VANCOMYCIN CAPSULES 125 MG

MODULE 2.4

NON-CLINICAL OVERVIEW

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[redacted]

Abbreviations:

AUC	Area under Curve
DDR	D-alanyl-D-alanine Residue
H/h	Hour
Im/im	Intramuscular
Ip/ip	Intrapulmonary
Iv/iv	Intravenous
MIC	Minimum Inhibitory Concentration
MRSA	Meticillin-Resistant Staphylococcus Aureus
PATC	Pulmonary Antibiotic Tissue Concentrations
PFD	Perfluorodecaline
PLV	Partial Liquid Ventilation
RNA	Ribonucleic Acid
SPCs	Summaries of Product Characteristics
VRSA	Vancomycin-Resistant Staphylococcus Aureus

2.4.1 OVERVIEW OF NONCLINICAL TESTING STRATEGY

In anticipation of the patent expiry, the principle objective was to develop a generic product with formulation and pharmaceutical properties as close as possible to the original Innovator product.

The innovator product is marketed in Europe by [redacted], as Vancocin Matrigel Capsules 125 mg containing 125 mg Vancomycin base. Vancocin Matrigel Capsules are used orally for the treatment of staphylococcal enterocolitis and pseudomembranous colitis due to Clostridium difficile.

The proposed Vancomycin Capsules 125 mg also contain the same active ingredient and are indicated for the same therapeutic indications as the innovator product.

According to the Article 10.1 of European Directive 2001/83//EEC, as amended, the applicant shall not be required to provide the results of pre-clinical tests and of clinical trials if he can demonstrate that the medicinal product is a generic of a reference medicinal product which is or has been authorised under Article 6 for not less than eight years in a Member State or in the Community.

A generic medicinal product authorised pursuant to this provision shall not be placed on the market until ten years have elapsed from the initial authorisation of the reference product. The 'ten year rule' applies and, in accordance with these provisions, therefore, the Applicant has not conducted clinical studies in support of this Application.

The proposed product contains the same active substance in the same concentration as the currently authorised product. Since Vancomycin is expected to have its pharmacological action within GIT for the said indication, it is not significantly absorbed from the normal gastrointestinal tract (i.e. it acts locally in GIT). Section 5.1.8 of the Note for Guidance on the Investigation of Bioavailability and Bioequivalence (CPMP/EWP/QWP/1401/98, EMEA 2001) describes exemption from in vivo bioequivalence studies for locally acting drug (after oral, nasal, inhalation, ocular, dermal, rectal, vaginal, etc. administration). Thus, in accordance with this guidance, the applicant is not required to submit a bioequivalence study.

The Summaries of Product Characteristics (SPCs) for the proposed Vancomycin Capsules 125 mg is based on very closely on the UK SPCs for Vancocin Matrigel Capsules 125 mg [redacted] and there are no significant differences in the contraindications, precautions and warnings.

A detailed discussion and critical review of all the published pre-clinical literature data on Vancomycin is therefore not warranted in this Overview as essential similarity to a product with an established and favorable risk-benefit profile has been demonstrated.

2.4.2 PHARMACOLOGY

The bactericidal actions of many antibiotics are associated with the inhibitions of cell wall biosynthesis that result from the binding of the antibiotics to enzymes and intermediates along the cell wall biosynthetic pathway. Also when cell wall biosynthesis is inhibited, the natural ongoing autolysis of the cell wall is no longer in balance with the insertion of new cell material. Hydrolytic cleavage of peptidoglycan glycosidic and peptide bonds proceeds until the mechanical properties of the cell wall that are responsible for osmotic protection of the protoplast membrane are lost and the membrane is damaged. The role of autolysins in the actions of penicillin and vancomycin has been amply demonstrated by the loss of the lethal actions of the antibiotics when cell wall autolysins are inhibited. Vancomycin forms strong complexes with gram-positive bacterial cell walls and lipid soluble cell wall intermediates and peptidoglycan precursors in the protoplast membrane. The inhibition of peptidoglycan biosynthesis by vancomycin in cell-free systems is restricted to its complexing with lipidsoluble intermediates in the membrane fraction. However, in the whole cell, part of the bactericidal effect of vancomycin may also be associated with the strong binding of vancomycin to the cell wall. The binding of highly charged vancomycin molecules to the amphoteric polyelectrolyte gel-like cell wall is likely to result in changes in the three dimensional arrangements of the cell wall polymers and in the contacts between the cell wall and protoplast membrane, which could affect the relative rates of cell wall autolysis and the insertion of new cell wall material. The activity of the major cell wall autolysin, N-acetylmuramoyl- L-alanine amidase (in Bacillus licheniformis and B. subtilis), has been shown to be related to the recognition of cell wall teichoic and teichuronic acids and to more subtle structural features of the cell wall.[1]

Mechanism of Action

Vancomycin is a tricyclic glycopeptide antibiotic derived from Amycolatopsis orientalis. The bactericidal action of vancomycin results primarily from inhibition of cell-wall biosynthesis. In addition, vancomycin may alter bacterial cell membrane permeability and RNA synthesis. There is no cross-resistance between vancomycin and other classes of antibiotics.[2]

Vancomycin inhibits the synthesis of peptidoglycan in membrane preparations from *Gaffkya homari* with uridine diphosphate-*N*-acetylmuramyl (UDP-Mur-NAc)-pentapeptide as substrate, but not with either UDP-MurNAc-tetrapeptide or UDP-MurNAc-tripeptide. These results are correlated with the complex formation between the antibiotic and the peptide subunit. It is concluded that the formation of a complex between vancomycin and a postulated cell wall acceptor or between vancomycin and the enzymes involved in peptidoglycan synthesis does not contribute to the inhibitory action of this antibiotic. The mechanism of vancomycin action on peptidoglycan synthesis is clearly different from that of moenomycin and bacitracin. In the presence of these antibiotics, peptidoglycan synthesis is inhibited with both UDP-MurNAc-pentapeptide and -tetrapeptide as substrates. In addition, these results provide additional insight into the mechanism of phospho-MurNAc-pentapeptide translocase.[3]

Vancomycin is active against most gram-positive bacteria, including streptococci, staphylococci, corynebacteria, clostridia, listeriae, and *Bacillus* species. Vancomycin does not interact with or block any enzyme involved in cell wall synthesis as do beta-lactam antibiotics; it physically

blocks the important substrates for cell wall-synthesizing machinery, i.e., the D-alanyl-D-alanine residue (DDR) of lipid II precursor. Thereby, it inhibits utilization of the substrates by glycosyltransferase (a cell wall synthesis enzyme) to produce the nascent peptidoglycan chain.[4]

Drug Resistance:

A pathogenic vancomycin-resistant Staphylococcus aureus (VRSA) isolate (MIC > or =64 microg ml(-1)) was obtained from a [redacted] in [redacted] Species identification was confirmed by Gram staining, standard biochemical tests and PCR amplification of the nuc gene, which encodes the thermostable nuclease that is highly specific for S. aureus. The VRSA isolate was also resistant to beta-lactams (amoxicillin, ampicillin, cefepime, cefotaxime, cefuroxime, cephalexin and meticillin), chloramphenicol, streptomycin, macrolides (erythromycin and roxithromycin), clindamycin, rifampicin and trimethoprim-sulfamethoxazole. However, the isolate was susceptible to gentamicin (an aminoglycoside) and ciprofloxacin (a fluoroquinolone). The resistance to vancomycin was inducible in vitro, because the MIC of vancomycin increased from [redacted] nitially to [redacted] during culture of this VRSA strain in the presence of vancomycin. The VRSA isolate contained a large plasmid (approximately 53.4 kb) and four small plasmids of approximately 6, 5.5, 5.1 and 1.5 kb. The large plasmid of approximately 53.4 kb harboured the vancomycin-resistance genes vanHAX, which was confirmed by PCR amplification using the same plasmid as template and, separately, primers specific for the 2.61 kb vanHAX gene cluster, vanH (969 bp), vanA (1032 bp) and vanX (609 bp). The VRSA isolate was also positive for mecA. Vancomycin resistance was successfully transferred from this VRSA donor to a vancomycin-sensitive recipient S. aureus clinical isolate by a broth mating procedure. The MIC of vancomycin for the transconjugant was [redacted], as against [redacted] for the parent strain. Nucleotide sequencing of the PCR product showed partial homology with van genes of an enterococcal transposon Tn1546-like element. This is believed to be the first [redacted] S. aureus isolate that has been shown to be phenotypically vancomycin-resistant, presumably due to a vanHAX analogue.[5]

Vancomycin has been the most reliable therapeutic agent against infections caused by meticillin-resistant *Staphylococcus aureus* (MRSA). However, in [redacted] the first MRSA to acquire resistance to vancomycin, was isolated from a [redacted] patient. The patient had contracted a post-operative wound infection that was refractory to long-term vancomycin therapy. Subsequent isolation of several vancomycin resistant *S aureus* (VRSA) strains from [redacted] has confirmed that emergence of vancomycin resistance in *S aureus* is a global issue. A certain group of *S aureus*, designated hetero-VRSA, frequently generate VRSA upon exposure to vancomycin, and are associated with infections that are potentially refractory to vancomycin therapy. Presence of hetero-VRSA may be an important indicator of the insidious decline of the clinical effectiveness of vancomycin in the hospitals. Vancomycin resistance is acquired by mutation and thickening of cell wall due to accumulation of excess amounts of peptidoglycan.[6]

2.4.3 PHARMACOKINETICS

[redacted] assessed the pharmacokinetic disposition of vancomycin and ciprofloxacin in rabbits before the efficacy of these compounds in experimental staphylococcal endocarditis was compared. Ciprofloxacin was given in single intravenous bolus doses of [redacted] and [redacted] and also in a multiple-dose regimen of [redacted]. Vancomycin was given in a similar manner in single doses of [redacted] and in a multiple-dose regimen of [redacted] Serum was sampled frequently after injections and analyzed by microbiologic assay for drug concentration. The pharmacokinetic parameters of clearance and steady-state volume of distribution were calculated by compartment-independent methods. These studies revealed that clearance of ciprofloxacin was reduced significantly after multiple doses (7.42 ± 0.85 [standard deviation] versus [redacted] $P < 0.01$). Although the half-life and volume of distribution increased after multiple dosing, the differences were not statistically significant. The disposition of vancomycin following single doses was significantly altered after the 25-mg/kg dose compared with the [redacted] dose. Half-life, clearance, and volume of distribution changed from [redacted] respectively. The disposition of ciprofloxacin was not altered with increases in dose size, and the disposition of vancomycin was not altered after multiple doses.[7]

[redacted]. examined the pharmacokinetics and pulmonary antibiotic tissue concentrations (PATC) of gentamicin and vancomycin after intrapulmonary administration of a perfluorodecaline (PFD)–gentamicin and a PFD–vancomycin emulsion during partial liquid ventilation (PLV). PLV was initiated in [redacted] healthy rabbits and [redacted] surfactant- depleted rabbits. The animals were randomized to receive either [redacted] gentamicin and 15 mg/kg vancomycin intravenously, or [redacted] gentamicin intrapulmonary, or [redacted] vancomycin intrapulmonary. Antibiotic plasma levels were measured after [redacted] min, and hourly thereafter. After [redacted] animals were sacrificed and lungs were removed to evaluate PATC and histology. PATC were significantly higher after intrapulmonary administration of both gentamicin and vancomycin. In healthy rabbits, peak plasma concentrations were lower and [redacted] plasma concentrations were higher after intrapulmonary administration, whereas plasma concentrations were not different in surfactant- depleted rabbits. There were no differences in lung histology, hemodynamics, lung mechanics, or gas exchange between the treatment groups.[8]

In one experiment, vancomycin ([redacted]) was given im, iv or ip and three rats were sacrificed at [redacted] min after injection. Renal kinetics of vancomycin after either a [redacted] dose or after [redacted] repeated doses given at [redacted] intervals was also studied. The rats were killed at predetermined intervals after the first injection. The results showed pharmacokinetics of a single dose of vancomycin ([redacted]) given iv revealed that the compound was eliminated with a serum half-life of [redacted] The area under the curve was [redacted] After im or ip administration of the same dose serum half-life was [redacted] and [redacted] (ip) and the area under the curve [redacted] (im) and [redacted] (ip) mg-h/1. The amount of vancomycin per g of kidney, however, remained almost constant during the first [redacted] After an ip dose of [redacted] renal concentrations of more than [redacted] were found until the sixth day. Doses of [redacted] given nine times ip led to renal accumulation (peak [redacted] renal tissue) and gradual decline

after the last doses. Intramuscular administration of identical doses resulted in similar renal kinetics.[9]

2.4.4 TOXICOLOGY

Acute and Chronic Toxicity

The nephrotoxicity, as measured by urinary cell and enzyme excretion, of vancomycin was studied in rats. The lowest daily iv dose inducing significantly increased cell elimination was [redacted] Intramuscular administration caused less effect probably due to incomplete absorption from the intramuscular injection site, since intramuscular dosages of [redacted] daily led to lower renal tissue concentrations than the same doses given iv. Nephrotoxicity of vancomycin increased when combined with tobramycin and was reduced when combined with D-glucaro-[redacted] lactam, a beta-glucuronidase inhibitor. Vancomycin accumulated in renal tissue during repeated administration.[9]

The influence of vancomycin on tobramycin nephrotoxicity was assessed in male Fischer rats. Treatment groups included controls receiving diluent and groups receiving vancomycin alone at a dosage of [redacted] (body weight) per day, tobramycin alone at a dosage of [redacted] day, and a combination of vancomycin and tobramycin at the above dosages. All regimens were injected on a twice-a-day schedule. The animals were sacrificed on days [redacted] When compared with controls, animals receiving vancomycin alone exhibited no detectable renal toxicity. Compared with the case with controls, tobramycin alone was toxic, as manifested by lower mean animal weights, increased blood urea nitrogen concentrations on days [redacted] ($P < 0.005$), increased serum creatinine concentrations on days [redacted] ($P < 0.005$), and the presence of renal cortical tubular necrosis and regeneration. When compared with tobramycin alone, the combination of vancomycin and tobramycin caused earlier and more severe toxicity. By day [redacted] the magnitude of weight loss, the rise in blood urea nitrogen, and the increase in serum creatinine concentration were all greater in the rats given the combination of vancomycin plus tobramycin than in the animals given tobramycin alone ($P < 0.005$). In addition, there was more proximal tubular necrosis and regeneration in rats given vancomycin plus tobramycin compared with those given tobramycin alone. In this animal model, vancomycin alone caused no detectable renal injury, tobramycin alone produced minimal proximal tubular damage, and the combination of vancomycin and tobramycin resulted in a greater degree of kidney injury than observed with tobramycin alone.[10]

Carcinogenesis, Mutagenesis, Impairment of Fertility

No long-term carcinogenesis studies in animals have been conducted.

At concentrations up to [redacted] µg/mL, vancomycin had no mutagenic effect *in vitro* in the mouse lymphoma forward mutation assay or the primary rat hepatocyte unscheduled DNA synthesis assay. The concentrations tested *in vitro* were above the peak plasma vancomycin concentrations of [redacted] µg/mL usually achieved in humans after slow infusion of the maximum recommended dose of [redacted] g.

Vancomycin had no mutagenic effect *in vivo* in the Chinese hamster sister chromatid exchange assay [redacted] mg/kg IP) or the mouse micronucleus assay [redacted] mg/kg IP).[11]

No definitive fertility studies have been conducted.[11]

Teratogenic Effects

Pregnancy Category B: The highest doses of vancomycin tested were not teratogenic in rats given up to [redacted] mg/kg/day IV ([redacted] mg/m^2 or 1 times the recommended maximum human dose based on $mg/m^{2)}$ or in rabbits given up to [redacted] mg/kg/day IV ([redacted] mg/m^2 or 1.1 times the recommended maximum human dose based on mg/m^2). No effects on fetal weight or development were seen in rats at the highest dose tested or in rabbits given [redacted] mg/kg/day [redacted] mg/m^2 or [redacted] times the recommended maximum human dose based on mg/m^2).[11]

2.4.5 ENVIRONMENTAL RISK ASSESSMENT

The Vancomycin Capsules 125 mg proposed for marketing are generically-equivalent to the capsules that will be prescribed interchangeably with, and instead of, other products containing Vancomycin already on the market in the Europe. The introduction of this product in the market is unlikely to result in any significant increase in the combined total sales volumes for all the pharmaceutical products containing Vancomycin and thus would not be expected to have an adverse effect upon the environment *per se*.

For this reason, a formal environmental risk assessment is not considered to be necessary.

2.4.6 INTEGRATED OVERVIEW AND CONCLUSIONS

Vancomycin Capsules 125 mg have been shown to be essentially similar to the UK Innovator product (Vancocin Matrigel Capsules). Both proposed and Innovator products contain the same active ingredient at the same strength.

The SPCs for the proposed Vancomycin Capsules 125 mg are entirely consistent with those of the innovator Vancomycin Capsules in the Europe, and the proposed and innovator products are expected to be prescribed interchangeably.

Vancomycin is a tricyclic glycopeptide antibiotic derived from Amycolatopsis orientalis. The bactericidal action of vancomycin results primarily from inhibition of cell-wall biosynthesis. In addition, vancomycin may alter bacterial cell membrane permeability and RNA synthesis. There is no cross-resistance between vancomycin and other classes of antibiotics.[2]

Vancomycin had no mutagenic effect *in vitro and in vivo*. No effects on fetal weight or development were seen in rats. Nephrotoxicity of vancomycin increased when combined with tobramycin. Vancomycin regimen is well-tolerated and effective treatment for staphylococcal enterocolitis and pseudomembranous colitis due to Clostridium difficile.

The approval of a Marketing Authorization for the proposed Vancomycin Capsules 125 mg is considered to be entirely justified on this basis.

2.4.7 References