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

**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 the genus (Fig. 1). In vitro studies have shown that the active moiety of the drug, Debio 1450, 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 development and physiology. There is growing evidence that changes in composition of the microbiota due to antibiotics can result in an increased susceptibility to diseases like diabetes, obesity and neurologic disorders. 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.

**METHODS**

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 Bacteroides, Porphyromonadaceae and Rikenellaceae families
- Firmicutes (between 44.3 and 56.4%) with Lachnospiraceae, Lactobacillaceae and Ruminococcaceae families

On days 5, 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.

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 the 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 on 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 (Akamaspora 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 5.5). For the groups treated with Clindamycin and Linezolid, the Shannon index values were lower for days 2. 10 and 17 than for day 2 –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).

**RESULTS**

Microbial populations present in fecal samples were determined using next generation high throughput sequencing. Total RNA was extracted from formalin-fixed paraffin-embedded tissue using the NucleoSpin RNA kit (Macherey-Nagel). 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 Units) and taxonomic classification of sequences were obtained using a bioinformatic pipeline based on Mothur software. For each taxon, an analysis of variance was conducted for repeated measurements across the treatment groups using the Dunnett test. This was achieved by computing the post hoc test and adjusting p-values using the Tukey adjustment for multiple testing.

**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.

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. jejuni and predisposes the host to infection and associated diseases. 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. Due to the difference in composition, the observed variations in bacteria 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 antibiotic therapy 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.

**REFERENCES**

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