CHAPTER II

REVIEW OF LITERATURE

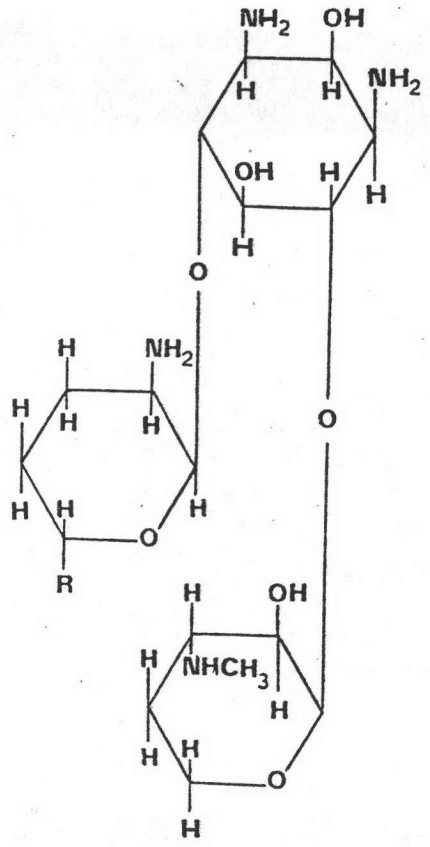
I. REVIEW OF GENTAMICIN

Physico-Chemical Properties (9)

Gentamicin sulphate is the sulphate salt of antibiotic substance produced by growth of Micromonospora purpurea. Gentamicin is a mixture of gentamicin C_1, gentamicin C_2 and gentamicin C_{1A}. Some commercial samples may contain significant quantities of minor components, i.e. gentamicin C_{2A} and gentamicin C_{2B}.

Chemical name of gentamicin C_{1A} is *O*-3-deoxy-4-*C* methyl-3-(methylamino)-*β*-L-arabinopyranosyl-(1⟶6)-*O*-[2,6-diamino-2,3,4,6-tetradeoxy-α-D-*erytho*-hexopyranosyl-(1⟶4)-2-deoxy-D-streptamine.

Structural formula:



C_{1A} = R = CH_2NH_2

C_2 = R = $CH(CH_3)NH_2$

C_1 = R = $CH(CH_3)NH(CH_3)$

Molecular weight:

Gentamicin C_1 = $C_{21}H_{43}N_5O_7$ (M.W. = 477.6)

Gentamicin C_2 = $C_{20}H_{41}N_5O_7$ (M.W. = 463.6)

Gentamicin C_{1A} = $C_{19}H_{39}N_5O_7$ (M.W. = 449.5)

Description :

White to almost white powder,odorless.

Solubility :

Freely soluble in water, 0.1 N hydrochloric acid, 0.1 N sodium hydroxide (>1 gm/ml in each of these aqueous

media), insoluble in alcohol and most other organic solvents

Melting range :

Melt with decomposition between 200°C and 250°C (10)

Stability :

Gentamicin is stable in light, air and heat

Incompatibility :

Gentamicin sulphate loses potency in plastic disposable syringe (11), Carlson (12) reported that gentamicin lost potency in serum stored for assay up to 63 days at -60°C. Gentamicin is inactivated by various beta-lactam antibiotic in vitro by interaction with the beta-lactam ring. In the treatment of serious gram-negative, penicillin or cephalosporin antibiotic is often used concomitant with gentamicin.

Results of the inactivation could be divided into three groups (13,14) :

1. Cefazolin and cefamandole cause little inactivation

2. Nafcillin, cephapirin and cefoxitin cause moderate inactivation.

3. Penicillin, ampicillin, carbenicillin and ticarcillin cause marked inactivation

Inactivation mechanism involves the nucleophilic opening of the penicillin beta-lactam ring, which then combines with an amino group of gentamicin resulting in

the formation of a biologically inactive amide. The rate of in vitro inactivation depends upon temperative time, the composition of medium and concentration. This usually is not a problem in vivo, because both drugs are eliminated quickly enough that there is insufficient time for inactivation (15).

Pharmacological Properties

Gentamicin is one of aminoglycoside antibiotic which is usually used in treatment of gram-negative bacterial infections. Perform culture and sensitivity testing showed that gentamicin is active against *Escherichia coli*, *Klebsiella species*, *Enterobacter species*, *Citrobacter species*, *Pseudomonas aeruginosa* and *Proteus species*. Gram-positive bacterial infections which gentamicin may be used concomitantly with other anti-infective agents are *Streptococus faecalis* and *Staphylococcus species* (16,17).

1. Mechanism of action

Basic actions of gentamicin are irreversible binding primarily to the 30 S subunit of bacterial ribosomes, blocking the recognition step in protein synthesis and causing misreading of the genetic code. Minimum inhibitory concentrations have been reported to range from 0.06 to 8 ug/ml for gram-negative bacteria. In the group of gram-positive organisms, most strains of *Staphylococus aureus* are sensitive to gentamicin with

minimum inhibitory concentrations being reported within the range of 0.12 to 1 μg/ml. It is more active in alkali (18-20). Reynolds, A.V., et al described that activity of gentamicin is poor against anaerobic bacteria or other bacteria under anaerobic conditions (21).

2. Therapeutic Efficacy

The goal of optimum antimicrobial in therapy is to achieve the best possible action against the target micro-organism with a minimum risk of unwanted side effect. Ideally, a therapeutic range should be defined for all types and severity of disease because the result derived from one study when applied to an assortment of patients may lead to undertreatment of some patients and excessive medication in others (22,23).

The clinical efficacy of gentamicin in the treatment of serious infections is related to the peak concentrations that could be achieve clinically in plasma. Jackson,G.G.,et al described that peak serum concentrations higher than 4 to 5 μg/ml are considered optimal for treatment of gram-negative bacteria early in therapy (24,25). Higher peak concentrations are recommended in patients with gram-negative pneumonias and burn wound sepsis (26,27). 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 (2). 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 (28,2). Wenk, M., et al recommened 5 to 10 µg/ml for serum level monitory of gentamicin in severe gram-negative infections in patients with normal renal function (23).

Different therapeutic range should be selected in different treatments since therapeutic range may be associated with variable factors such as diagnosis of disease, disease severity, subject, age and concomitant drugs (29-31,5).

3. Adverse effect and toxicity

Gentamicin or other aminoglycoside affected on the blood, ear, kidney, electrolyte homeostasis, mental state, nervous, eye, immune function and neuromuscular system (18). But the two most frequent toxic effects occurring are irreversible ototoxicity and reversible nephrotoxicity. Both therapeutic and toxic effect of gentamicin are related to dose and serum levels of the drug. Accumulation of gentamicin occurs predominantly when concentrations in tissue are high and diffusion back into the blood stream is slow. Gentamicin will be accumulated in the inner ear (19). Ototoxicity is the result of vestibular or cochlear sensory damage.

It has been suggested that ototoxicity occurs more frequently with gentamicin when peak concentration exceed 10 to 12 µg/ml. It can occur even after discontinuation

of therapy. Because destroyed or damaged cochlear hair cells are unable to regenerate, ototoxicity is often irreversible and is cumulative in nature being related to the dose and duration of therapy (23). Not only ototoxicity but nephrotoxicity were also correlated with serum peak concentration measured after serum creatinine was already rising (increase in serum creatinine > 0.5 mg/dl). Approximately 8 to 20% of patients who receive gentamicin for more than several days will develop mild renal impairment that is almost always reversible (32,33).

Trough concentration has been reported to associated with nephrotoxicity by many investigators and the nephrotoxic trough concentration of gentamicin has been reported to be above 2 µg/ml (34,35,2). Wenk, M., et al concluded that there are important differences between nephrotoxicity and ototoxicity with respect to clinical management. Firstly, in most cases nephrotoxicity seems to be reversible, and secondly the manifestation of this side effect can be readily detected by a simple laboratory test. It therefore believe that the value of therapeutic drug monitoring in preventing nephrotoxicity is limited. Instead, it is recommended that frequent monitoring of the serum creatinine and an appropriated dosage adjustment in the presence of renal failure is performed to avoid ototoxicity.

Pharmacokinetic Study in Man

1. Absorption

Gentamicin is poorly absorbed from the gastro-intestinal tract but it is rapidly absorbed after intramuscular injection or subcutaneous injection. Intramucular injection results in peak concentration in plasma after 30 to 90 minutes and the peak level appears approximately 30 minutes after complete intravenous infusion (18,19). A problem of intramuscular (IM) absorption after repeated injection into the same site might be questioned if peak concentrations are lower than expect and result in more variation in serum concentration. It is best to be avoided, because intramuscular administration is never completely reliable in critically ill patients (36), intravenous infusions of 30-60 minutes are reported to be safer (16).

Parenteral administered gentamicin diffuses mainly into extracellular fluids, little amount goes into the cerebrospinal fluid and it is poorly diffused into eye. Gentamicin can cross the placenta but only small amount have been reported in breast milk. Crosby, S.S., et al (37) found that systemic absorption in patients with pneumonia who were given gentamicin (1 patient) or tobramycin (9 patients) by endotracheal instillation was ranged from 1.5 to 3.4 %.

2. Distribution

Gentamicin is widely distribution in extracellular fluids including cross placenta barrier. Measurable concentrations are found in bile, synovial fluid, renal lymph, sputum, bronchial secretions and pleural fluid (38,17). Binding to plasma protein is low. 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 (19,2). Peak serum concentrations may be changed from usual in patients whose extracellular fluid volume is change, which may occur during sepsis (dehydration), congestive heart failure, fever, liver disease, edema or ascites, burn, obese and malnutrition. In addition, newborn infants have a large extracellular fluid, frequently in the range of 50 to 70 % of their body weights (39-41,16).

Changing in the distribution volume is independent of any change in renal function but will influence the drug's half-life if total body clearance remains constant within the patient. On the other hand, when the volume increase, the drug's half-life will also increase. Several methods for predicted gentamicin levels show estimated volume of distribution value of normal renal function patients as follows:

Vd = 0.24 L/kg of BW : Culter (42)

Vd = 0.25 L/kg of BW : Sawchuk and Zaske (43)

Vd = 0.26 L/kg of BW : Hull and Sarubbi (36)

(BW = lean body weight or actual weight)

3. **Excretion**

Gentamicin does not appear to be metabolized. It was excreted by glomerular filtration, virtually unchanged from urine. Small amount of drug have found in bile and may represent an additional route of elimination. Approximately 53 to 98% of a single intravenous dose is excreted in urine within 24 hours. (39)

Culter, R.E., et al (42), McHenry, M.C., et al (44) indicated that there was relationship between the loss of gentamicin and the endogenous creatinine, the serum creatinine concentration and the half life ($t_{1/2}$) of gentamicin. Measurement of the endogenous creatinine clearance depend on accurate collection of urine specimens which may be difficult in some cases of seriously ill patients. The $t_{1/2}$ of gentamicin could be estimated roughly by multiplying the value of serum creatinine concentration (mg/100 ml) by four (44).

The serum half-life of gentamicin is between 2 to 3 hours in patients with normal renal function, and longer in young infants (immature renal system). Schentag, J.J. and Jusko, W.J. (45) indicated that the rate of gentamicin disappearance from body is considerably longer than from

plasma. The half-life for tissue-bound gentamicin has been estimated to range from 30 to 700 hours. For this reason gentamicin can be detected in the urine for 10 to 209 days after dosage is discontinued.

Because of the narrow range between therapeutic and toxic serum levels and the drug was mainly eliminated by kidney, patients' with renal impairment will have high peak gentamicin levels. The rate of renal elimination influences serum levels and $t_{1/2}$ 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 regimen of dosage adjustment in patient, the clinician often use the serum creatinine concentration or the creatinine clearance as an index of excretory function such as Hull and Sarubbi's method (3), Chan's method (4), Rule of eight method (46), Dettli's method (5).

The estimated creatinine clearance, which is based on the serum creatinine concentration and employs modifications for age, sex and body weight provides a reasonable accurate index of renal glomerular function for the individual patient. Example of these several methods are as follows (47-49) :

Cockcroft and Gault's method (1976)

$$\text{CrCl (male)} = \frac{(140 - \text{age}) \times \text{BW(kg)}}{72 \times \text{Scr(mg/dl)}} \quad \text{ml/min}$$

$$CrCl(female) = \frac{(140-age)\times BW(kg)}{72\times Scr(mg/dl)} \quad (0.85) \quad ml/min$$

Jellife's method (1973) : for 70 kg person

$$CrCl(male) = \frac{98-0.8\ (Age - 20)}{Scr} \quad ml/min/1.73m^2$$

$$CrCl(female) = \left[\frac{98 - 0.8\ (Age - 20)}{Scr}\right] \quad (0.90) \quad ml/min/1.73m^2$$

Schwatz's method (1976)

This method is used particularly for children.

$$CrCl = \frac{0.55\ body\ length\ (cm)}{Scr} \quad ml/min/1.73m^2$$

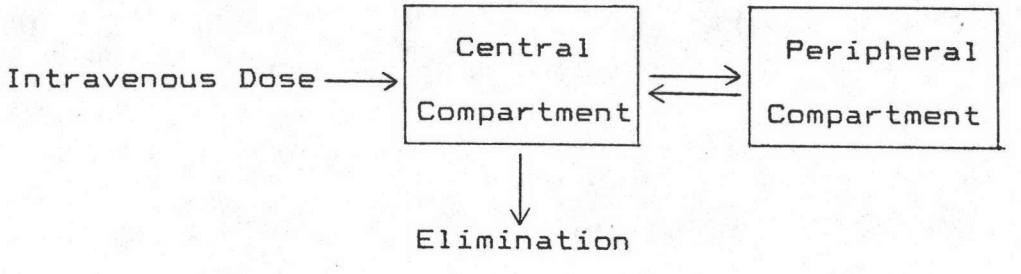
Bjornsson, T.D., et al's method (1983)

This method is shown in Figure A. (Appendix D)

Pharmacokinetic model of gentamicin

Pharmacokinetic models, initially developed to describe the behaviour of drug in man, can also be used to predicted the serum drug concentrations that will result from a dosing regimen.

Drug accumulation into the deep tissue compartment occurs during gentamicin therapy. The amount of drug accumulated depends on dosage and duration of treatment.



Evans, W.E., et al (50) 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 significant ($p < 0.05$), while concentrations predicted by the two-compartment model were not significantly different from measured values ($p > 0.1$). Gibaldi, M. and Weintraub, H. (51) described that inappropriate mathematics model applied to blood level or urinary excretion data is a problem in pharmacokinetic study. Two-compartment model was reported to be the best to describe plasma levels of gentamicin after n^{th} dose, since the apparent half-life of this drug has increased in an asymptotic manner with increasing dose numbers, while a one-compartment model is all that is apparently needed in describing the plasma levels of the drug after the first dose. On the other hand, the net fraction of the drug distributed from the central compartment to a peripheral compartment may be so small at steady-state that the existence of the compartment may not be apparent, whereas its existence is quite apparent after a single dose (51).

Therefore, a one-compartment model is reported to be much more practical for computing dosage regimens and predicted serum concentration of gentamicin (52-55,35,43). Example of some equations commonly used to calculate

gentamicin serum levels and its dosage regimen are in Appendix C (Equations 6-10 and 11).

II. REVIEW OF FLUORESCENCE POLARIZATION IMMUNOASSAY TECHNOLOGY (FPIA TECHNOLOGY TDx^R ANALYZER SYSTEM (56-59).

The TDx^R Analyzer system is a fully automated system, developed from fluorescence polarization immunology assay technology to measure therapeutic drug and hormone levels.

The principles of fluorescence polarization were first developed by Perrin in the 1920, later by Uleber in 1953 and the first application to the antigen-antibody reaction was described by Dandliker and Feigen in 1961.

The principle reagents for FPIA are fluorescent-labeled antigen (tracer) and antibodies specific for the antigen. From Figure B.1 and B.2 (Appendix D) show that, the light source is changed to be linearly polarized light. Figure B.3 (Appendix D) shows that, the blue light is the linearly polarized which is used to excite the tracer.

The TDx^R Analyzer system uses a competitive binding immunoassay methodology to allow fluorescent-labeled antigen and patient antigen to compete for binding site on the antibody molecules. When the fluorescent-labeled antigen (tracer) is bound to the antibody, the

fluorophore (tracer bound to antibody) is constrained from rotating between the time light is absorbed and emitted. So that, when plane polarized blue (linearly polarized light) is used to excite the fluorescent-labeled antigen, the fluorophore is still highly polarized upon emission (shows in Figure B.4 and B.5; Appendix D). On the other hand, when the fluorescent-labeled antigen is free, its rotation is much greater; molecules are more randomly oriented and the emitted light is in different plane, polarization is lost (shown in Figure B.6; Appendix D).

Fluorescence polarization is a direct measure of bound and free fluorescent-labeled antigen in a competitive binding immunoassay. The fluorescent-labeled antigen compete for antibody binding site with the antigen (unlabeled analyte) in sample. When competitive binding occur, the fluorescent-antigen complex becomes a part of the very large antibody molecule; the free fluorescent-labeled antigen (tracer) is small in comparison (shown in Figure B.7; Appendix D). 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 (shown in Figure B.8; Appendix D). Therefore, if a patient sample contains a low concentration of antigen, when reaction reaches steady-state, there will be high bounding of fluorescent-labeled antigen with antibody (fluorophore) and low

bounding of antigen in patient sample with antibody in the reaction mixture. Polarization will be high (shown in Figure B.9; Appendix D). On the other hand, if a patient sample contains a high concentration of antigen, after the competitive binding reaction reaches steady-state, there will be very low bound fluorescent-labeled antigen to the antibody and it will be free to rotate which the emitted light (green light) will be in a different plane. So, polarization will be low (shown in Figure B.10; Appendix D).

The precise relationship between the concentration of antigen (unlabeled drug) and polarization is established by generation a standard curve. Standards with known amounts of drug are read and the polarization are recorded. When an unknown is read, its concentration is estimated by interpolating between standards. Figure C.1 and C.2 (Appendix D) showed the major components of the totally automated, bench-top, fluorescence polarization analyzer.

There are specific reagents of TDx^{R} Analyzer system for assay of each drugs. Before using new reagent pack for assaying of each drugs, assay calibration must be made and memorized in TDx^{R} Analyzer system. Concentration of specimens are determined by referring to the stored curve. A weighted log-logit tape curve fit is used. Verify the calibration acceptability by using three criteria.

1. The polarization error (PERR) or percent error (ERR) values must be within the acceptable range for each drugs, i.e.,

PERRS = 3.5 (for gentamicin)

2. The root mean squared error (RMSE) must be within the acceptable range for each drugs, i.e.,

RMSE = 2.0 (for gentamicin)

3. Each level of control that is run must be within the acceptable range indicate for each drugs.

An example of the calibration printout is shown in Figure D (Appendix D).

Assaying sample specimen; serum sample (>50 µl) is placed in the sample side of the dual-chamber sample cup. Sample cup and cuvette are placed in the carousel. Loading the carousel and drug reagent pack in the instrument.

Set up is automatic. The bar-code on drug reagent pack is read and processing is retrieved from non-volatile memories. The number of sample to be processed is determined by an optical sensor, and all reagent volumes are checked by a liquid-level sensor on the tip of the probe. The cuvettes are warmed to 35^{o}C by an air heater controlled through an infrared sensor mounted in the optics modules. This typically requires less than 30

seconds. Temperature is maintained at 35 $\pm$ 0.5^{o}C with forced air heat.

A blank-corrected polarization values are calculated and concentration of specimens are determined by referring to the stored curve. The hard-copy report is made with the printer. Concentration of samples are reported by Assay Printout (shown in Figure E; Appendix D).

Every time of assay, we can check the integrity of samples and reagents by using Assay control along with the samples.

Serum sample (or Calibrator, Control) > 50 µl

→ 20 µl of sample was mixed with 700 µl of buffer in dilution well of the sample cup

↓

25 µl of the dilute and 25 µl of pretreatment solution were added to cuvette along with buffer to a total volume of 975 µl

↓

A blank reading was then made when the cuvette reaches the fluorimeter

↓

25 µl of antibody, 25 µl of tracer, and a volume of diluted sample equal to that used in the second revolution were added with buffer to bring the total volume to 1.95 ml

↓

Incubation 3 minutes

↓

Reading the values from TDx^{R} analyzer