CHAPTER 3
AZITHROMYCIN
A MACROLIDE ANTIMICROBIAL

3.1 Introduction and background

Antimicrobial drugs play a critical role in the treatment and fight against microbial organisms, responsible for infections and bacterial related diseases. Macrolides comprise a specific group of antimicrobial drugs that have been used in the treatment and prevention of microbial infections for around six decades now (Amsden, 1996:57; Retsema & Fu, 2001:S3). The term, macrolide, had its origin from its chemical structure, consisting of a macrocyclic lactone ring (usually 12, 14, or 16 atoms), with amino sugars attached to the ring. Most macrolides (except for several compounds that are isolated from *Micromonospora* species and other compounds that are semi-synthetically derived from erythromycin A) are directly derived from the *Streptomyces* species and are therefore known to be among the largest groups being isolated from natural products (Čulić *et al*., 2001:209; Kanfer *et al.*, 1998:256; Retsema & Fu, 2001:S3; Shinkai *et al*., 2008:394; Weisblum, 1998:29).

3.2 Classification of macrolides

Erythromycin, the first macrolide antibiotic, also referred to as the first generation of macrolides, was introduced for clinical use in 1952. Since then, the macrolide group of antibiotics has rendered a four-generation chemical change, due to structural evolution (Weisblum, 1998:29). The amount of carbon atoms in the macrocyclic lactone ring is the guideline used for the classification of these active compounds. Those macrolide compounds that are considered to be of importance generally have either a 14-membered, 15-membered, or 16-membered macrocyclic lactone ring. The 14-membered macrolides (first generation) are erythromycins (A, B, C, D, E, F), oleandomycin, roxithromycin, dirithromycin, clarithromycin and flurithromycin. The macrolides that contain a 16-membered lactone ring (second generation) are josamycin, rosaramicin, rokitamycin, kitasamycin, mirosamicin, spiramycin and tylosin. Azithromycin is the only 15-membered macrolide and can be referred to as a third generation macrolide. The ketolides, also

characterised by a 14-membered lactone ring, are classified as the fourth generation of macrolide antibiotics (Čulić *et al*., 2001:209; Kanfer *et al.*, 1998:256; Miroshnyk *et al*., 2008:232; Retsema & Fu, 2001:S3; Shinkai *et al*., 2008:394; Weisblum, 1998:29).

3.3 Azithromycin

Azithromycin (Figure 3.1), also referred to as an azalide and a member of the macrolide family of antibiotics, was first approved for clinical use in 1992 (Kremer, 2002:174). This azalide represents an added range of antimicrobial activity in comparison with erythromycin and related macrolides. The most important differences between azithromycin and other macrolides can be explained with reference to their pharmacokinetics and pharmacodynamics, microbiology, interactions with other active drugs, the safety of this azalide and the overall cost of a full regimen. Azithromycin is also better tolerated and has proven to cause less adverse effects than other macrolides. This antimicrobial drug has three known solid state forms, i.e. an anhydrous-, a monohydrate- and a dihydrate form (Abu-Gharbieh *et al*., 2004:212; Amsden, 1996:57; Čulić *et al*., 2001:209; Gandhi *et al*., 2002:183; Retsema & Fu, 2001:S4).

3.3.1 Structural aspects of azithromycin

Since azithromycin is semi-synthetically derived from erythromycin (Figure 3.2), it is structurally related to erythromycin. The chemical structure of azithromycin (Figure 3.1) differs from erythromycin (Figure 3.2) through methyl substitution of a nitrogen atom at the C9 carbon atom in the lactone ring (indicated in red in Figure 3.1, compared to Figure 3.2). The azithromycin structure has a 15-membered lactone ring and erythromycin a 14 membered lactone ring. Azithromycin has carbon, oxygen and a nitrogen atom included in its 15-membered lactone ring, whereas the other macrolides only include carbon and oxygen in their lactone ring structures. The presence of the nitrogen atom in the lactone ring results in azithromycin having a dibasic chemical behaviour, compared to the other known macrolides having monobasic chemical behaviour. Chemical behaviour is the key to the altered cellular dynamics of the macrolides (Amsden, 2001:S11; Gandhi *et al*., 2002:183; Retsema & Fu, 2001:S4).

Anhydrous azithromycin (Figure 3.1) has a molecular mass (MM) of 749 g/mol, the monohydrate (Figure 3.3) a MM of 767.02 g/mol, and the dihydrate (Figure 3.3) a MM of

785.02 g/mol (Gandhi *et al*., 2002:183; Hoepelman & Schneider, 1995:146; Miroshnyk *et al*., 2008:237; Retsema & Fu, 2001:S4; USP, 2010).

The dihydrate (Figure 3.3) is the most stable solid form of azithromycin. An increase in hydration from the anhydrous form to higher hydrated forms will hence increase the solid state stability of azithromycin (Gandhi *et al*., 2002:179-183; Hoepelman & Schneider, 1995:146; USP, 2010).

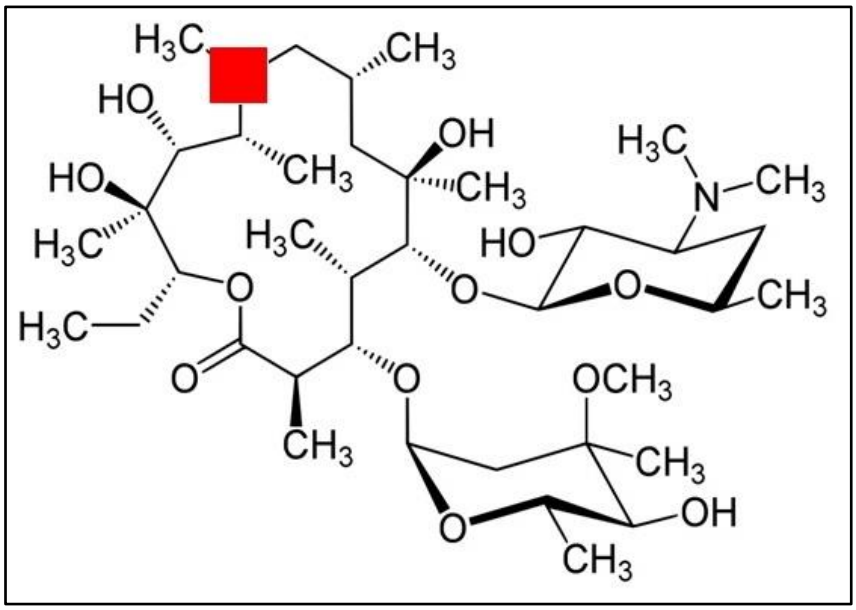

Figure 3.1 Chemical structure of azithromycin base (anhydrous form) (Adapted from Wikipedia, 2010).

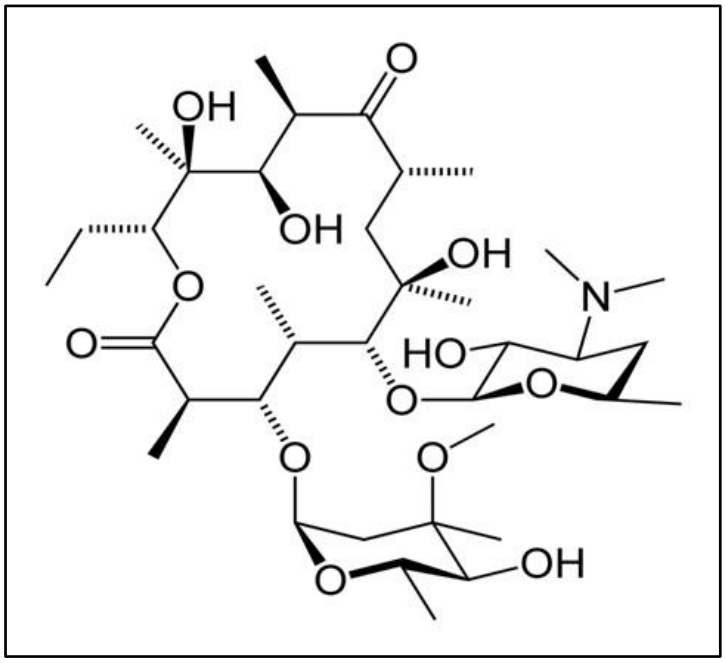

Figure 3.2 Chemical structure of erythromycin (Adapted from Wikipedia, 2010).

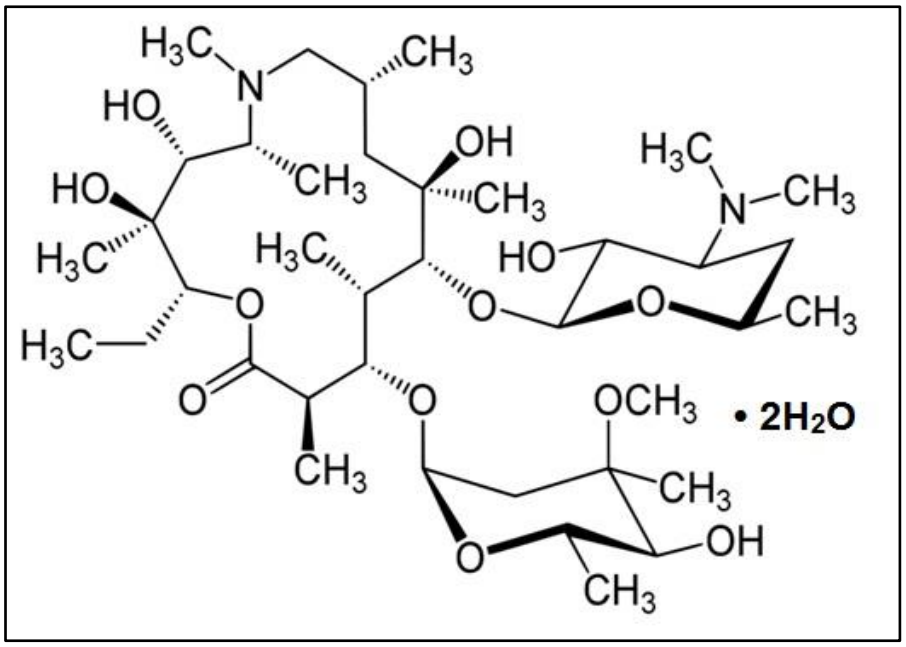

Figure 3.3 Chemical structure of azithromycin dihydrate (Adapted from Wikipedia, 2010).

3.3.2 Spectrum of pharmacological activity

Azithromycin exhibits a broad spectrum of antimicrobial activity. Atypical organisms, as well as *Mycobacterium avium complex* (MAC) organisms, are also inhibited by the activity of azithromycin. Azithromycin is predominantly bacteriostatic, but it may gradually act bactericidal if maintained at high concentrations for the duration of treatment. Although

erythromycin has more antimicrobial activity against *staphylococci* and *streptococci* infections (i.e. gram positive bacteria), azithromycin shows improved potency against gram-negative microbes. Azithromycin is known to sustain high tissue concentrations at the infected area, due to targeted delivery by white blood cells, thus resulting in concentration values exceeding the minimum inhibitory concentration (MIC), which may be sustained for about 10 days, once the final dosage has been administered. Some of these gram-negative microbes include *N. ghonorrhoeae*, *B. catarrhalis* and *H. influenzae.* Amsden (1996:60) states that, compared to clarithromycin, azithromycin is as much as eight-fold more effective against the gram-negative *H. influenzae* (Amsden, 1996:60; Amsden, 2001:S11; Hoepelman & Schneider, 1995:145; Reisner, 1996:124; Sood, 1999:27).

3.3.3 Mode of action

Azithromycin is active against microbes through interference of the microbial ribosome function and protein synthesis. It binds to the 50S ribosomal sub-unit of the bacterial ribosome, more specifically to the 2058 - 2062 region found in the 23S ribosomal RNA within the 50S sub-unit, where azithromycin obstructs the process reactions of transpeptidation and translocation. Azithromycin is known to be mostly bacteriostatic, however it presents as bactericidal, when used for treating infections that relate to *Streptococcus pyogenes*, *Streptococcus pneumoniae*, or *Hemophilus influenzae* (Hoepelman & Schneider, 1995:147; Kremer, 2002:174; Sood, 1999:27).

3.3.4 Indications of azithromycin

Azithromycin is a broad spectrum, antimicrobial agent that is commonly prescribed for adults and paediatrics, due to its relative safety and its improved efficacy against gram-negative microbes. According to Hoepelman and Schneider (1995:145), azithromycin is generally prescribed to treat bacterial infections in the upper and lower respiratory tract, for skin and soft tissue related infections and also for sexually transmitted diseases (Hoepelman & Schneider, 1995:145; Kremer, 2002:174; Reisner, 1996:124).

The following organisms can be treated with a course of azithromycin:

- **Gram-positive aerobes:** *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Streptococcus aureus*, the Viridans group streptococci and *Neisseria gonorrhoeae*.

- **Gram-negative aerobes:** *Bordetella pertussis*, *Hemophilus influenzae*, *Moraxella catarrhalis* and *L. monocytogenes*.
- **Other known pathogens:** *Chlamydia trachomatis*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Legionella pneumophila*, *B. burgdorferi*, *Treponema pallidum*, *Campylobacter jejuni*, MAC, *Toxoplasma, Prevotella bivia, Escherichia coli, Salmonella* species*, Yersinia entercolitica and Shigella* species (Amsden, 2001:S11; Hoepelman & Schneider, 1995:145; Kremer, 2002:174; Sood, 1999:27).

3.3.5 Physical properties of azithromycin

3.3.5.1 Appearance

Azithromycin generally is a white or almost white crystalline powder (USP, 2010).

3.3.5.2 Solubility

Azithromycin is very poorly soluble in water. However, it is freely soluble in several solvents, including dehydrated alcohol and dichloromethane. The solubility of azithromycin in water increases with a decrease of its hydration state. This implies that the monohydrate will be more soluble in water than the dihydrate and that that the anhydrous form will be the most water soluble. The relatively fast anhydrous to hydrated form conversion complicates the solubility of the anhydrous state. This hindered ability to determine its solubility is also applicable to the monohydrate, but to a lesser extent. Since the monohydrate also converts into the more stable dihydrate, the solubility of the monohydrate will correlate with that of the dihydrate, due to the conversion to the stable hydrate within 48 hours (Gandhi *et al*., 2002:182; Hoepelman & Schneider, 1995:146; USP, 2010).

3.3.5.3 Water content

According to the USP (2010), the labelled water content of an anhydrate must not exceed 2.0 % of water, if analysed with Karl Fischer titration. Furthermore, the monohydrate will have a water content that varies between 1.8 - 4.0 % and a dihydrate will present with a water content of between 4.0 - 5.0 % (USP, 2010).

3.3.5.4 Theoretical weight loss with drying

When azithromycin (dihydrate or monohydrate) is exposed to adequately high temperatures to initiate the dehydration process, a theoretical weight loss value is given for the amount of water molecules being lost with drying. For a dihydrate this theoretical weight loss is 4.59 %, whereas a monohydrate theoretically represents a 2.3 % weight loss (Gandhi *et al*., 2002:179; USP, 2010).

3.3.5.5 Melting point

The melting point varies for the different hydrated solid forms of azithromycin. The anhydrous form of azithromycin has a melting point at 113 - 115˚C and its dihydrated form at approxiamtely 126˚C. The melting points of dihydrates may vary, as a result of the different physico-chemical properties of dihydrates being prepared using different solvents, or preparation methods (Vippagunta *et al*., 2001:4). According to Gandhi *et al.* (2002:179), the crystals of the hydrated solid state of azithromycin melt at temperatures within the range of 120 - 130˚C (Gandhi *et al*., 2002:179; USP, 2010). Rodriquez *et al.* (2001) recorded an endothermy of 143.7 ± 0.5°C, with the heat of fusion being 54, 6 ± 1.6 J/g (heated at 10°C/min) for their patented azithromycin dihydrate. A clathrate form of azithromycin (Suh *et al*., 2007:10), was found to melt at 130°C with an endothermic peak at 150.8°C and heat capacity of 104.42 J/g. In contrast, a monohydrate resulted in an endothermic peak at 145.44°C and heat capacity of 137.37 J/g. The dihydrate was found to have an endothermic peak at 142.72°C with a heat capacity of 160.15 J/g (Suh *et al*., 2007:10).

3.3.5.6 Stability in acidic environment

Azithromycin is described as being more stable in an acidic environment, than its predecessor, erythromycin. This improved acid stability is mainly due to the chemical addition of a methyl substituted, nitrogen atom to the structure. According to Sood (1999:27), azithromycin is six times more tolerable of acid (pH 2.0) than erythromycin, i.e. azithromycin degrades six times slower in acid having a pH of 2.0 at 37˚C. At pH 2, degradation of erythromycin commences just 3.5 seconds following exposure to such acidic environment, while azithromycin only starts degrading after 20 minutes (Gandhi *et al*., 2002:175; Kremer, 2002:174; Retsema & Fu, 2001:S4; Sood, 1999:27).

3.3.5.7 Iso-electric point of azithromycin

The iso-electric point is the pH at which the positive and negative charges of a molecule are equal, i.e. the point at which there is no electrical charge for the molecule at this specific pH. The iso-electric point for azithromycin is 8.74, which may change with a change in temperature (USP, 2010).

3.3.6 Pharmacokinetic properties

Azithromycin is unique in the sense that its altered chemical structure results in improved pharmacokinetic properties. It is tissue selective and shows an extensive uptake of drug into the cellular compartments. This accumulated cellular azithromycin is retained for a prolonged period of time, where it is gradually released to maintain high tissue concentrations. The pharmacokinetics of azithromycin is the main reason for the effective implementation of the once daily dosage regimen over a period of 3 - 5 days (Amsden, 2001:S11).

3.3.6.1 Absorption and metabolism

Azithromycin is quickly accumulated *via* fibroblasts, monocytes, polymorphonuclear lymphocytes and macrophages. Rapid absorption can also be related to the active transport of azithromycin to infection sites *via* phagocytic cells. Although the majority of azithromycin remain in their unchanged form, the possibility of azithromycin being changed or metabolised is also minimalised, due to its rapid absorption and targeted delivery. However, absorption of azithromycin is largely influenced by the presence of food in the stomach and in the first part of the small intestine. The absorption of azithromycin can be reduced by up to 50 % when administered immediately following a meal. Azithromycin has a systemic half-life of more than 60 hours. The tissue concentration of azithromycin sustains its therapeutic level for several days, allowing for once daily azithromycin administration over a treatment period of 3 - 5 days (Amsden, 1996:57; Amsden, 2001:S12; Hoepelman & Schneider, 1995:146; Kremer, 2002:174; Niederman, 2005:S141; Reisner, 1996:122).

Azithromycin has no inducing or inactivating effects on the hepatic cytochrome P450 enzyme. Therefore, the most absorbed azithromycin stays unmetabolised in its azithromycin base (anhydrous) form. If azithromycin undergoes biotransformation, it primarily relates to either *N*-demethylation, or hydroxylation of the desosamine sugar, or

O-demethylation of the macrocyclic ring (9a position), or deconjugation of the cladinose sugar, or hydroxylation of the aglycone rings. The bile fluid contains approximately 50 % of azithromycin related material and may host as many as 10 metabolites, all of which display no relevant antimicrobial actions (Hoepelman & Schneider, 1995:146; Kremer, 2002:174; Reisner, 1996:122).

3.3.6.2 Distribution

Of all macrolides, azithromycin exhibits the most widespread tissue distribution, due to its high selectivity for tissue. The rapid distribution of azithromycin results in high and efficient concentrations that can be sustained. White blood cells, responsible for carrying azithromycin, will either release the accumulated drug when it reaches the microbes, or it will phagocytise the microbes being present. The achieved intracellular and extracellular potencies of azithromycin are due to the absorption, accumulation and gradual drug release by the participating white blood cells. These accumulated high concentrations of azithromycin will result in the prolonged inhibition, or fatality of the microbes at the infection site (Čulić *et al*., 2001:212; Niederman, 2005:S141).

Accumulation of azithromycin in fibroblasts can ultimately result in an intracellular:extracellular ratio of more than 3700:1. This intracellular azithromycin is gradually released (almost 70 % after 48 hours) into the extracellular area. Together with a pH partition, the intracellular release of azithromycin will result in the extensive volume of distribution, the increased and delayed concentration of intracellular and extracellular azithromycin, serum concentrations being reduced, whilst, importantly, achieving the terminal half-life of more than 60 hours. This explains the origin of the dosage regimen that consists of once daily azithromycin (500 mg) administration over 3 - 5 days, depending on the pharmacological indication (Amsden, 1996:59; Čulić *et al*., 2001:212; Niederman, 2005:S141).

According to Amsden (1996:58), the volume of distribution for azithromycin is 100 L/kg. These tissue concentrations can be up to 100 times higher than the serum concentrations. The bioavailability of azithromycin after a single, orally administered dose (500 mg) is 37 %. Despite the extensive tissue distribution, azithromycin lacks the ability to penetrate the central nervous system to a large extent. Azithromycin is 7 - 50 % protein bound within the human body (Amsden, 1996:58; Hoepelman & Schneider, 1995:152; Kremer, 2002:174; Niederman, 2005:S141; Reisner, 1996:123; Sood, 1999:27).

3.3.6.3 Elimination

Azithromycin has an extensive elimination half-life when compared to other antimicrobial drugs (e.g. β–lactam antimicrobials). The majority of administered azithromycin doesn't undergo any metabolic changes whilst present in the human body. This macrolide is mainly excreted in its unchanged form through the bile and faeces. Almost 50 % of material related to azithromycin is found in the bile fluid. A small portion of unchanged azithromycin (± 6 %) is eliminated in urine. This portion of unmetabolised azithromycin can be detected in urine samples over a period of two weeks following an administered, single oral dose (Hoepelman & Schneider, 1995:153; Kremer, 2002:174; Niederman, 2005:S141; Reisner, 1996:123).

3.3.7 Drug interactions

In comparison with the other macrolide group antimicrobials, azithromycin is the drug for which a smaller number of drug interactions have been reported. The serum levels of azithromycin are negatively influenced by the presence of antacids. Antacids that contain aluminium and magnesium should be avoided, as they reduce the peak serum level of azithromycin. After administration of a full dose of azithromycin, almost 24 % less azithromycin reaches the serum in the human body. Azithromycin has been associated with higher serum theophylline concentrations and also digoxin toxicity. The anti-coagulation effect of Coumadin® (Warfarin sodium tablets) has been potentiated by treatment with azithromycin. As mentioned, when azithromycin is administered in conjunction with a meal, the absorption of azithromycin is decreased by almost 50 %. It is hence recommended that azithromycin be taken 1 hour prior to a meal, or alternatively 2 hours after the meal (Kremer, 2002:175; Reisner, 1996:126; Rubinstein, 2001:S75).

3.3.8 Adverse effects

It is known that azithromycin present with fewer adverse effects than those being experienced with other macrolides. Adverse effects that are often experienced, as with other macrolides, are gastro-intestinal disturbances, such as diarrhoea, nausea and abdominal pain. However, not more than 1 % of patients experience adverse effects to such an extent that the discontinuation of the use of azithromycin, as primary treatment agent, is recommended. Very few cases of adverse hepatic effects, due to azithromycin usage, have been reported, whilst haematological adverse effects, resulting from treatment with azithromycin, are uncommon. In very rare cases (< 1 %), patients have

reportedly exhibited mild symptoms of skin rash (Abu-Gharbieh *et al*., 2004:211; Kremer, 2002:175; Reisner, 1996:126; Rubinstein, 2001:S72).

3.3.9 Toxicity

In the event that a patient is allergic to a macrolide, or shows signs of drug sensitivity when treated with a macrolide, the patient must be advised not to take any antimicrobial agents from the macrolide group. When a patient is diagnosed with septic shock or sepsis, the use of azithromycin should be avoided, because of the patient's low serum levels (Sood, 1999:28).

3.3.10 Safety

Azithromycin can be administered with safety to patients known to have hepatic status problems, since this azalide has no altering effect on hepatic enzymes. Azithromycin is also safe for prescription to patients with impaired renal function. In terms of safety during pregnancy, azithromycin is considered a category B antimicrobial drug and may therefore be prescribed to pregnant women (Amsden, 1996:67; Hoepelman & Schneider, 1995:154).

3.3.11 Cost of treatment

The total cost of treatment may be negatively influenced by poor patient compliance, especially if the antimicrobial dosage regimen comprises 10 - 14 days of treatment. If the patient does not comply with the dosage regimen, microbial infection could persist, whilst resistance against the antimicrobial drug may also develop. Consequently, the cost of treatment can escalate, due to repeated visits to clinics or doctors, whilst other antimicrobial regimens may have to be prescribed to treat the continuing infection. Although azithromycin is an expensive antimicrobial drug, the once daily dosage regimen over 3 - 5 days has proven to be more cost effective than other antimicrobials that require the standard 10 - 14 days treatment regimens (Amsden, 1996:67).

3.3.12 Bacterial resistance to azithromycin and other macrolides

Antimicrobial resistance has become an aspect of grave concern in the health sector. Resistance to azithromycin may originate from different factors and variables.

Inappropriate use of antimicrobials is one major variable that results in the development of resistance to these drugs. Scenarios categorised as inappropriate use include:

- The use of antimicrobials when the infection is viral based.
- Inadequate length of the treatment regimen.
- The incorrect use of a broad spectrum antimicrobial, when a more selective antimicrobial would be better suited as treatment regimen.
- Poor patient compliance (Niederman, 2005:S141).

One of the most prevalent factors causing microbial resistance against azithromycin is its increased prescription rate, according to Karlowsky *et al*. (2009:378).

As mentioned, the other significant factor causing resistance is poor patient compliance. When patients fail to complete the full course of antimicrobial treatment (or if daily doses are missed), the microbes are continually exposed to low drug concentrations and are thus not being influenced in an inhibiting or lethal way. This prolonged exposure to sub-optimal concentrations ultimately results in the microbes developing resistance against the respective antimicrobial drugs (Niederman, 2005:S141).

Due to the long treatment regimen required for erythromycin and related macrolides, patient compliance becomes a much more defining factor. Gastro-intestinal adverse effects are commonly experienced when treated with macrolides, which may contribute towards the patient terminating antimicrobial treatment, hence resulting in the microbes being exposed to ineffective drug concentrations and ultimately in resistance against the specifically prescribed macrolide. Studies show that most microbes that are resistant against erythromycin are also resistant against azithromycin, clarithromycin and roxithromycin. Lately it has become evident that *Streptococcus pneumoniae* is becoming more resistant against, and thus less susceptible to macrolides and β-lactam antimicrobials. Two mechanisms harboured by microbes are accountable for the development of macrolide resistance. The first is where the microbes use an efflux pump mechanism to defend themselves against the onslaught of the macrolide, whereas the second mechanism can simply be described as ribosomal modification (Bergman *et al*., 2006:3646; Chu, 1999:467; Garau, 2001:S34; Karlowsky *et al*., 2009:378; Niederman, 2005:S141; Retsema & Fu, 2001:S6).

3.4 Conclusion

Azithromycin, one of the world's bestselling antimicrobials, belongs to the macrolide group and exhibits extensive antimicrobial activity. Its spectrum of antimicrobial activity includes both gram-positive and gram-negative microbes. Azithromycin is important in the prevention and treatment of microbial infections in infants and patients having weakened immune systems. Because of its broad spectrum of activity against microbial infections, azithromycin is more likely to be prescribed than erythromycin. Azithromycin is derived from the erythromycin macrolide. One of the main differentiating characteristics between these two drugs is the improved stability of azithromycin under acidic conditions (e.g. stomach acid) that hence offers a tremendous advantage when used in oral dosage forms in humans. Since less azithromycin is degraded, a higher percentage of active drug is available to reach the small intestine for absorption by the systemic circulation (Kanfer *et al.*, 1998:256; Kremer, 2002:174; Reisner, 1996:124).

Azithromycin has three known solid state forms, i.e. an anhydrous (basis) form and a monohydrate and dihydrate. The dihydrate is the most stable of the two hydrated forms. Azithromycin is very poorly soluble in water, which negatively impacts on its absorption in the human body. Ultimately, the reduced absorption of azithromycin results in its oral bioavailability being only 37 %, thus requiring higher doses to be administered to achieve the necessary antimicrobial effect. The total cost of treatment may escalate, whilst it may also lead to microbial resistance against azithromycin (Amsden, 1996:59; Čulić *et al*., 2001:212; Gandhi *et al*., 2002:175; Niederman, 2005:S141).

3.5 References

ABU-GHARBIEH, E., VASINA, V., POLUZZI, E. & DE PONTI, F. 2004. Antibacterial macrolides: a drug class with a complex pharmacological profile. *Pharmacological Research*, 50:211-222.

AMSDEN, G.W. 1996. Erythromycin, clarithromycin, and azithromycin: are the differences real? *Clinical Therapeutics*, 18(1):56-72.

AMSDEN, G.W. 2001. Advanced-generation macrolides: tissue-directed antibiotics. *International Journal of Antimicrobial Agents*, 18:S11-S15.

BERGMAN, M., HUIKKO, S., HUOVINEN, P., PAAKKARI, P., SEPPÄLÄ, H. & FINNISH STUDY GROUP FOR ANTIMICROBIAL RESISTANCE. 2006. Macrolide and azithromycin use are linked to increased macrolide resistance in *Streptococcus pneumoniae*. *Antimicrobial Agents and Chemotherapy*, 50(11):3646-3650.

CHU, D.T.W. 1999. Recent developments in macrolides and ketolides. *Current Opinion in Microbiology*, 2:467-474.

ČULIĆ, O., ERAKOVIĆ, V. & PARNHAM, M.J. 2001. Anti-inflammatory effects of macrolide antibiotics. *European Journal of Pharmacology*, 429:209-229.

GANDHI, R., PILLAI, O., THILAGAVATHI, R., GOPALAKRISHNAN, B., KAUL, C.L. & PANCHAGNULA, R. 2002. Characterization of azithromycin hydrates. *European Journal of Pharmaceutical Sciences*, 16:175-184.

GARAU, J. 2001. The clinical impact of macrolide resistance in pneumococcal respiratory infections. *International Journal of Antimicrobial Agents*, 18:S33-S38.

HOEPELMAN, I.M. & SCHNEIDER, M.M.E. 1995. Azithromycin: the first of the tissue-selective azalides. *International Journal of Antimicrobial Agents*, 5:145-167.

KANFER, I., SKINNER, M.F. & WALKER, R.B. 1998. Analysis of macrolide antibiotics. *Journal of Chromatography A*, 812:255-286.

KARLOWSKY, J.A., LAGACÉ-WIENS, P.R.S., LOW, D.E. & ZHANEL, G.G. 2009. Annual macrolide prescription rates and the emergence of macrolide resistance among

Streptococcus pneumonia in Canada from 1995 to 2005. *International Journal of Antimicrobial Agents*, 34:375-379.

KREMER, C.J. 2002. Azithromycin: a new macrolide. *Primary Care Update Ob/Gyns*, 9:174-175.

MIROSHNYK, I., MIRZA, S., ZORKY, P.M., HEINÄMÄKI, J., YLI-KAUHALUOMA, J. & YLIRUUSI, J. 2008. A new insight into solid-state conformation of macrolide antibiotics. *Bioorganic & Medicinal Chemistry*, 16:232-239.

NIEDERMAN, M.S. 2005. Introduction. *International Journal of Antimicrobial Agents*, (26) suppl. 3:S141-S142.

PECHÈRE, J.C. 2001. New perspectives on macrolide antibiotics. *International Journal of Antimicrobial Agents*, 18:S93-S97.

REISNER, D.P. 1996. Uses of macrolide antibiotics in obstetrics and gynecology. *Primary Care Update Ob/Gyns*, 3:122-127.

RETSEMA, J. & FU, W. 2001. Macrolides: structures and microbial targets. *International Journal of Antimicrobial Agents*, 18:S3-S10.

RODRIQUEZ, R.A., CRUZADO RODRIQUEZ, M.C., DIAZ TEJO, L.A., BORREL BILBAO, J.I., NOMEN RIBE, R. & SEMPERE CEBRIAN, J. 2001. Polymorphic form of azithromycin dihydrate and preparation method thereof. Patent: WO 01/87912 A1. 21 p.

RUBINSTEIN, E. 2001. Comparative safety of the different macrolides. *International Journal of Antimicrobial Agents*, 18:S71-S76.

SHINKAI, M., HENKE, M.O. & RUBIN, B.K. 2008. Macrolide antibiotics as immunomodulatory medications: proposed mechanisms of action. *Pharmacology & Therapeutics*, 117:393-405.

SOOD, S.K. 1999. Macrolides: clarithromycin and arithromycin. *Seminars in Pediatric Infectious Diseases*, 10(1):23-30.

SUH, K.W., KIM, G.J., YOON, S.M., SEONG, M.R. & LEE, G.S. 2007. Clathrate of azithromycin hydrate with 1,2-propyleneglycol: method for the manufacture thereof and pharmaceutical composition comprising same. Patent: US 7,205,394 B2. 12 p.

UNITED STATES PHARMACOPEIA. 2010. http://www.uspnf.com. Date of use: 12 Feb. 2010.

USP **see** UNITED STATES PHARMACOPEIA.

VIPPAGUNTA, S.R., BRITTAIN, H.G. & GRANT, D.J.W. 2001. Crystalline solids. *Advanced Drug Delivery Reviews*, 48:3-26.

WIKIPEDIA. 2010. http://en.wikipedia.org Date of use: 16 Mar. 2010.

WEISBLUM, B. 1998. Macrolide resistance. *Drug Resistance Updates*, 1:29-41.