Effect of dalbavancin on the normal intestinal microflora

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Objectives: Dalbavancin is a new lipoglycopeptide antibiotic active in vitro against most Gram-positive bacteria. It is administered parenterally as a weekly regimen, is eliminated both in urine and faeces, and has \( t_{1/2} \) in plasma of 8.5 days. Investigating the impact of antibiotics on endogenous microflora is important since alteration of the balance may facilitate colonization by new potentially pathogenic strains or enable microorganisms in the normal flora to develop resistance. The purpose of the present study was to investigate the effect of administration of dalbavancin on the intestinal flora of healthy subjects.

Methods: Six women and six men, 18–40 years, received a single 30 min intravenous infusion of 1 g dalbavancin. Plasma and faeces were collected over several weeks for determination of dalbavancin concentration and analysis of faecal flora. Faecal specimens were cultured on non-selective and selective media. Different colony types were counted, isolated in pure culture and identified to genus level. All new colonizing bacteria were tested for susceptibility to dalbavancin.

Results: Plasma dalbavancin concentrations at 2, 21 and 60 days after administration were 35.8–208.7, 3.9–22.1 and 0.5–2.9 mg/L, respectively. The faecal concentrations of dalbavancin were 6.8–73.4 mg/kg on day 5 and 7.4–26.4 mg/kg on day 14. Dalbavancin was not detectable in faeces on day 60. There was some impact on numbers of enterococci and \( \text{Escherichia coli} \) and no changes in numbers of lactobacilli, clostridia and bacteroides. No \( \text{Clostridium difficile} \) strains were recovered. No new colonizing aerobic and anaerobic bacteria resistant to dalbavancin were found.

Conclusions: Dalbavancin has no major ecological effect on the human normal intestinal microflora.

Keywords: antibiotics, clinical trials, pharmaceutical products

Introduction

Dalbavancin is active in vitro against most Gram-positive bacteria, including staphylococci, streptococci and enterococci, corynebacteria and anaerobes.\(^1\)\(^2\) It is active against methicillin-susceptible \( \text{Staphylococcus aureus} \), methicillin-resistant \( S. \) \( \text{aureus} \) and coagulase-negative staphylococci such as \( \text{Staphylococcus epidermidis} \), \( \text{Staphylococcus haemolyticus} \) and others. In experiments conducted in different laboratories, the MICs for at least 90% of isolates varied from 0.06 to 0.12 mg/L for staphylococcal species. Dalbavancin is highly active against \( \text{Streptococcus pneumoniae} \), including isolates resistant to penicillin, and against vancomycin-susceptible enterococci and some classes of vancomycin-resistant enterococci. Clinical studies have recently shown that dalbavancin, administered once weekly, is effective in the treatment of Gram-positive infections.\(^3\)\(^4\) Dalbavancin is excreted both in urine and faeces. The human normal microflora is relatively stable at each ecological habitat under normal circumstances and acts as a barrier against colonization by potentially pathogenic microorganisms. Disturbances in the normal microflora may occur due to changes in diet, radiation or administration of antimicrobial agents. Administration of antimicrobial agents can cause several adverse effects on the microflora.\(^5\) Careful investigation of the impact of antibiotic treatment on the endogenous microflora is of importance since alteration of the endogenous flora balance, qualitatively and/or quantitatively, may facilitate colonization by new potentially pathogenic strains or may enable microorganisms already present in the normal flora to develop resistance.

The purpose of the present study was to investigate the effect of systemic administration of dalbavancin on the intestinal flora of healthy subjects.

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Materials and methods

Subjects

A total of 12 subjects (6 women and 6 men; 18–40 years) participated in the study. All subjects included in the study had normal findings from physical examination, electrocardiogram and laboratory tests (including haematological and biochemical parameters, hepatitis and human immunodeficiency virus serological tests, tests for drug abuse, urinalysis and negative pregnancy test). Exclusion criteria were: regular use of medications; abuse of alcoholic beverages; symptoms of significant illness within 3 months before the study period; history of gastrointestinal, liver or kidney disease potentially interfering with absorption, metabolism or excretion of drugs; history of CNS disorders; allergy or hypersensitivity to the study drug; antibiotic treatment within 3 months before the study period; and pregnancy. Written informed consent was obtained from all subjects prior to the study. The study was approved by the Ethics Committee of the Karolinska Institute, Stockholm, Sweden, and the Medical Products Agency, Uppsala, Sweden.

Drug administration

All subjects received a single 1 g dose of dalbavancin as a 30 min intravenous infusion.

Sampling of specimens

Blood was collected for assessment of dalbavancin plasma pharmacokinetics. A total of 13 blood samples, 10 mL of blood each, were drawn into heparinized tubes at the following times: pre-infusion (prior to dose), end of infusion and 6 h post-start of infusion. In addition, blood samples were drawn on days 2, 3, 5, 7, 14, 21, 28, 35, 42 and 60, at the same clock time on each day as the time the infusion was started on day 1. Faeces were collected throughout the investigation to study the excretion of dalbavancin and for microbiological assessment. Faecal samples were collected prior to study drug administration and on days 2, 5, 14, 28/35 and 60. The collected faecal samples were returned to the clinic within 4 h of defecation.

Determination of dalbavancin plasma and faecal concentrations

Plasma and faecal samples were stored at −70°C until analysis for dalbavancin concentrations. The concentrations were measured by HPLC with atmospheric pressure chemical ionization tandem mass-spectrometry detection. The plasma and faeces assays had lower limits of quantification of 0.5 mg/L and 2 mg/kg, respectively.

Processing of faecal specimens for microbiological analysis

The faecal specimens were suspended in pre-reduced peptone–yeast extract medium, diluted to 10⁻⁷ and inoculated on non-selective and selective media. The following agar media were used: blood agar (Kemila, Lab M, Bury, UK) for total aerobes and anaerobes, CLED agar (Merck, Darmstadt, Germany) for detection of Enterobacteriaceae, Enterococcus faecalis ATCC 29213, Bacteroides fragilis ATCC 25285, Bacteroides thetaiotaomicron ATCC 29741 and Eubacterium lentum ATCC 43055. The agar plates were incubated at 37°C aerobically for 24 h or anaerobically for 48 h. The breakpoint for resistance was ≥4 mg/L.

Statistical methods

Descriptive statistics were calculated for the values estimated for the faecal specimens as log numbers of microorganisms/g faeces and faecal concentrations of dalbavancin.

Results

Dalbavancin concentrations in plasma and faeces

The plasma concentrations are shown in Table 1. The concentrations were as follows: on day 2, 3.8–20.8 mg/L (mean value 100 mg/L); on day 21, 3.9–22.1 mg/L (mean value 11.1 mg/L); and on day 60, 0.5–2.9 mg/L (mean value 1.3 mg/L). Table 2 shows the dalbavancin concentrations in faeces. No measurable concentrations were found on day 0 and 60, while the concentrations on day 5 were in the range 6.8–73.4 mg/kg, mean value 22.1 mg/kg, and on day 14 7.4–26.4 mg/kg, mean value 13.2 mg/kg.

Effect of dalbavancin on the aerobic intestinal microflora

Figure 1 presents the effect of dalbavancin on the aerobic intestinal microflora. There was some impact on the numbers of enterococci and Escherichia coli. The enterococci were identified to the species level. A total of 12 subjects were colonized with fusobacteria, veillonella agar (Difco) for cultivation of Veillonella coccii, egg-yolk agar (Oxoid, Basingstoke, UK) for cultivation of clostridia and taurocholate-cycloserine-cefoxitin-fructose agar (peptone from casein/peptone peptone no. 3 40 mg/mL, sodium hydrogen phosphate 5 mg/mL, potassium dihydrogen phosphate 1 mg/mL, sodium chloride 2 mg/mL, magnesium sulphate 0.2 mg/mL, Bacto agar/agar-agar 20 mg/mL, taurocholic acid 1 mg/mL, neutral red 0.03 mg/mL, 15% fructose, Clostridium difficile supplement D-cycloserine, cefoxitin) for detection of C. difficile. The aerobic agar plates were incubated for 48 h at 37°C and the anaerobic plates for 48 h at 37°C in GasPak anaerobic jars (BBL). After incubation, different colony types were counted, isolated in pure culture and identified to the genus level. All isolates were analysed according to Gram reaction and cell and colony morphology, followed by different biochemical tests. An API-20E test kit (BioMérieux, Marcy l’Etoile, France) was used for the identification of Enterobacteriaceae. The anaerobic microorganisms were identified by gas-liquid chromatography of metabolites from glucose. The lower limit of detection was 10² microorganisms per gram of faeces.

Determination of MICs for dalbavancin

All new colonizing bacteria were isolated from each subject’s treatment samples to determine the MICs of dalbavancin during the investigation period by the agar dilution method according to NCCLS guidelines. The medium used for the determination of MIC for bifidobacteria and lactobacilli was Brucella agar supplemented with 5 µg haemin and 1 µg vitamin K per mL and 5% laked sheep blood. The following reference strains were used: S. aureus ATCC 29213, Enterococcus faecalis ATCC 29212, Bacteroides fragilis ATCC 25285, Bacteroides thetaiotaomicron ATCC 29741 and Eubacterium lentum ATCC 43055. The agar plates were incubated at 37°C aerobically for 24 h or anaerobically for 48 h. The breakpoint for resistance was ≥4 mg/L.
Enterococcus faecium, 10 subjects with E. faecalis and 5 subjects with Enterococcus durans. No specific changes in colonization of the different enterococcal species were found. There were increased numbers of Klebsiella pneumoniae on day 5 in Patient 2, of Enterobacter cloacae on day 28 in Patient 5, of E. cloacae on day 14 in Patient 7 and of K. pneumoniae on day 14 in Patient 12. No impact was observed on the number of yeasts. No new colonizing bacteria resistant to dalbavancin were observed.

Effect of dalbavancin on the anaerobic intestinal microflora

The effect of dalbavancin on the anaerobic intestinal microflora is shown in Figure 2. There were no significant changes on the numbers of lactobacilli, clostridia and bacteroides and no new colonization with resistant anaerobic bacteria. No C. difficile strains were recovered.

Dalbavancin susceptibility tests

No new colonizing aerobic and anaerobic bacteria resistant to dalbavancin (MIC ≥ 4 mg/L) were found.

Discussion

Knowledge about the interaction between antibiotics and the normal intestinal microflora is crucial when the clinician chooses agents for treatment of bacterial infections. Antibiotics associated with minor ecological disturbances should be preferred to avoid the risk of development of resistant bacteria and transfer of resistant elements between bacteria.9,10 The increase of infections caused by Gram-positive bacteria and the rise of antibiotic-resistant enterococci, staphylococci and streptococci have prompted the need for the development of new agents active against these bacteria.11 Among the new agents are linezolid, oritavancin, telavancin, daptomycin and dalbavancin with good activities against most Gram-positive bacteria.12 Clinical studies have also shown the usefulness of these antibiotics in the treatment of Gram-positive infections. Two of these agents, linezolid and dalbavancin, have been investigated for their ecological effect on the intestinal human microflora. Linezolid caused a significant suppression of enterococci and a marked increase of Klebsiella...
strains. The numbers of bifidobacteria, lactobacilli, clostridia and bacteroides decreased significantly. No \textit{C. difficile} strains were recovered, which may be explained by the linezolid faecal concentrations found in the intestine during administration.\textsuperscript{13}

In the present investigation, dalbavancin had some ecological impact on the aerobic and anaerobic microflora with changes in the numbers of enterococci and \textit{E. coli} during 14 days. The concentration of dalbavancin in the faecal specimens was higher than the breakpoint for susceptibility, which may account for the effect on the aerobic bacteria. \textit{C. difficile} is susceptible to dalbavancin\textsuperscript{1} and therefore no strains were isolated due to the faecal concentration of dalbavancin. McNulty \textit{et al.}\textsuperscript{14} have also shown that the use of narrow-spectrum agents is effective in lowering the occurrence of \textit{C. difficile} infections. In two patients, no faecal concentrations of dalbavancin were found on day 2 and day 5, respectively, which may be due to several reasons as mentioned below.

The mechanism inactivating or binding dalbavancin in the intestine may explain why no marked effect on the microflora during and after the administration was found. New animal and human studies to investigate the proposed mechanism are therefore planned.

In conclusion, dalbavancin did not have any major ecological effect on the normal human intestinal microflora.
Dalbavancin and intestinal microflora

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Transparency declarations
None to declare.

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