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DAV131, an Oral Adsorbent-Based Product, Fully Protects Hamsters Against Moxifloxacin-Induced *Clostridium difficile* Lethal Infection

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Abstract

Background. *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 the protective effect of DAV131 in a moxifloxacin-induced CDI hamster model.*

Methods. *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 15 animals were orally administered 1,800 mg/kg/day DAV131, from 6 hours before the first moxifloxacin injection until three days after the last one. 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 60% survival at day 4, 13% at day 5, 7% at day 6, and 0% at day 7. In contrast, all animals in both DAV131 treated groups exhibited 100% survival until the end of the experiment at day 12, with no signs of morbidity. Fecal titers of C. difficile were 300-fold lower in DAV131 treated animals, 24-48 hours after infection as compared to moxifloxacin alone and were undetectable by 72 hours. Recoverable moxifloxacin levels were approximately 20-times lower in animals given DAV131 over the course of the study.*

Conclusion. *Oral DAV131 fully prevented moxifloxacin-induced lethal CDI with both regimens tested; the protective effect of DAV131 most probably results from the effective adsorption of residual antibiotic in the gut, and prevention of gut microflora disruption. This is, to our knowledge, the first demonstration that a preventive strategy can protect against CDI if 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

When patients are treated with antibiotics, either orally or parenterally, part of the antibiotic reaches the colon in its active form, and can severely impact the bacterial population of the gut. This may result in the colonization of the intestine, and *in situ* proliferation of undesirable and potentially pathogenic bacteria. Such is the case with the anaerobic toxigenic bacterium *Clostridium difficile*, which is responsible for diarrhea (CDAD) which may progress into severe and potentially lethal conditions such as pseudomembranous colitis or toxic megacolon.

A characteristic of this condition is that patients that have had a first episode are at a much higher risk of developing a recurrence, either spontaneously a few weeks after the end of treatment, or during treatment with systemic antibiotics for an unrelated infection, most often respiratory or urinary. CDAD and most of all, recurrence of CDAD in high risk patients, is a known growing medical need worldwide which is observed in hospitals but also in the community, particularly in nursing homes (1), with increased frequency and severity. Most antibiotics can lead to CDAD, but clindamycin, cephalosporins and fluoroquinolones are considered as major risk factors (2).

Da Volterra has been developing a novel adsorbent-based product, DAV132, which limits the alterations of the colonic commensal flora during antibiotic treatments by adsorbing unwanted residual antibiotics in the lower intestine before they reach the colon. In this work, we have investigated, using the hamster animal model, whether such a product (DAV131 adapted for rodents), when co-administered with moxifloxacin, could prevent the onset of CDAD induced by the antibiotic treatment.

Methods

Male Golden Syrian hamsters, (80-120 g) were housed one to a cage with free access to food and water in accordance with NIH guidelines. They were treated subcutaneously (SC) once per day with the indicated amounts of moxifloxacin on days 1-5, and challenged with 10^4 spores of *C. difficile* strain UNT-103-1 (non-epidemic, cdtB-) on day 3 approximately 4h after moxifloxacin dosing. The protective effect of DAV131 was assessed by additionally treating animals twice or thrice per day orally with DAV131 on days 0-8. Feces were collected daily 6, 12 and 24h after moxifloxacin administration for the determination of fecal moxifloxacin levels (microbiological assay using *B. subtilis* ATCC 6633 as the indicator organism), and viable *C. difficile* counts (by plating the 24h fecal samples on selective agar media). Animals were examined at least three times per day for the duration of the experiment, and those judged to be in a moribund state were euthanized.

Results

The objective of this study was to evaluate if the DAV131 adsorbent-based product could prevent the onset of CDAD induced by antibiotics in the well-established hamster model (3, 4). Moxifloxacin was selected as the inducing antibiotic, as it has been observed to be a significant risk factor for CDAD in humans.

Determination of optimal antibiotic dosage for CDAD induction. Animals were treated SC with increasing doses of moxifloxacin (n=5 per group), and the development of the disease was assessed by monitoring their survival. A dose-response relationship was observed between moxifloxacin doses, animal mortality (Fig 1A) and *C. difficile* titers in feces (Fig 1B).

Mortality was observed beginning 24 hours post-infection with 20%, 80% and 60% survival for the 40, 30 and 20 mg/kg dose groups. At the end of the study on day 10, there was no survival in the 40 mg/kg dose group and 20% survival in the 20 and 30 mg/kg dose groups. In these groups, high fecal *C. difficile* titers were measured 24 hours after infection.

100% survival was observed in the 5 and 10 mg/kg dose groups over the end of study on day 10. Approximately 3 $\log_{10}$ lower counts of fecal *C. difficile* were measured, as compared with high dosage groups; these animals did not appear to develop CDAD.

Protective effect of DAV131 on CDAD induced by moxifloxacin subcutaneously-dosed at 30 mg/kg. Animals (n=15 per group) administered moxifloxacin alone exhibited a rapid mortality following oral infection by *C. difficile* spores, with 60% survival at day 4, 13% at day 5, 7% at day 6, and 0% at day 7 (Fig 2A). In contrast, all animals in both DAV131-treated groups exhibited 100% survival until the end of the experiment at day 12, with no signs of morbidity or ill-effects; all animals appeared to gain weight at a comparable rate from day 0 through day 12 (not shown).

The fecal counts of viable *C. difficile* at 24-48 hours after infection are presented in Fig 2B: a mean titer of 6.09 $\log_{10}$ CFU was detected in hamsters treated with moxifloxacin alone. In contrast, animals administered DAV131 alone, or co-treated with moxifloxacin and DAV131 exhibited 300 – 800x lower counts (3.45 – 3.70 $\log_{10}$ CFU); these differences were found statistically significant ($p<0.05$) by a 1-way ANOVA and Bonferroni's Multiple Comparison Test. In addition, *C. difficile* was undetectable from 72 hours post-infection in fecal samples from all DAV131-treated animals (data not shown).

Mean fecal moxifloxacin levels in animals administered antibiotic alone ranged 8.5 – 60.2 µg/g with an overall mean sample concentration of 37.2 µg/g (Fig 2C). In contrast, animals co-administered with DAV131 exhibited fecal moxifloxacin concentrations ranging 0.5 – 3.8 µg/g (mean of 1.6 µg/g and 1.92 µg/g, for 900 mg/kg bid, and for 600 mg/kg tid, respectively). The difference in fecal moxifloxacin levels for groups receiving DAV131 treatment compared to moxifloxacin alone were statistically significant ($p<0.05$) by a 1-way ANOVA and Bonferroni's Multiple Comparison Test.

Fig1: Induction of CDAD in hamsters by moxifloxacin administration. Groups of 5 animals were treated subcutaneously once a day for 5 days with the indicated amounts of moxifloxacin (5, 10, 20, 30 or 40 mg/kg), and challenged on day 3 with *C. difficile* spores.

1A: Survival of animals: recorded at the end of each day.
1B: Mean viable counts of *C. difficile* in feces. For the groups treated with 20, 30 and 40 mg/kg of moxifloxacin, due to the high mortality as of day 5, the data shown for days 5-7 correspond to fecal samples collected from 1 or 2 animals only. The limit of detection (LOD) was 2.75 $\log_{10}$ CFU/g of feces.

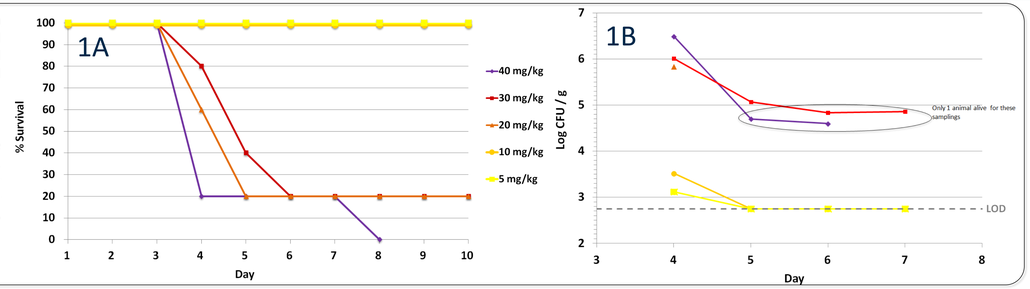
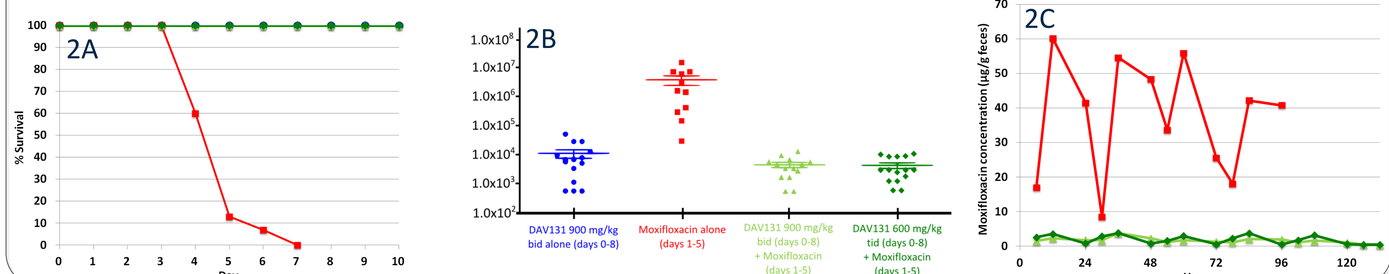


Fig 2: Preventive effect of DAV131 on the induction of CDAD by moxifloxacin. Groups of 15 animals were dosed subcutaneously once per day with 30 mg/kg moxifloxacin on days 1-5 (groups 2-4); animals in groups 3 and 4 were additionally treated on days 0-8 with 900 mg/kg bid, or 600 mg/kg tid DAV131. Animals in group 1 only received 900 mg/kg DAV131 bid on days 0-8. All animals were challenged on day 3 with 10^4 *C. difficile* spores.

2A: Animal survival. **2B:** Mean and individual fecal viable *C. difficile* counts recorded 24 hours post-infection. **2C:** Mean fecal concentrations of moxifloxacin.

Group		SC moxifloxacin on days 1-5	Oral DAV131 on days 0-8	*C. difficile* inoculation on day 3
1	DAV131 900 mg/kg bid alone (days 0-8)	-	900 mg/kg bid	10^4 spores
2	Moxifloxacin alone (days 1-5)	30 mg/kg/day	-	10^4 spores
3	DAV131 900 mg/kg bid (days 0-8) + Moxifloxacin (days 1-5)	30 mg/kg/day	900 mg/kg bid	10^4 spores
4	DAV131 600 mg/kg tid (days 0-8) + Moxifloxacin (days 1-5)	30 mg/kg/day	600 mg/kg tid	10^4 spores



Conclusion

The study confirms that moxifloxacin induces the development of CDAD in hamsters following ingestion of *C. difficile* spores, presumably by affecting composition of the normal bacterial flora, thereby allowing germination of *C. difficile* spores and resulting in the observed mortality. This work shows that the development of the disease and subsequent mortality is dose dependent, requiring a sufficient dose and thereby gastrointestinal antibiotic levels, to result in the disease state.

Protective effect of DAV131 in the moxifloxacin-induced hamster CDAD model was evaluated with two regimens of administration, either 900 mg/kg bid or 600 mg/kg tid (1800 mg/kg/day for 9 days).

In both regimens, co-administration of DAV131 resulted in:

- **lower mean fecal levels of moxifloxacin than for animals treated with moxifloxacin alone (approximately 20x lower)**
- **lower *C. difficile* mean fecal titers than for animals treated with moxifloxacin alone (approximately 300x lower at 24–48 hours after infection)**
- **100% survival of infected animals.**

Hence, DAV131 demonstrates excellent potential for the prevention of antibiotic-induced *C. difficile* associated diarrhea and warrants further investigation to verify the relationship between adsorption of intestinal antibiotic residues, effect on normal intestinal flora (microbiome), and outgrowth of *C. difficile* organism causing production of toxins and active disease.

References

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