COMPARATIVE EFFICACIES OF DAPTOMYCIN AND VANCOMYCIN FOR TREATMENT OF *CLOSTRIDIUM DIFFICILE*-ASSOCIATED DIARRHEA IN HAMSTERS

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ABSTRACT

Clostridium difficile-associated colitis is an increasing cause of morbidity and mortality in hospitalized patients, with high recurrence rates following conventional therapy. Daptomycin, a non-absorbed lipopeptide antibiotic which inhibits the biosynthesis of cell wall peptidoglycan, has proved a rapid bactericidal activity *in vitro* against *C. difficile*.
We evaluated the efficacy of daptomycin: 1) against a set of reference and clinical *C. difficile* strains collected from two French hospitals by determining the minimal inhibitory concentrations (MICs) and 2) in a hamster model of *C. difficile*-associated colitis, reproducing that observed in humans.
For *in vivo* experiments, hamsters received clindamycin by oral route five and one days before being infected with the reference strain VPI-10463. Daptomycin (50 mg/kg), vancomycin (50 mg/kg) or isotonic saline (for untreated controls) were then given orally in a single dose or multiple dose for five consecutive days beginning either 24 hours after the challenge with *C. difficile* or since the onset of the disease. The respective efficacies of both antibiotics was evaluated by Kaplan Meier survival estimates, weight gain, delays to the onset of the disease or to death, and the observation of recurrence within 30 days post infection in case of survival.
In epidemiological approach, the MIC_{50} and MIC_{90} for 120 strains were 0.38 and 0.75 mg/L respectively [range: 0.047-1.5]. The MIC for *C. difficile* VPI-10463 was 0.5 mg/L.
Oral treatment with a single dose of both antibiotics produced a similar effect with prolonged survival of hamsters, however, although death invariably occurs within 7 to 20 days after those of untreated animals.
Oral treatment with a multiple dose of both antibiotics for five consecutive days showed that daptomycin was as effective as Vancomycin, as reference treatment, in delaying death in hamsters. However, it has been observed fewer recurrences at 30 days among animals treated with daptomycin than in those treated with Vancomycin.
No emergence of resistant strains or increasing MICs of daptomycin has been detected during all *in vivo* experiments.
Altogether, our results indicate that Daptomycin is an effective treatment for clindamycin-associated colitis in hamsters and suggest that it may be a valuable alternative in humans.

INTRODUCTION

Clostridium difficile is the primary bacteria causing hospital-acquired diarrhea. *Clostridium difficile* infections (CDI) leads to increase overhead linked to significant increase in morbidity and mortality and prolonged length of hospital stays. Since 2003, CDI are more frequent (41,400 cases/year in France), more severe (18% of complications, and 3% of deaths/year).

Despite treatment of CDI based on Metronidazole or Vancomycin orally administrated, relapses are common occurring in nearly 20 % of all cases and in some series in as many as 50%.
Moreover, although no resistant strains were described, recent data indicate that a failure to respond to Metronidazole, the usual first-line agent, is now more common, raising concerns that the current treatment approach may be inadequate.

Daptomycin is the first agent of cyclic acidic lipopeptide which inhibits the biosynthesis of cell wall peptidoglycan. Daptomycin, nonabsorbed when administered orally, has proved a rapid bactericidal activity *in vitro* against *C. difficile* with making it ideally suited for used as alternative treatment for CDI.

We carried out a combining epidemiological and experimental approache in order to evaluate the efficacy of Daptomycin in comparison to those of Vancomycin, as reference treatment.
Because several studies have demonstrated the effect of calcium concentration in the activity of Daptomycin, we first tested different blood culture media for the determination of MIC *in vitro*.

MATERIEL AND METHODS

Effect of the Calcium on the determination of MIC of Daptomycin:
The influence of different blood culture media on minimal inhibitory concentration (MIC) determination, including the concentration of Calcium was first evaluated in double blind in two distinct laboratories using six clinical isolates and five reference strains of *C. difficile*. *Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 29212) were used as controls. The blood agar medium tested were: conventional sheep blood agar media (COS, bioMérieux), trypticase soy agar with 5% horse blood (TSH, bioMérieux), Mueller Hinton blood agar (BD and Biorad).

Bacterial isolates and in vitro susceptibility testing:
A total of 120 clinical toxigenic *C. difficile* isolated from patients hospitalized in two French Hospital in 2010 and before 2007 and five reference strains of *C. difficile* (VPI-, 630, 6425, R20291, 79-685) were tested against daptomycin according to the current EUCAST recommendations. MICs were determined by E-test method on blood agar media.

Detection of the selection of resistant strains
CCTa media including 1mg/L daptomycin were used for isolating resistant strains from stool specimens and caecal contents of dead animals. The MIC of daptomycin were systematically determined for each isolate during all the *in vivo* experiments.

Animals
Female Golden Syrian Hamsters (80-100g, Charles River) were housed in individualized cages with free access to sterile food and water. Bedding were changed daily.
Infection
Hamsters were conditioned with two doses of clindamycin (50mg/kg) administered orally, five and one days before being infected by gavage of ~200 spores of the hypervirulent *C. difficile* VPI-10463 (D0).
Antibiotic treatments
Various schemes of treatment by oral route were applied to the 3 groups of animals: untreated control (sterile isotonic saline solution), vancomycin (50 mg/kg of body weight), and daptomycin (50 mg/kg of body weight). The single dose schema consists in the administration of both antibiotic or sterile isotonic saline solution 24 hours after infection. Multiple dose schemes consists in the administration of both antibiotic or isotonic saline solution for five consecutive days since D1 after infection or from the onset of the CDI (first time for which CDI could be suspected according to weight loss (compare to the initial weight at D0), diarrhea, wet tail, loss of activity).
Criteria of efficacy
Animals were weighted and observed twice a day to determine the delays for first clinical manifestation of CDI including weight loss, loss of activity, modification of the consistence of stool, and mortality.

RESULTS of *in vitro* experiments

Effect of calcium on the determination of MICs of daptomycin

The influence of the concentration of Calcium has been assessed by testing four usually used blood agar medium (TSH, COS, and two Mueller Hinton).
No significant differences in MICs for clinical and reference strains (R20291, 79-685, 630, VPI-10463, 6425) were observed regardless the different medium tested or the laboratory where the assays were performed.

Epidemiological study

The MIC_{50} and MIC_{90} for 120 strains were 0.38 and 0.75 mg/L respectively [range: 0.047-1.5].
The MIC for *C. difficile* VPI-10463 was 0.5 mg/L
No significant differences were observed regardless the origin or the period of isolation of the strains

RESULTS of *in vivo* experiments

Effect of a single dose of Vancomycin or Daptomycin

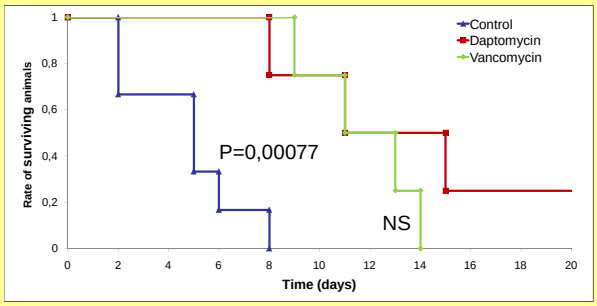

Figure 1: Kaplan-Meier estimates of survival following a single dose of both antibiotics

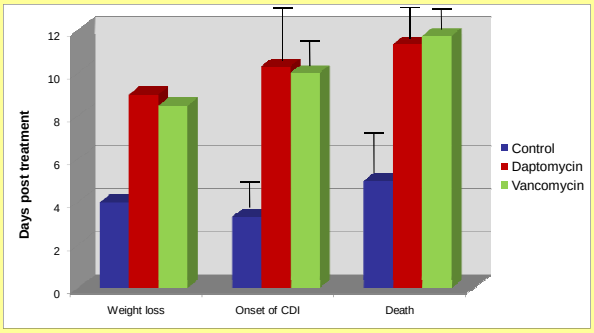

Figure 2: Delays for first clinical manifestations of CDI including weight loss and death following a single dose of both antibiotics.

-The result of a single dose of both antibiotics, orally administrated 24 hours after infection, delayed significantly the onset of *C. difficile*-induced colitis in hamsters and the occurrence of death (p=0,00077, Log Rank test) (Figures 1 and 2).

- However, a single of both of them was not sufficient to prevent death that invariably occurs within 7 to 20 days after those of untreated animals.

- We did not observe differences in the respective efficacies of Daptomycin and Vancomycin (as reference treatment), after the administration of a single dose that minimizes the differences in terms of pharmacokinetics.

Antibiotic efficacies following various scheme of multiple doses treatment

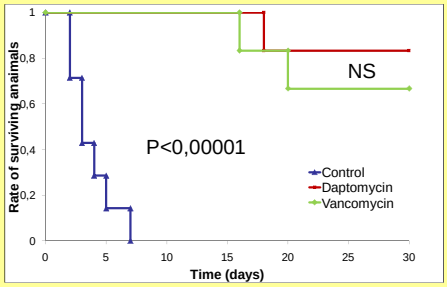

Figure 3: Kaplan-Meier estimates of survival following five consecutive days of treatment started 24 hours after infection

Multiple doses of Daptomycin and Vancomycin, started as soon as 24 hours after infection, prevents *C. difficile-induced* colitis in hamsters (P=0.0064, Log Rank Test, Figure 3). Although no significant differences between the respective efficacies of Daptomycin and Vancomycin were observed, recurrences within 30 days post infection occurred in 20% and 40% respectively.

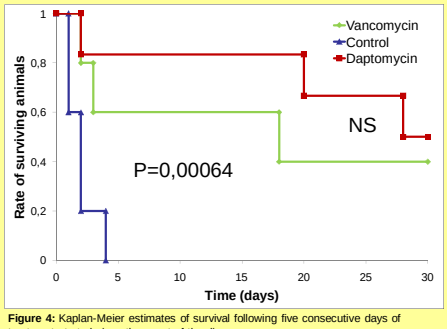

Figure 4: Kaplan-Meier estimates of survival following five consecutive days of treatment started since the onset of the disease.

Multiple doses of Daptomycin and Vancomycin started since the onset of ICD, cures animals from *C. difficile-induced* colitis in hamsters (P<0.00001, Log Rank Test, Figure 4). However, this therapeutic scheme led to early mortality not reported with the previous assay and recurrences occurred with both antibiotics.
This clearly shows the interest to start an adapted treatment as soon as possible in order to prevent treatment failure or recurrence.

No emergence of resistant strains or increasing MICs of daptomycin has been detected during all the *in vivo* experiments.

CONCLUSIONS

Incidence of CDI is increasing whereas they seem to become less responsive to antibiotics. In this context, the validation of new therapeutic alternatives may be required.
Altogether, our results indicate that Daptomycin presents an intrinsic activity against *C. difficile* with low MICs against a large collection of strains from various origin. Moreover, compared to Vancomycin as reference treatment, Daptomycin appears to be an effective treatment to prevent or to cure clindamycin-associated colitis in hamsters and suggests that it may be a valuable therapeutic alternative in humans.