THE STAPHYLOCOCCAL-SPECIFIC ANTIBIOTIC DEBIO 1450 MINIMIZES DISTURBANCE TO THE GUT MICROBIOTA IN MICE

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INTRODUCTION

Debio 1450 is an antimicrobial drug candidate currently in Phase II clinical development for intravenous and oral treatment of staphylococcal infections. It inhibits staphylococcal FabI, an enzyme essential for fatty acid synthesis in this genus[1] (Fig. 1). *In vitro* studies have shown that the active moiety of the drug, Debio 1452, specifically targets staphylococcal species and has no relevant activity on other genera.

The human intestinal microbiota is regarded as a well-balanced community, not only regulating gut function, but also playing a beneficial role during normal homeostasis, modulating the host's immune system as well as influencing host development and physiology[2]. There is growing evidence that changes in composition of the microbiota due to antibiotics can result in an increased susceptibility to diseases like obesity, diabetes mellitus and neurologic disorders[3].

The aim of the present study was to compare the effects of Debio 1450 versus three broad-spectrum antibiotics commonly used in the clinic to treat staphylococcal infections on the mouse intestinal microbiota using a metagenomics approach.

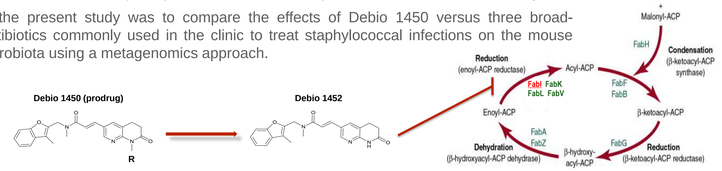
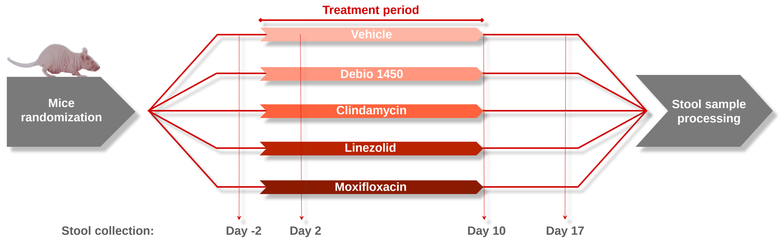


Figure 1: Mode of action of Debio1450 (adapted from[8])

METHODS



Groups of 5 CD-1 mice were treated orally for 10 days twice daily with either Debio 1450 (65 mg/kg), Clindamycin (100 mg/kg), Linezolid (100 mg/kg), or Vehicle, or once daily with Moxifloxacin (65 mg/kg). Dose levels were selected based on equivalent surface area dosage conversion factors in order to represent the human equivalent dose levels (300 mg every 6 hours for Clindamycin; 600 mg twice daily for Linezolid; 400 mg daily for Moxifloxacin; 320 mg twice daily for Debio 1450).

Microbial populations present in fecal samples were determined using next generation high throughput sequencing.

Total DNA was extracted from stool samples using a phenol-chloroform method optimized for this kind of matrix. For the library construction, PCR amplification was performed using universal primers targeting the V3-V4 region of the 16S rDNA gene. The sequencing was performed using an Illumina MiSeq platform.

Clustering in OTUs (Operational Taxonomic Unit) and taxonomic classification of sequences were obtained using a bioinformatic pipeline based on Mothur software[4]. For each taxon, an analysis of variance was conducted for repeated measurements (repeated ANOVA) including treatment, day, and treatment-by-day interaction as fixed factors in the statistical model and with a rank transformation in case of non normal distribution of data.

In this statistical model, post-hoc tests were conducted:

- to perform pairwise between-group comparisons using Tukey adjustment for multiple testing.
- to conduct within-group analysis by comparing pre- and post-treatment values in each treatment group, using Dunnett adjustment for multiple testing.

As multiple hypotheses are tested simultaneously, an FDR (False Discovery Rate) adjustment was used to correct p-values of the ANOVA model.

RESULTS

Microbiota composition:

On day -2, at the phylum and family level, no statistically significant differences were observed between groups (Fig.2). The two main phyla were:

- *Bacteroidetes* (between 41.7 and 50.3%) with *Bacteroidaceae*, *Porphyromonadaceae* and *Rikenellaceae* families
- *Firmicutes* (between 44.3 and 54.5%) with *Lachnospiraceae*, *Lactobacillaceae* and *Ruminococcaceae* families

On days 2, 10 and 17, no statistically significant differences were noted between Vehicle and **Debio 1450** treated groups. The two most abundant phyla were the same as the ones observed on day -2. In contrast, the microbiota composition changed dramatically under treatment with the three comparator antibiotics.

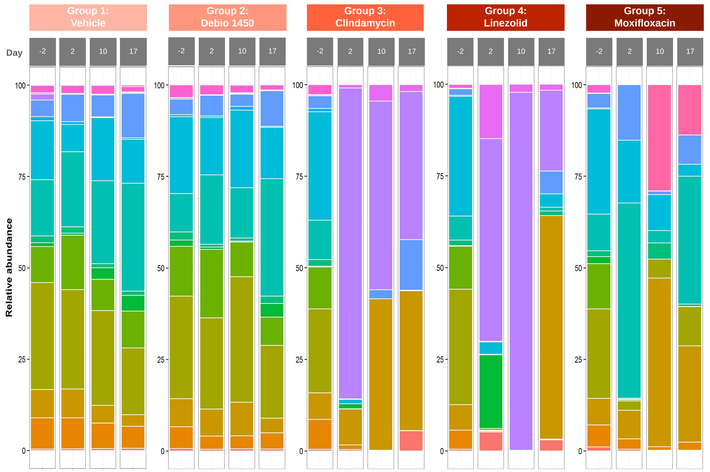


Figure 2: Evolution of relative abundance of each taxon at the family level (averages per day)

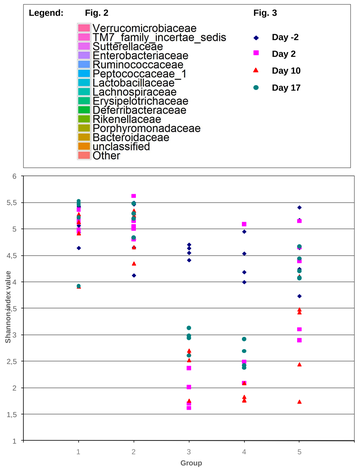


Figure 3: Classification by groups of Shannon indexes

From day 2 of **Clindamycin** treatment, the initial microbiota composition changed in favor of a large majority of *Enterobacteriaceae* (*Escherichia-Shigella* genus). At the end of treatment, the two major families were *Enterobacteriaceae* (*Escherichia-Shigella*) and *Bacteroidaceae* (*Bacteroides* genus).

During **Linezolid** treatment, the *Enterobacteriaceae* (*Escherichia-Shigella*) became the major family (from 0.1% on day -2 to 97.6% on day 10). Seven days after the end of treatment, the relative abundance of *Enterobacteriaceae* decreased again, whereas the *Bacteroidaceae* (*Bacteroides*) increased.

At the beginning of the **Moxifloxacin** treatment, there were also significant changes in the microbiota composition: increase of *Lachnospiraceae* and *Ruminococcaceae* families (from 10.0% to 53.3% and from 3.9% to 15.2% respectively). At the end of treatment (day 10), the proportion of *Verrucomicrobiaceae* (*Akkermansia* genus) and *Bacteroidaceae* (*Bacteroides*) increased whereas that of *Lachnospiraceae* decreased.

Diversity:

The diversity index (Shannon) of the samples was calculated at OTU cutoff of 0.03 distance. Communities that are numerically dominated by one or a few species exhibit low diversity values while communities where abundance is distributed equally amongst species exhibit high diversity values.

For groups treated with Vehicle and Debio 1450, the Shannon index values were similar (near 5.0). For the groups treated with Clindamycin and Linezolid, the Shannon index values were lower for days 2, 10 and 17 than for day -2 (near 2.5). This decrease was not clearly observed in the group treated with Moxifloxacin, except on day 10 (Fig. 3).

Diversity was:
- similar in all groups before treatment,
- similar in Vehicle and Debio 1450 treated groups for all time points,
- lower after comparator antibiotic treatment (groups 3, 4 and 5).

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5 J. Yao, *et al.* (2016) *Antimicrob Agents Chemother*
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DISCUSSION

Radical changes in the microbiota were observed for all mice in response to the three broad-spectrum antibiotics over the course of the treatment and post treatment. In contrast, there were no statistically significant differences in the microbiota composition between the Debio 1450 treated group and the Vehicle group at the phylum level. These findings are in line with a recent publication showing that a 10-day treatment with the active moiety Debio 1452 has only minor impact on the mouse intestinal microbiota[5].

Differences in the taxonomy distribution appeared from day 2 and persisted for up to 7 days post treatment in the three broad-spectrum antibiotics groups. However, the global microbiota composition on day 17 was closer to the baseline profile (day -2) for the Moxifloxacin-treated group compared to the Clindamycin- and Linezolid-treated groups.

Broad-spectrum antimicrobial agents, such as the antibiotics used in this study, upset the balance of the host microbial community and it is commonly accepted that gut flora disruption allows for the proliferation of pathogenic species like *C.difficile* and predisposes the host to infection and associated disease[6].

Antibiotics have adverse effects on human microbiota but host-associated factors like diet, health status, or the microbial community itself modulate the impact of antibiotics[7]. Due to the difference in composition, the observed variations in mice could be of different nature and intensity in the human gut and the effect of this promising molecule needs to be confirmed in a clinical study.

CONCLUSIONS

While the three broad-spectrum comparator antibiotics led to major profile variations, Debio 1450 did not cause relevant changes in the gut microbiota. Bacterial diversity and balance appeared to be preserved during and after treatment.

This result supports the development of targeted antibiotherapy to treat staphylococcal infections as minimizing microbiota disturbance is expected to reduce antibiotic-associated complications such as diarrhea, colitis, *C. difficile* infections or candidiasis and to limit the impact on all physiological processes governed by the gut inhabitants.

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