifloxacin. Triangles, dots and squares represent the observed concentrations in studies 1, 2 and 3, respectively. Whiskers represent 10th and 90th percentiles. The star represents data below the limit of quantification. BID, *bis in die*, TID, *ter in die*.



Supplementary Figure S3.

Boxplots of the fecal free moxifloxacin concentrations measured at D_3 in the 80 hamsters treated with DAV131A daily doses of 600 or 1200 mg/kg/day (no administration 10 hours before the first administration of moxifloxacin), according to the schedule of DAV131A administration. Triangles, dots and squares represent the observed concentrations in studies 1, 2 and 3, respectively. Whiskers represent 10^{th} and 90^{th} percentiles. "Before", administration at H_{-4} before moxifloxacin and H_1 after moxifloxacin administration; "Together", administration at H_0 and H_5 after moxifloxacin administration; "After", administration at H_2 and H_7 after moxifloxacin administration.