1. BACKGROUND

The intestinal microbiota forms a vast reservoir of bacteria and provides a non-leaf defense mechanism (symbiotic resistance) by inhibiting the growth in the gut of pathogenic gut microbiota present or acquired daily and preventing antibiotic-associated diarrhea, and occurrence of Clostridioides difficile infection (CDI).

We developed an oral antibiotic-based product, DAV132, to be associated with antibiotics in order to prevent these effects [Gough et al., 2015].

We report the results of protection of the gut microbiome in human volunteers treated with oral moxifloxacin (MOX), with or without a protection challenge. As such, the study was designed specifically aimed at altering multiple bacterial species in vivo in a human gut model and in vivo in hamsters.

2. METHODS

2.1 Randomized clinical trial in human healthy volunteers

This randomized, controlled, double-blind, placebo-controlled, non-blinded trial was designed to evaluate the influence of DAV132 administration on the fecal concentrations of MOX. The primary endpoints were the differential counts of Bifidobacterium and Lactobacillus by multiplex PCR and the fecal health index of the volunteers. Written informed consent was given by all healthy volunteers before enrollment (IIT) (NCT02269521).

All healthy volunteers were randomized and treated with MOX (n=14), MOX-DAV132 (n=14), DAV132 alone (n=14) and negative control without intervention, n=8. MOX was administered orally, once a day, from Day 0 to Day 35. DAV132 and negative control administration, started from Day 9 to Day 35 and negative control, started from Day 9 to Day 35, respectively. Data sets were analyzed until Day 15 and Day 35.

DAV132 was assayed in an aqueous phosphate buffer (pH 7.4) in all samples collected and assessed with phosphate buffer (pH 7.4). The assay was performed in triplicate on a set of samples from each volunteer (n=14). A DAV132 content >100 µg/mL was considered a positive result. A DAV132 content >100 µg/mL was considered a positive result.

2.2 In vitro gut model of C. difficile infection

DAV132 (316x) adapted format as described was assessed in a scenario in vitro gut model (immunocompromised on an in vitro isolated with a different strain of Clostridioides difficile (ATCC 6984) and a different strain of C. jejuni (ATCC 8118). Bacterial counts, toxin production and MOX concentrations were measured over time.

2.3 In vivo hamster model of C. difficile infection

DAV132 (316x) adapted format as described was assessed in a scenario in vitro gut model (immunocompromised on an in vitro isolated with a different strain of Clostridioides difficile (ATCC 6984) and a different strain of C. jejuni (ATCC 8118). Bacterial counts, toxin production and MOX concentrations were measured over time.

3. RANDOMIZED CLINICAL TRIAL IN HEALTHY HUMAN VOLUNTEERS

3.1. Moxifloxacin fecal and plasma pharmacokinetics

DAV132 associated with MOX reduced by 99% free MOX fecal concentrations (Fig. 1). The ratio of the means of AUC_{12h} for the DAV132 group were 0.0059, 0.95 CI 0.0011:0.0030 (p<0.01) (Fig. 1a) and 0.0030, 0.95 CI 0.0011:0.0030 (p=0.01) (primary endpoint).

MOX plasma concentrations were maintained on Day 1 and on Day 5 (Fig. 2). On Day 5, the ratio of the means of Log AUC_{12h} for the DAV132 group were 0.0030, 0.95 CI 0.0011:0.0030 (p<0.01).

3.2. Metagenomic analysis of the gut microbiome

DAV132 associated with MOX prevented the MOX-induced disturbance of the microbiome in terms of microbial gene richness (Fig. 3). In fact, MOX, without DAV132, increased the number of bacterial genera relative to the control, and thereby the abundance of certain bacterial genera was found to be significantly higher in the DAV132 group than in the MOX group.

3.3. Safety

DAV132 was given during 7 days without any adverse effects.

Before treatment (baseline), 43% of subjects were colonized with Gram-negative bacteria resistant to quinolones and 14% with Gram-positive bacteria resistant to fluoroquinolones. After the start of treatment, acquisition of resistant bacteria in subjects not colonized at baseline was similar in all treatment groups.

Heats were rare in all groups. No C. difficile was isolated at any time in any subject.

4. SIMULATED CLOSTRIDIUM DIFFICILE INFECTION IN AN IN VITRO HUMAN GUT MODEL

DAV132 associated with MOX prevented the infection of C. difficile (Fig. 4a, 4b). MOX alone caused a marked disturbance of the microbiota, notably to Bacteroides, lactate fermenting enterobacteria, lactobacilli and bifidobacteria populations (Fig. 4a). Co-inoculation of DAV132 with MOX prevented the majority of perturbations of the gut microbiota (Fig. 4b).

DAV132 co-administration with MOX delayed C. difficile spore germination and vegetative proliferation, with cytopathicity production further delayed, likely due to reduced microbial disturbance. DAV132 prevented emergence of C. difficile with reduced susceptibility to MOX (not shown).

5. IN VIVO HAMSTER MODEL OF CLOSTRIDIUM DIFFICILE INFECTION

DAV132 associated with MOX showed a dose-dependent reduction of MOX fecal levels (Fig. 5a) and C. difficile counts in feces (Fig. 5b). C. difficile spores were ingested by DAV132-protected animals against MOX-induced lethal C. difficile (Fig. 5c).

DAV132 and MOX were administered at 300 mg/kg/DAV132 and 200 mg/kg MOX were protected at 600 mg/kg and above. Protected animals had reduced MOX fecal concentrations and undetectable C. difficile in feces.

6. CONCLUSION

Association of DAV132 with moxifloxacin protected healthy humans against gut microbiome disruption, by removing free moxifloxacin from the feces without altering its plasma pharmacokinetics.

In vitro results in a human gut model and in vivo results in a hamster model suggest that DAV132 should be protected against antibiotic-induced Clostridioides difficile infection.

These results warrant further clinical development of DAV132 to protect the intestinal microbiome, and thereby to prevent antibiotic-related diarrhea and occurrence of Clostridioides difficile infection, in patients receiving oral or parenteral antibiotics.