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DAV131, an oral adsorbent-based product, exerts a dose-dependent protection of hamsters against moxifloxacin-induced *Clostridium difficile* lethal infection

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ABSTRACT

Objectives: Antibiotic treatments greatly impact gut microflora which can result in potentially severe, sometimes lethal *Clostridium difficile* infection (CDI); prevention strategies would be highly welcome. DAV131, a new adsorbent-based product, significantly reduces the level of residual antibiotics reaching the colon in several animal models. Here, we report an escalating dose study of the protective effect of DAV131 in a moxifloxacin-induced CDI hamster model.

Methods: Male Syrian hamsters were administered 30 mg/kg moxifloxacin subcutaneously once per day for 5 days, and infected orally at day 3 with 10^4 *C. difficile* UNT-103-1 spores. Groups of 10 animals were orally administered 100, 300, 600 or 900 mg/kg DAV131 twice per day and were continued for three days after moxifloxacin cessation. Fecal levels of moxifloxacin and viable *C. difficile* counts were respectively determined using a bioassay and standard plating methods.

Results: Animals administered moxifloxacin alone exhibited rapid mortality upon ingestion of *C. difficile* spores, with 90% survival at day 4, 50% at day 5, 30% at day 6, and 0% at day 7. Whereas the lowest DAV131 dose of 100 mg/kg bid did not protect the animals, doses of 300 mg/kg bid and greater were highly protective. Regimens of 600 and 900 mg/kg DAV131 bid, enabled for the total protection of animals until the end of the experiment at day 22, with no signs of morbidity, nor detectable counts of *C. difficile* in feces. At 300 mg/kg, 80% of the animals were protected from lethal outcome despite some *C. difficile* pathogen remaining detectable in the feces for a transitory period of 5 days.

Conclusion: Oral DAV131 exhibited a dose-dependent protection of hamsters against moxifloxacin-induced lethal CDI. The protective effect corresponded to the limitation of *C. difficile* expansion in the gut, concomitant with the effective adsorption of the antibiotic by DAV131. This study clearly shows that DAV131 constitutes a preventive strategy that can protect against CDI when applied concomitantly with the causative antibiotic treatment. The development of this promising strategy for the prevention of CDI in humans (code name DAV132) is under way.

INTRODUCTION

Treatment by most antibiotics can lead to CDI by perturbing the colonic commensal flora, thereby allowing colonization of the intestine by *C. difficile*. Amongst antibiotics, clindamycin, cephalosporins and fluoroquinolones are considered as the predominant risk factors (1, 2). CDI and most of all, recurrence of CDI in high risk patients, are medical conditions with increased frequency and severity worldwide. The prevention of CDI episodes and CDI relapses would therefore be a major medical progress, associated with improved quality of life for patients, and a decrease of public health costs.

Da Volterra (Paris, France) has been developing a novel adsorbent-based product, which limits the alterations of the colonic commensal flora during antibiotic treatments by adsorbing unwanted antibiotic residues in the lower intestine before they reach the caecum and colon. The protective effect of DAV131 in the moxifloxacin-induced hamster *Clostridum difficile* Associated Disease (CDAD) model has already been demonstrated at a high dose of the product (3). Here, we report the results of a dose-dependent study of the protective effect of DAV131.

RESULTS: Preventive effect of DAV131 on the induction of *C. difficile* lethal infection by moxifloxacin treatment.

METHODS

- Male Syrian hamsters were housed in accordance with NIH guidelines and were examined at least three times per day. Those judged to be in a moribund state were euthanized.
- Animals were administered either moxifloxacin 30 mg/kg from Day 1 to Day 5, moxifloxacin 30 mg/kg from Day 1 to Day 5 plus DAV131 at different doses from Day 1 to Day 8, or DAV131 alone at 900 mg/kg bid from Day 1 to Day 8, as shown in Fig 1.
- All animals received an inoculation of *C. difficile* spores (10^4 spores of non-epidemic *C. difficile* UNT-103-1 (TcdA+, TcdB+, cdtB–, REA J strain) at Day 3.
- Feces were collected daily into three pools: 0-6 hr, 6-12 hr and 12-24 hr. Fecal moxifloxacin levels were determined by microbiological assay (*B. subtilis* ATCC 6633 as the indicator organism) from Day 1 to Day 6 on all the samples. Viable *C. difficile* counts were determined by plating the 12-24 hr fecal samples on selective agar media from Day 2 to Day 12.

Fig 1: Experimental groups

Experimental groups	Treatments: Moxifloxacin (Days 1-5)	Oral DAV131 (Days 1-8)	*C. difficile* (Day 3)
"Moxifloxacin control" (n=10)	30 mg/kg	-	10^4 spores
"DAV131 100 mg/kg" (n=10)	30 mg/kg	100 mg/kg bid	10^4 spores
"DAV131 300 mg/kg" (n=10)	30 mg/kg	300 mg/kg bid	10^4 spores
"DAV131 600 mg/kg" (n=10)	30 mg/kg	600 mg/kg bid	10^4 spores
"DAV131 900 mg/kg" (n=10)	30 mg/kg	900 mg/kg bid	10^4 spores
"DAV131 control" (n=9)	-	900 mg/kg bid	10^4 spores

Fig 2: Survival of hamsters following inoculation of *C. difficile* spores

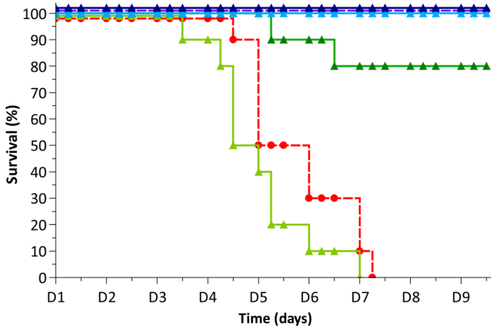


Fig 3: Viable *C. difficile* counts on Day 4

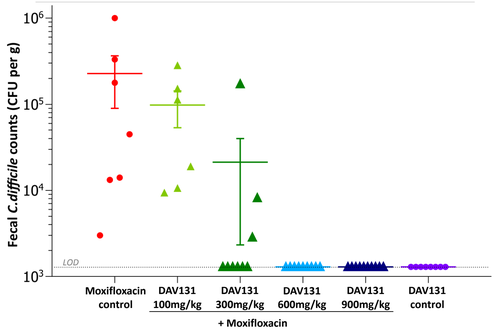


Fig 4: Evolution of body weight

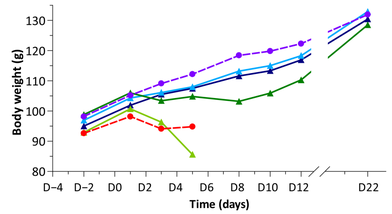
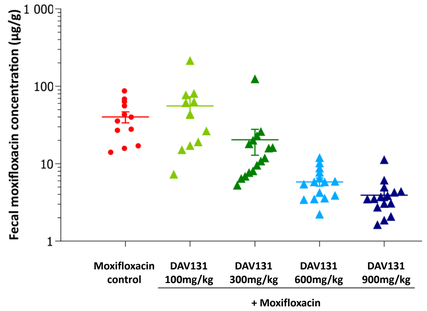


Fig 5: Mean fecal moxifloxacin concentrations



The development of the disease was assessed by monitoring hamster survival (Fig 2), body weight (Fig 4), and measuring viable *C. difficile* counts in feces (Fig 3).

Hamsters receiving only DAV131 without moxifloxacin treatment did not present any detectable C. *difficile* counts in feces, gained weight steadily and all survived.

As previously shown (3, 4), moxifloxacin treatment allowed colonization by *C. difficile*, as attested by high fecal *C. difficile* titers measured 20 hours after infection (Day 4), and caused rapid mortality (survival of 50% at Day 5 and 0% at Day 7).

When co-administered with moxifloxacin, the lowest DAV131 dose (100 mg/kg bid) did not protect the animals which exhibited elevated *C. difficile* counts between infection and death.

On the contrary, animals receiving 600 and 900 mg/kg bid DAV131 were entirely protected with 100% survival up to Day 22, no signs of morbidity nor detectable *C. difficile* counts in feces throughout the study; their body weight evolution was similar to control animals that received no antibiotic treatment.

Animals treated with 300 mg/kg bid DAV131 experienced 80% survival. Interestingly, some of the animals that survived exhibited a transient elevation of fecal *C. difficile* counts (as attested for day 4 on Fig 3), as well as a concomitant pause in weight gain (Fig 4); eventually, viable *C. difficile* counts returned to baseline, the animals resumed weight gain and survived until the end of the experiment. These data suggest that this dose level could be close to the lower limit of efficacy for the DAV131 adsorbent in this model.

Fecal levels of moxifloxacin were also monitored for each fecal sample during the first 6 days of the study; Fig 5 shows the average moxifloxacin concentration in each group, for all of the 3 daily fecal samples. In the group receiving 100 mg/kg bid DAV131 presenting 100% mortality, fecal moxifloxacin concentrations were similar to the control group receiving moxifloxacin alone. In contrast, fecal moxifloxacin concentrations were considerably reduced in the groups that experienced total protection by DAV131. Interestingly, in the group presenting 80% protection, fecal moxifloxacin concentrations were intermediate between these two extremes.

CONCLUSION

The results of this study confirm that, when co-administered with moxifloxacin, DAV131 can prevent the onset of *Clostridium difficile* Associated Disease in hamsters thereby protecting animals from death. This protection is correlated with the elimination, through adsorption, of moxifloxacin residues in the gut and with the absence of colonization of the gut by *C. difficile*. In addition, our study shows the dose-dependency of this protective effect on animal survival, as well as on fecal concentrations of moxifloxacin.

In conclusion, we confirm that DAV131 represents an excellent preventive means to protect against CDI, if applied concomitantly with the causative antibiotic treatment.

The development of this promising strategy for the prevention of CDI in humans (with code name DAV132) is on-going. The first trial in healthy volunteers of DAV132 is presented in poster P0248 at ECCMID 2014.

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