Extended protection by DAV131A against antibiotic-induced *Clostridium difficile* infection in hamsters

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1. BACKGROUND

Treatment with antimicrobials is the primary risk factor contributing to the development of *C. difficile* infection (CDI), which ranges from a mild self-limiting disease to the severe, life-threatening condition called pseudomembranous colitis. Among the antimicrobials most often implicated are clindamycin, fluoroquinolones (such as moxifloxacin), and cephalosporins (such as ceftriaxone) (1, 2).

Antibiotics induce the development of (CDI) presumably by affecting the composition of the intestinal microbiota, thereby allowing germination of *C. difficile* spores, proliferation of the bacteria, production of cytotoxins and development of enterocolitis (3).

Da Volterra is developing the DAV132 product (DAV131A being the formulation for rodents), an adsorbent-based therapy designed to adsorb antibiotic residuals reaching the caecum and colon, thereby preventing their deleterious effects on the microbiota, including the development of *C. difficile*.

We previously showed that DAV131A could protect hamsters against CDI induced by moxifloxacin (4). Here, we investigated if DAV131A could also protect hamsters against CDI after treatment with clindamycin or ceftriaxone.

2. METHODS

2.1 Moxifloxacin (MXF)-, ceftriaxone (CRO)- and clindamycin (CLI)-induced *C. difficile* infection models in hamsters

- Male Syrian hamsters (n=10 per group) were administered 30 mg/kg MXF or 90 mg/kg CRO subcutaneously oad for 5 days (D1 to D5). Alternatively, they were given a single subcutaneous administration of 10 mg/kg CLI at D2. All animals received an inoculation of 10^4 *C. difficile* spores (non-epidemic UNT-103-1 strain, TcdA+, TcdB+, cdtB–, vancomycin MIC 2 µg/mL, MXF MIC 16 µg/mL, CLI MIC>256 µg/mL, CRO MIC 128 µg/mL) administered orally at D3 in animals treated with MXF and CRO, whereas animals to be treated with CLI were infected with spores on D1. Animals were orally administered 100, 300, 600 or 900 mg/kg DAV131A twice a day from D1 to D8 for animals treated with MXF and CRO, and from D2 to D8 for animals treated with CLI. Control groups received either antibiotic alone (MXF, CLI or CRO control), or DAV131A without antibiotic (DAV131A control).
- Survival was assessed over time. Feces were collected to determine free MXF, CRO and CLI concentrations by microbiological assay (*B. subtilis* ATCC 6633, *E. coli* ATCC 25922 and *M. luteus* ATCC9341 as the indicator organisms for MXF, CRO and CLI, respectively).

2.2 Statistical analysis

- For each studied antibiotic, a global Fisher exact test was performed to compare the survival at D12 between DAV131A treatment groups and control group. When significant, paired comparisons of survival for each DAV131A treatment group versus the control group were performed, with p values corrected using the Benjamini-Hochberg's method.
- The log-transformed antibiotic concentrations were compared between DAV131A treatment groups and control group using an ANalysis Of VAriance (ANOVA). When significant, Dunnett's tests were performed in order to compare antibiotic concentrations in each DAV131A treatment group versus the control group. All tests were 2-sided with a type-I error of 0.05.

3. DAV131A PROTECTS HAMSTERS FROM MOXIFLOXACIN-INDUCED *C. DIFFICILE* INFECTION *(PREVIOUS RESULTS)*

- When co-administered with MXF, DAV131A treatment at 600 or 900 mg/kg bid fully protected animals from MXF-induced lethal CDI (100% survival vs 0% survival in the absence of DAV131A over 12 days) (**Fig. 1**). Animals treated with 300 mg/kg bid DAV131A exhibited 80% survival, whereas the lowest DAV131A dose (100 mg/kg bid) did not protect the animals.
- The protection effect of DAV131A was associated with a dose-dependent reduction of free MXF fecal levels (**Fig. 2**).

Figure 1: Hamster survival over D1-D12 (%).

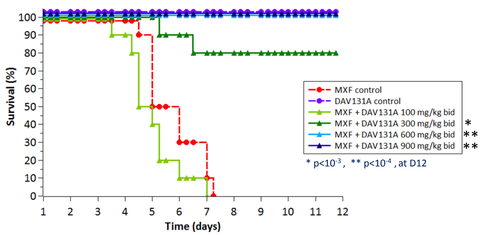
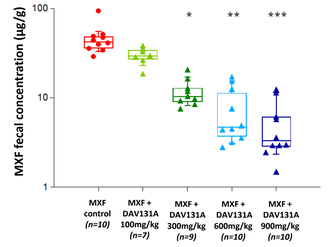


Figure 2: Moxifloxacin (MXF) fecal concentrations on D3 (box plots: median and quartiles; whiskers: 0.1 and 0.9 percentiles; dots: individual D3 values)



4. DAV131A ALSO PROTECTS HAMSTERS FROM CLINDAMYCIN-INDUCED *C. DIFFICILE* INFECTION

- When administered at 900 and 600 mg/kg bid, DAV131A protected 100% of hamsters from CLI-induced lethal CDI, whereas 30% of protection was achieved with 300 mg/kg bid (**Fig. 3**).
- This protection against lethality was associated with a dose-dependent reduction of free CLI concentrations in feces (**Fig. 4**).

Figure 3: Hamster survival over D1-D13 (%).

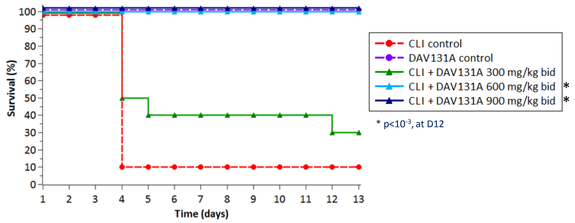
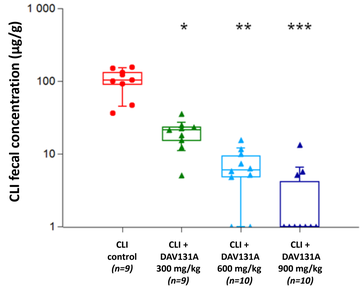


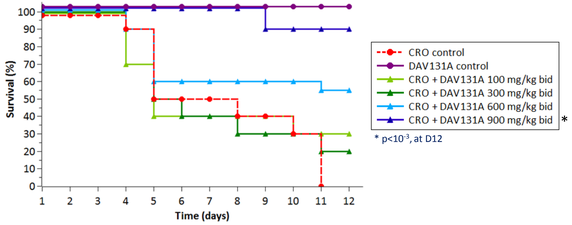
Figure 4: Clindamycin (CLI) fecal concentrations on D2 (box plots: median and quartiles; whiskers: 0.1 and 0.9 percentiles; dots: individual D2 values)



5. DAV131A ALSO PROTECTS HAMSTERS FROM CEFTRIAXONE-INDUCED *C. DIFFICILE* INFECTION

- DAV131A protected 90% of hamsters from CRO-induced lethal CDI, when administered at 900 mg/kg bid (**Fig. 5**). At the dose of 600 mg/kg bid, 55% of the animals survived. Lower doses were less protective, with 20% and 30% of survival at 300 and 100 mg/kg bid, respectively.
- CRO was hardly detectable in feces, not enabling the analysis of the effect of DAV131A on fecal CRO concentrations. This is probably due to an intestinal degradation of CRO by endogenous β-lactamases produced by the intestinal microbiota.

Figure 5: Hamster survival over D1-D12 (%).



6. CONCLUSION

- **Oral DAV131A exhibited a dose-dependent protection of hamsters against CLI- and CRO-induced lethal CDI, as it did with MXF. For CLI, as we had shown for MXF, protection is correlated with the elimination of free antibiotic residues in the gut through their adsorption by DAV131A. Although we were unable to measure free CRO concentrations in feces, we assume that DAV131A exerts protection against CRO-induced CDI by the same mechanism.**
- **This study clearly shows that DAV131A constitutes the first preventive strategy that can potentially protect against CDI when applied concomitantly with diverse causative antibiotic treatments. The development of this promising strategy for the prevention of CDI in humans (named DAV132) is ongoing.**

References

1. McCusker M. et al., *Emer Infect Dis* 2003;9(6):730-733
2. Johnson S. et al., *J Infect* 2009;58:403-410
3. Theriot C. M. et al., *Annu Rev Microbiol* 2015,69:445-461
4. de Gunzburg J. et al., Poster 760, IDWeek 2015.

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