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Dose-effect and safety of DAV132, an activated charcoal based product, when given with oral moxifloxacin on free moxifloxacin fecal concentrations and intestinal microbiota diversity: a randomized controlled trial in 144 healthy volunteers

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1. BACKGROUND

After systemic antibiotic treatment, part of the antibiotic reaches the colon. Antibiotics are known to alter the gut microbiota (Sullivan et al., Lancet Infect Dis 2001). Clinical manifestations of antibiotic-induced dysbiosis include antibiotic-associated diarrhea (AAD) and *C. difficile* infection (CDI) (Khanna et al., Expert Rev Gastroenterol Hepatol 2016).

DAV132 is an oral product to be associated with antibiotics to protect the gut microbiota. It delivers the powerful adsorbent activated charcoal to the late intestine (de Gunzburg et al., J Clin Pharmacol 2015). In a previous randomized controlled study, DAV132 (22.5 g/d) reduced by over 99% the exposure of the gut microbiota to the fluoroquinolone moxifloxacin (MXF) without interfering with its systemic exposure, resulting in protection of the intestinal microbiota from MXF-induced dysbiosis in healthy volunteers (HVs) (de Gunzburg et al., J Infect Dis 2018).

This new clinical trial was undertaken to investigate the DAV132 dose-effect on free MXF fecal concentrations and bacterial diversity of the gut microbiota.

2. METHODS

- **Design.** Randomized controlled trial, open-labeled for investigators and subjects, blinded for analytical and microbiological evaluations, in 12 parallel treatment groups of HVs, to evaluate the effect of different DAV132 dose regimens on the decrease in free MXF fecal concentrations between D1 (start of treatment) and D16 (11 days after the end of MXF treatment).
- **Approvals.** The study was conducted according to Good Clinical Practice and ethical considerations (declaration of Helsinki) and approved by the Ethics Committee "CPP SUD-EST IV" (Lyon, France) and the French Health Authorities (ANSM). Written informed consent to participate was given by all subjects before inclusion. ClinicalTrials.gov NCT02917200.
- **Main inclusion criteria.** Male and female volunteers, aged 18-60, BMI 18.5-30 kg/m^2, considered as healthy (medical history, physical examination, normal ECG, vital signs, blood biological values).
- **Treatments**
  - Moxifloxacin (MXF) 400 mg oad, orally from D1 to D5
  - DAV132 from 2 to 22.5 g/d (corresponding to 1.02 to 15.34 g of activated charcoal), given in 2 or 3 intakes per day, orally from D1 to D7
  - CTRL (control group; gellified water): tid, orally from D1 to D7
- **Randomization** 1:1 to one of 12 treatment groups:
  - Group A: MXF + CTRL
  - Groups B to K: MXF + DAV132 from 2 to 22.5 g/d, bid or tid regimens
  - Group L: CTRL
- **Samples.** Fecal samples were taken daily from D1 to D9, at D12, D16 and D37 to measure free MXF concentrations by LC/MS/MS and analyze the gut microbiome by sequencing the V3-V4 regions of 16S rRNA genes. Plasma MXF concentrations were measured at D1 (0.5, 3, 8, 24 hours after MXF administration) and at D5 (0.5, 3, 8, 24, 48, 72 hours after MXF administration).
- **Statistics**
  - **Primary endpoint: area under the time curve from D1 to D16 ($AUC_{D1\text{-}D16}$) of free MXF concentrations in feces.** Differences of log AUC between groups receiving MXF were assessed by a global ANOVA. If significant, pairwise group comparisons using the Dunnett's test were performed between each MXF + DAV132 group and the MXF + CTRL group.
  - **Secondary endpoints.** The $AUC_{D1\text{-}D16}$ of several indices of α or β bacterial diversity were analyzed. First global ANOVA between all groups were performed. If significant, two series of pairwise group comparisons were performed using Dunnett's test: first between each MXF + DAV132 group and the MXF + CTRL group; second between each MXF + DAV132 group and the CTRL group.
  - MXF plasma PK parameters were estimated using a population approach and individual $AUC_{0\text{-}24h}$ and Cmax at D1 and D5 were derived. Differences of log AUC and log Cmax at D1 and D5 between groups receiving MXF were assessed by a global ANOVA. If significant, pairwise group comparisons using the Dunnett's test were performed between each MXF + DAV132 group and the MXF + CTRL group.

3. RESULTS

3.1. Disposition and baseline characteristics of subjects

- Intent-to-treat set (ITTS): 144 subjects completed the study (N=12 in each group).
- Per protocol set (PPS): 143 subjects had sufficient compliance to treatment (N=11 in group H, N=12 in each other group).
- Safety set (SS): 148 subjects received at least one dose of study product.

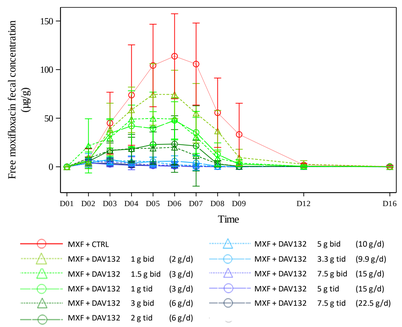
Table 1: Baseline characteristics of subjects (ITTS, N=144)

Age (years), mean ± SD (range)	38.4 ± 12.70 (19-60)
Gender, n	54M/90F
BMI (kg/m^2), mean ± SD (range)	24.0 ± 3.1 (18.5-30.1)
Antibiotic use (past 3 months), n	0
Hospitalisation (past 3 months), n	0
Fecal colonization with *C. difficile*, n	0
Fluoroquinolone-resistant enterobacteria in feces, n (%)	29 (20.1%)

3.2. Effect on moxifloxacin fecal pharmacokinetics

- Free MXF fecal concentrations in group A (**Fig. 1**) were within the expected range.
- **Primary endpoint: $AUC_{D1\text{-}D16}$ of free MXF fecal concentrations.** Administration of DAV132 with MXF led to a dose-dependent decrease in free MXF fecal concentrations ($p=10^{-51}$; **Fig. 1**).
- The decrease was significant at DAV132 doses ranging from 3 g/d to 22.5 g/d ($p=0.002$ for 3 g/d and $p=10^{-12}$ for doses greater or equal to 6 g/d). The decrease was huge: about 95-98% with DAV132 at 10 g/d or higher, about 83-85% with DAV132 at 6 g/d, and about 56-61% at 3 g/d. There were no marked differences between bid and tid regimens.

Figure 1: Free moxifloxacin fecal concentration (µg/g)/ Plots of mean ± SD over time per treatment group (PPS, N=143; LLOQ 0.040 µg/g)



3.3. Effect on the intestinal microbiota diversity (16S rRNA gene profiling)

- **α-diversity.** In the MXF + CTRL group (**Fig. 2 left**), the Shannon diversity index markedly decreased during the treatment period then tended to return progressively to baseline. In the CTRL group, it stayed roughly unchanged over time.
- DAV132 led to a dose-dependent prevention of the moxifloxacin-induced reduction of the Shannon index ($p=3.10^{-5}$) (**Fig. 2 right**). Pairwise comparisons were significant at DAV132 doses ranging from 10 g/d (tid regimen only; p=0.007 vs. MXF + CTRL; p=0.96 vs. CTRL) to 22.5 g/d (p=0.002 vs. MXF + CTRL; p=1 vs. CTRL).
- **β-diversity.** In the MXF + CTRL group (**Fig. 3 left**), Unweighted UniFrac distance from D1 markedly increased during the treatment period, then tended to decrease progressively. In the CTRL group, it stayed roughly unchanged after D3.
- DAV132 led to a dose-dependent prevention of the moxifloxacin-induced increase of the Unweighted UniFrac distance ($p=3.10^{-5}$) (**Fig. 3 right**). Pairwise comparisons were significant at DAV132 doses ranging from 6 g/d (bid and tid: p=0.003 vs. MXF + CTRL; bid: p=0.97 vs. CTRL; tid: p=0.83 vs. CTRL) to 22.5 g/d (p=0.026 vs. MXF + CTRL; p=0.35 vs. CTRL).

Figure 2: Change of Shannon index from D1 (α-diversity). Left: Mean ± SD over time per treatment group. Right: $AUC_{D1\text{-}D16}$ of the change from D1 by DAV132 dose regimen (PPS, N=143)

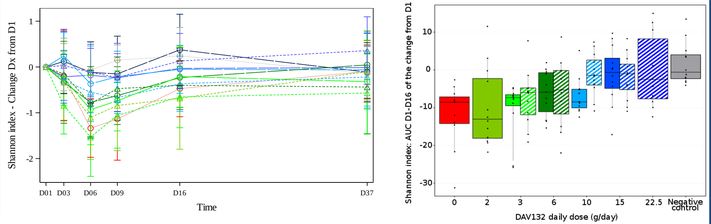
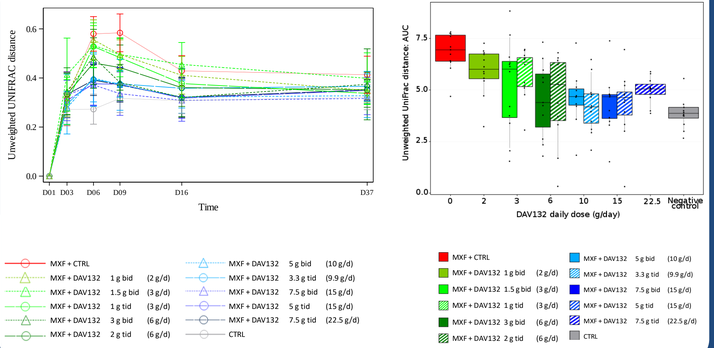


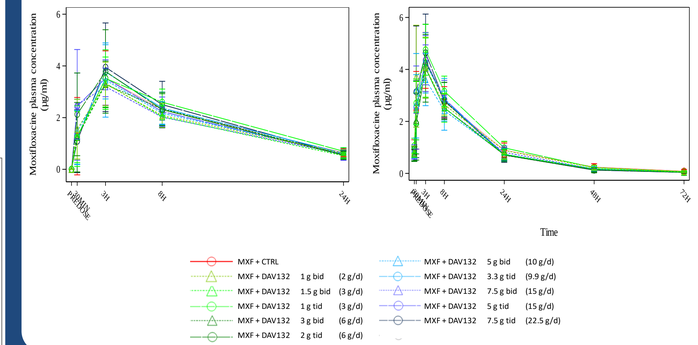
Figure 3: Unweighted UniFrac distance from D1 (β-diversity). Left: Mean ± SD over time per treatment group. Right: $AUC_{D1\text{-}D16}$ by DAV132 dose regimen (PPS, N=143)



3.4. Effect on moxifloxacin plasma pharmacokinetics

- The results of all the planned statistical tests (global ANOVA and pairwise group comparisons) did not show differences between MXF and MXF + DAV132 for the MXF $AUC_{0\text{-}24h}$ for Cmax , both at D1 (**Fig. 4 left**) and at D5 (**Fig. 4 right**).

Figure 4: Moxifloxacin plasma concentration (µg/g) at D1 (left) and D5 (right). Mean ± SD over time (24 or 72 hours) per treatment group (PPS, N=143; LLOQ 0.010 µg/mL)



3.5. Adverse events

- No serious adverse events (AEs) occurred.
- Out of the 123 subjects exposed to DAV132, 25 subjects had 30 AEs related to DAV132, of which 24 were also related to moxifloxacin, and all being gastrointestinal disorders: abdominal distension (n=15 subjects), abdominal pain (n=7), constipation (n=4), and feces discolored (n=3). All AEs were transient. None led to treatment discontinuation.
- Out of the 136 subjects exposed to moxifloxacin, 67 subjects had 137 AEs related to MXF, mainly gastrointestinal disorders (n=46 subjects) and nervous system disorders (n=40). Four subjects prematurely discontinued the study due to AEs related to MXF.

4. CONCLUSION

- We assessed the free moxifloxacin fecal concentration and the microbiome bacterial diversity to evaluate the clinical efficacy of DAV132 to protect the intestinal microbiota from the clinical manifestations of antibiotic-induced dysbiosis.
- The results demonstrated that the ability of DAV132 to prevent moxifloxacin-induced modifications of these parameters was dose-dependent. They also confirmed that DAV132 did not impact the systemic exposure to moxifloxacin in healthy volunteers, indicating that it will not impair the clinical use of the antibiotic.
- The optimal protection, based on the decrease in free antibiotic fecal concentrations and maintenance of bacterial diversity, requires high doses of DAV132.
- The use of moxifloxacin, which has well-known fecal and plasma pharmacokinetics, as a model helps anticipating the effects of DAV132 associated with various other antibiotics. These results are highly encouraging for further developments of DAV132 with other antibiotics and to prevent CDI.