CHAPTER II

REVIEW OF LITERATURE

Review of Gentamicin

1. Chemistry and Pharmacology

Gentamicin used commercially is a complex of structurally related gentamicin C_1, C_1a and C_2 derived from the fermentation of *Micromonospora purpurea*. The drug contains an aminosugar joined to two cyclic aliphatic rings by glycoside linkage. Gentamicin is water soluble, stable over a wide pH range, and relatively heat resistant (Weinstein et al, 1963). Gentamicins are bactericidal compounds that cause aberrant bacterial polypeptide synthesis by binding to specific receptor sites on the 30s subunit of bacterial ribosomes, blocking the recognition step in protein synthesis and causing misreading of the genetic code (Kastrup, 1987).

2. Antibacterial Spectrum and Therapeutic Efficacy

The antibacterial spectrum activity of gentamicin is active against *Enterobacter species, Escherichia coli, Klebsiella species, Proteus species, Citrobacte species, Salmonella species, Shigella species, Serratia species and Pseudomonas aeruginosa*. It has limited activity against some gram-positive bacterias

such as *Streptococus faecalis and Staphylococcas species* (American Medical Association, 1977; Kastrup, 1987). The clinical efficacy of gentamicin in the treatment of serious infection is related to the peak concentrations that could be achieved clinically in plasma. Jackson and Riff (1971) described that peak serum concentrations higher than 4 to 5 µg/ml are considered optimal for treatment of gram-negative bacteria early in therapy. Higher peak concentrations are recommended in patients with gram-negative pneumonias and burn wound sepsis (Noon et al, 1974;Zaske, 1980a). Peak concentration exceeding 12-15 µg/ml and trough concentration above 2 to 3 µg/ml are associated with a higher incidence of toxic effect (Barza and Lauermann, 1978). Thus, peak concentrations of 8 to 10 µg/ml are recommended for severe infections, while peak of 5 to 8 µg/ml generally adequate for less severe infections (Wallace, Gesy and Gorecki, 1981). Wenk, Vozeh and Follath (1984)recommended 5 to 10 µg/ml for serum level monitoring of gentamicin in severe gram-negative infections in patients with normal renal function.

3. Toxicity

Gentamicin has two most frequent toxic efffects, irreversible ototoxicity and reversible nephrotoxicity. It can cause renal damage as well as damage to both the cochlear and vestibular portions of the eighth cranial nerve. The incidence of toxic effects can be related to the dosage of drug, length of treatment, pre-existing renal dysfunction and age of the patient (Appel and Neu,

1978). Ototoxicity is most likely to occur in patients with impaired renal function, especially those receiving gentamicin for longer periods or in larger dose than usually recommended (American Medical Association, 1977; Reynolds, 1989). Jackson and Arcieri (1971) reported the ototoxicity occured more frequently when peak concentration greater than 10 μg/ml. It may appear several days after discontinuation of the treatment. Since destroyed or damaged cochlear hair cells are unable to regenerate, ototoxicity is often irreversible and is accumulative with relation to the dose and duration of therapy (Wenk et al, 1984). Many investigators reported that trough concentration greater than 2 μg/ml causing nephrotoxicity (Burton, Vasko and Brater, 1985;Sarubbi and Hull, 1978; Zaske et al, 1982). Wenk et al (1984) concluded that there were important differences between nephrotoxicity and ototoxicity with respect to clinical management. In most cases nephrotoxicity seems to be reversible, and the manifestation of this side effect can be readily detected by a simple laboratory test. Therefore, it is believed that the value of the therapeutic drug monitoring in preventing nephrotoxicity is limited. Instead, it is recommended that frequent monitoring of the serum creatinine and appropriate dosage adjustment in the presence of renal failure should be performed to avoid ototoxicity.

4. Pharmacokinetic

4.1 Absorption

Gentamicin is poorly absorbed when given orally, however, adequate serum levels are obtained with either intramuscular or intravenous administration. For intramuscular injection, peak serum concentrations usually are achieved within 30 to 90 minutes after the injection (Sande and Mandell, 1985;Reynolds, 1989). In patients with severe gram-negative sepsis, blood perfusion of the rate of drug absorption may be substantially reduced. Additionally, repeated injections at the same intramuscular site may impair absorption and result in more variation in serum concentrations.

Gentamicin can be administered intravenously by bolus injection, by 30 to 60 minutes intermittent infusion, or by continuous IV infusion. Zaske (1980b) concluded that intermittent infusions were safer and had therapeutic advantages.

4.2 Distribution

After dosing,gentamicin is widely distributed in extracellular fluids. Serum protein binding is low and range 0 to 20%. Peak concentrations may be lower than usual in patients whose extracellular fluid volume is expanded, which may occur during edema or ascites. In contrast, peak concentration may be higher than expected in obese people because their fat stores are poorly accessible to the drug. Measurable concentrations are

found in unobstructed bile, synovial fluid, renal lymph, sputum, bronchial secretions and pleural fluid (Barza and Lauermann, 1978;Kastrup, 1987). In average adults, volume of distribution for gentamicin is about 25-30% of patient's body weight and is approximate the volume of the extracellular fluid. However, newborn infants have a large extracellular fluid, frequently in the range of 50 to 70% of their body weights (Barza and Lauermann; Zaske, 1980b).

4.3 Excretion

Gentamicin is not metabolized and is excreted primarily by glomerular filtration. After a single dose, 40 to 65% is recovered in the urine within 24 hours, and ultimately almost 90% is excreted (Appel and Neu, 1978). Small amount of drug has been found in bile and may represent an additional route of elimination. The serum half-life of gentamicin is between 2 to 3 hours in patients with normal renal function. The serum half-life is longer in elderly and young infants with immature renal system but shorter in severely burned patients (Kastrup, 1987).

Applied Pharmacokinetic for Monitoring Serum Concentrations of Gentamicin

Therapeutic drug monitoring is measurement of drug level to optimise the dosage regimen for an individual patient. It has generated much interest during the last few years. The goal of gentamicin serum level monitoring

is not only to reduce the risk of nephrotoxicity and ototoxicity but also to avoid subtherapeutic levels in treating micro-organism infections. At present, considerable progress has been made to understand the pharmacokinetic behaviour of gentamicin, which helps the clinician to get the optimal dosage regimen for patients under different clinical conditions. The pharmacokinetic characteristics of aminoglycoside are relatively uncomplicated. The drugs are distributed into most of the extracellular fluid space, low protein bound and eliminated almost solely by glomerular filtration. As a result,their serum concentrations should be fairly easy to anticipate , the serum levels of gentamicin can be predicted by using pharmacokinetic equations.

1 Factors Influnece Predictable Serum Concentrations of Gentamicin (Barza and Lauermann, 1978 ; Wenk, Vozeh and Follath, 1984)

1.1 Altered Absorption Rates

A variable rates of absorption from intramuscular provides uncertain serum levels. Factors found to influence the absorption are site of injection, regional blood flow and prior infections in the same site.

1.2 Altered Distribution

The wide interpatient variations in distribution have effect on aberrate serum levels.

Changing in the distribution volume is independent of any change in renal function but will influnece the drug's half-life if total body clearance remains constant within the patient. On the other hand, when the volume increases, the drug's half-life will also increase.

1.3 Altered Renal Function

The rate of renal elimination influences serum level and half-life of gentamicin. In order to avoid drug accumulation, the size of the dose must be reduced, or time of the interval between dose must be extended. In making dosage adjustments in patients with renal insufficiency, the clinician often uses the serum creatinine concentration or the creatinine clearance as the index of excretory function. Culter et al (1972) and Mchenry et al (1971) concluded that there was relationship between the loss of gentamicin and the endogenous creatinine, the serum creatinine concentration and the half-life of gentamicin. Measurement of the endogenous creatinine clearance depends on accurate collection of urine specimens which may be difficult in some cases of seriously ill patients.

1.4 Other Variables Affecting Serum Levels.

Many investigators have attempted to explain other variables affecting serum levels such as age, obesity, haematocrit, major burns, interaction with β-lactam antibiotics and body temperature.

2. Monitoring Serum Concentrations of Gentamicin

2.1 Specimen Collection and Storage (Wenk, Vozeh and Follath, 1984)

Blood samples should be drawn into tubes without anticoagulant , because it can affect the assay performance. After separation, serum should be kept frozen if the samples are not immediately analysed. Gentamicin is very stable at room temperature, but frequently they are administered concomitantly with β-lactam and consequently the gentamicin can be inactivated by a chemical reaction with the β-lactam ring.

Trough concentration should be drawn immediately before the next dose. Since the trough is best represents distribution equilibrium between blood and tissue, trough concentration is more useful than peaks for prediction of tissue accumalution and for assessment of the risk of nephrotoxicity. Peak concentration should be taken after the rapid distributive phase is completed. Peak concentration should be drawn 30 to 120 minutes after intramuscular injection and at the end or approximately 30 minutes after complete intravenous infusion.

2.2 Assay Methods.

There are 5 several assays which are commonly used to measure aminoglycoside concentrations in

body fluid. Some of these assays include microbiological plate diffusion assay (bioassay), radioimmunoassay(RIA), enzyme immunoassay (EIA) , fluorescence immunoassay (FIA) , fluorescence polarization immunoassay (FPIA) and high-performance liquid chromatography (HPLC). The FPIA was chosen for this study.

Fluorescence Polarization Immunoassay(FPIA) and TDx^{R} Analyzer System (Abbott, TDX Training ; Jolley, stroupe, Wang et al, 1981;Jolley, Stroupe, Schwenzer et al, 1981; Popelka et al, 1981)

The principles of fluorescence polarization are the competitive binding immunoassay using fluorescence polarization to measure tracer-antibody binding directly. The polarization of fluorescent light emitted by fluorescein tracer increses as the tracer is bound to antibody. Fluorescence polarization is a direct measure of bound and free fluorescent-labeled antigen in a competitive binding immunoassay. The fluorescent-labeled antigen competes for antibody binding site with the antigen (unlabeled analyte) in sample. When competitive binding occurs, the fluorescent-antigen complex becomes a part of the very large antibody molecule; the free fluorescent labeled antigen (tracer) is small in comparison. Because of the rotational properties of molecules in solution, degree of polarization is directly proportional to the size of molecule. That is, polarization increases as molecular size increases. Therefore, if a patient sample contains low concentration of antigen,after the competitive binding reaction reaches

steady state, there will be highly binding of fluorescent-labeled antigen with antibody (fluorophore) and low binding of patient-sample antigen with antibody in the reaction mixture. So polarization will be high. On the other hand, if a patient sample contains high concentration of antigen, after the competitive binding reaction reaches steady state, there will be very low binding of fluorescent-labeled antigen to the antibody and it will be free to rotate which the emitted light will be in a different plane. So polarization will be low.

The TDx^{R} analyzer system is a fully automated system,developed from fluorescence polarization immunology assay technology to measure therapeutic drug and hormone levels. It uses a competitive binding immunoassay methodology to allow fluorescent-labeled antigen and patient antigen to compete for binding sites on the antibody molecules. There are specific reagents of TDx^{R} analyzer system for assay of each drug. Before using new reagent pack for assay of each drug, the assay calibration must be made and memorized in TDx^{R} analyzer system. Concentration of specimens are determined by referring to the stored calibration curve. A weighted log-logit tape curve lit is used.

2.3 Predicting Serum Concentrations of Gentamicin

Evan et al (1983) proposed a two-compartment pharmacokinetic model for dosing gentamicin in children and adolescents which had been

adjusted for tissue accumulation with continuous dosing. The difference between measured concentrations and values predicted by the one-compartment model was found significant ($p<0.05$), but not with the values predicted by the two-compartment model ($p>0.1$). However, the one-compartment model was reported to be much more practical for computing dosage regimens and predicting serum concentration of gentamicin.

Several methods have been proposed to calculate the optimal aminoglycoside dosage and dosing interval, including the nomograms described by Chan, Benner and Hoeprich (1972), Dettli (1974), Hull, Sarubbi and Hill (1976) and Sarubbi and Hull(1978).Individualized pharmacokinetic method appears to have an advantage over nomogram or empiric dosing for predicting the aminoglycoside dose necessary to achieve specific serum concentration. One of the individualized methods commonly used, the Sawchuk and Zaske (1976), has been implemented frequently for providing aminoglycoside dosing services. This method attempts to measure the distribution volume and elimination rate constant on a patient-by-patient basis. Using these pharmacokinetic values generated from measured serum concentration-time data, the clinician can determine the appropriate dose and dosage interval for each patient to achieve the desired peak and trough concentrations. Pharmacokinetic equations used to calculate gentamicin serum levels and its dosage regimen are shown in Appendix A

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2.4 Recommendations for Serum Level Monitoring of Gentamicin (Wenk, Vozeh and Follath, 1984)

Because of the wide variability of gentamicin serum concentration as discussed previously, serum level monitoring of gentamicin should be included as part of patient management in the following situations.

2.4.1 Severe gram-negative infections in patients with normal renal function in order to ensure bactericidal antibiotic levels. Additional blood samples are needed only in cases of prolonged aminoglycoside treatment (>7 days) or if a tendency of rising creatinine value is seen. Special attention must be paid to patients with reduced immunological defence mechanisms to avoid subtherapeutic levels, such as patients with agranulocytosis or under immunosuppresive therapy.

2.4.2 In patients with slight to moderate renal impairment , both peak and trough concentrations should be measured to control the adequacy of the dosage regimen. Trough levels repeated every 5 to 7 days will allow an early recognition of a tendency to abnormal drug accumulation.

2.4.3 In advanced renal failure, repeated serum level measurements are always indicated to avoid toxic concentrations and, at the same time, to maintain therapeutically effective bactericidal peak values. Especially in cases with a rapidly changing clinical condition, as frequently seen in patients with septicaemia, close monitoring with repeated blood

sampling is mandatory. Patients with a history of aminoglycoside toxicity are also included.

2.4.4 Gentamicin treatment in neonates and small children should probably also be included in this list.