CHAPTER 3

RESEARCH DESIGN AND RESEARCH METHODOLOGY

3.1 Research Question

Primary research question:

Is low dose oral erythromycin effective for treatment of feeding intolerance in preterm infants by reducing time required to achieve full enteral feeding ?

Secondary research question:

Are there any side effects associated with low dose oral erythromycin for treatment of feeding intolerance in preterm infants?

3.2 Objective of the study

To evaluate efficacy and safety of low dose oral erythromycin for feeding intolerance in preterm infants. We hypothesized that initial loading dose followed by low dose erythromycin would reduce feeding intolerance without any significant side effects.

3.3 Hypothesis

Research hypothesis:

Low dose oral erythromycin shorten the time to establish full enteral feeding in preterm infants with feeding intolerance

Statistical hypothesis: in survival function (*S*t)

Ho: $St_{(t)} = Sp_{(t)}$ at all t

Ha: $St_{(t)} \neq Sp_{(t)}$ at all t

Where:

*S*t = Probability of establishing full feeding in infants treated with EM at time t

*S*p = Probability of establishing full feeding in infants treated with placebo at time t

Null hypothesis: The time to establish full enteral feeding in preterm infants with feeding intolerance treated with low dose erythromycin **is not different** from those with similar condition treated with placebo.

Alternative hypothesis: The time to establish full enteral feeding in preterm infants with feeding intolerance with low dose erythromycin **is different** from those with similar condition treated with placebo.

3.4 Conceptual Framework

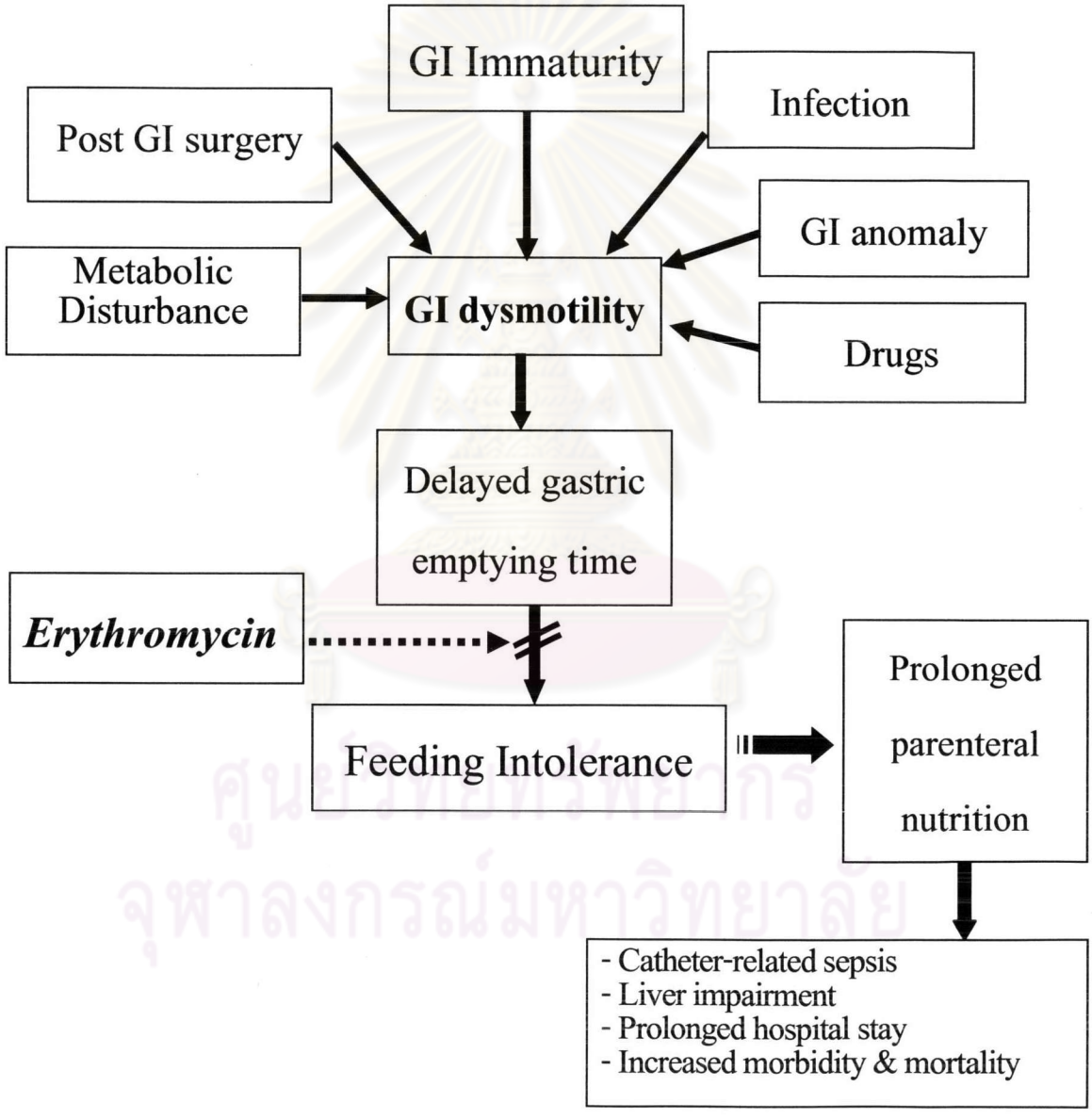

Figure 1. Conceptual framework

3.5 Research design

This study had been carried out as a randomized, double blinded, placebo controlled trial emphasis to determine the efficacy and safety of low dose erythromycin for preterm infants with feeding intolerance.

For treatment allocation, subjects were stratified by gestational age (<32, ≥ 32 weeks) since gestational age was the major influencing factor, and then were randomized using a block of size 4 to ensure the balance of subjects in each allocation. The allocation concealment was performed under an opaque sealed envelope to reduce allocation bias. The investigators, the patient care team, and the assessors were blinded to the treatment allocation in order to prevent outcome assessment bias. The patients who met the inclusion criteria are enrolled into the trial and all recruited patients were received a similar protocol of feeding and a similar protocol of discontinuing feeds in order to avoid a possibility of contamination and co-intervention.

3.6 Research methodology

Population

Target population: Preterm infants, born at less than 35 weeks of gestation or birth weight less than 1800 g, who develop feeding intolerance.

Sampled population: All preterm infants, born at less than 35 weeks of gestation or birth weight less than 1800 g, who were admitted consecutively at the neonatal intensive care unit and the nursery at Ramathibodi Hospital, Srinagarind Hospital, and Chiang-Mai University Hospital during the study period and met the following eligibility criteria.

Inclusion criteria:

1. Gestational age less than 35 weeks or birth weight less than 1800 g
2. Post natal age ≥ 5 days
3. Clinically stable condition, no hypotension, no recurrent severe episodes of hypoxemia or desaturation
4. Feeding intolerance defined as:
   - gastric residual > 50% of feed volume given over the previous 3 hours for at least 2 occasions during 48-hours period

Exclusion criteria:

Infants with the following condition were excluded:

1. lethal congenital anomalies
2. anatomical gastrointestinal abnormalities
3. necrotizing enterocolitis (suspected or proven) within 7 days before the onset of feeding intolerance
4. congenital cyanotic heart diseases
5. infant who received major gastrointestinal surgery within 2 weeks
6. suspected or proven sepsis at the onset of feeding intolerance or within 5 days of antimicrobial treatment
7. metabolic or electrolyte disturbances at the onset of feeding intolerance
8. any of the following medications within 24 hours before the onset of feeding intolerance; fentanyl, indomethacin, vercuronium

Randomization

Eligible infants were randomly assigned by a staff not involved in patient care team to either treatment group (erythromycin) or placebo group (manufactured placebo). Block of 4 randomization was employed. The allocation concealment was performed under an opaque sealed envelope to reduce allocation bias.

Intervention

Study Drug: Erythromycin ethyl succinate (Erysil, Siam Phamarceutical Ltd) was diluted to 40 mg/ml with sterile water and a loading dose of 10 mg (0.25ml) /kg/dose was given via an orogastric tube every 6 hours for 2 days followed by 4 mg (0.10ml) /kg/dose every 6 hours for another 5 days (total course 7 days).

Placebo: Manufactured placebo with the same ingredient but no active drug (erythromycin) was given at the same dose, intervals, and duration.

Both drug and placebo were prepared by an assigned staff not involved in patient care team, and were identified by code numbers.

All patients received similar standard neonatal care. They were monitored closely and allowed to withdraw from the study at any time. During the study period, the use of other prokinetic agents such as cisapride, domperidone, or metoclopramide were strictly prohibited. Electrocardiography was performed immediately before and on the forth day of treatment. Liver function tests were performed before and at the end of treatment.

Protocol for enteral and parenteral nutrition

Infant with birth weight less than 1500 g was started on parenteral nutrition on day 2 of life begining with 0.5 g/kg of amino acid and 0.5 g/kg of lipid with an increased increment of 0.5mg/kg/day up to a maximum of 3 g/kg/day of both amino acid and lipid.

Enteral feeding was initiated on day 3-4 of life if he or she was clinically stable. They were fed mother's milk whenever possible, but preterm infant formulas were also used according to their parents' preference. Enteral feeding was given through a nasogastric or orogastric tube as an intermittent bolus in 30-60 minutes, starting with 10-20 ml/kg/day with an increased increment of 10-15 ml/kg/day for infants < 32 weeks gestation and 15-20 ml/kg/day for infants $\geq$ 32 weeks gestation.

Protocol for feeding with various gastric residuals:

1. If a gastric residual is less than 15% of the previous feed volume, place back the gastric content and continue to feed with same volume of milk
2. If a gastric residual is between 15-30% of the previous feed volume, place back the gastric content and continue to feed with a deducted volume of milk with a volume of gastric residual.
3. If gastric residual is between 30-50% of the previous feed volume, place back the gastric content and hold that feed, then resume the next feed with the same volume.
4. If gastric residual is more than 50% of the previous feed volume, discard the gastric content and hold that feed, then resume the next feed with the same volume of milk of the previous day.

All infants were examined at least twice a day and closely monitored for occurrence of vomiting, diarrhea, abdominal distension, and volume of gastric residuals. Gastric aspirates were measured every 3 hours before the feed and also observed for any bile-stained content. Abdominal circumferences were measured at 12-hours interval before feeds, an increased in abdominal circumference of greater than 1.5 cm between the 12-hour interval was considered abnormal. Any episodes of vomiting / regurgitation and bile-stained aspirates during the study were recorded.

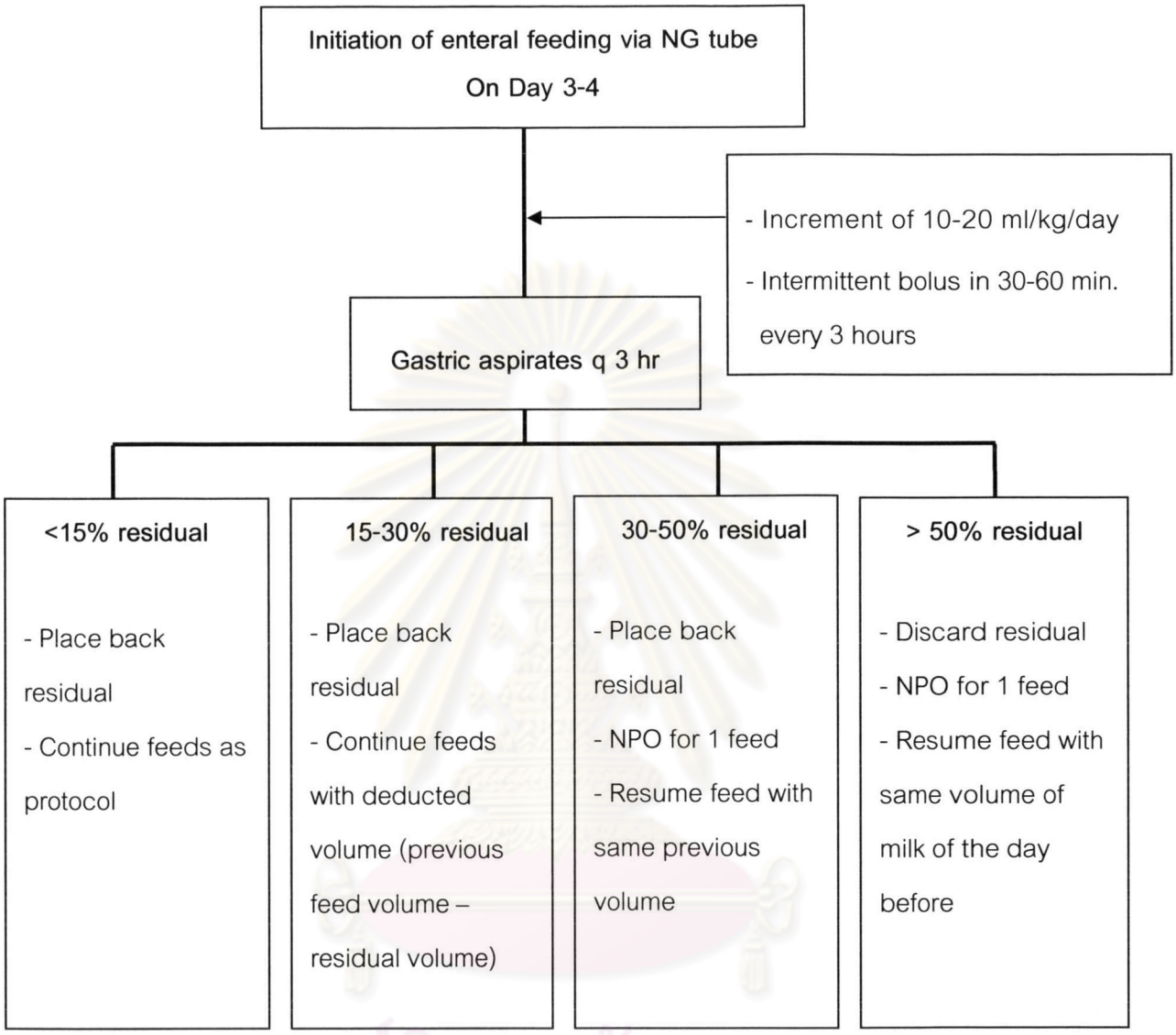

Figure 2. Feeding protocol

Guideline for stopping enteral feeding:

- vomiting occurs more than twice in 24 hours
- volume of gastric residuals exceeds half of the milk given in the previous feed on two occasions within 24 hours
- clinical signs and symptoms suggesting necrotizing enterocolitis or other ominous intra-abdominal pathologies.

An isolated bile-stained or bloody stained gastric aspirate with normal physical examination was not an indication for stopping feed.

The duration of holding feed was determined by the discretion of attending physician. Enteral feeding together with the study drug was resumed as soon as the above signs and symptoms subsided. The volume of resumed feeds started at the half volume of the feed before holding.

Monitoring:

All participants were monitored as a standard neonatal intensive / intermediate care. If there was any serious side effect related to the experimental drug, he or she would be withdrawn from the study immediately. Laboratory tests were performed for safety and early detection of complications; including

- Electrocardiography (EKG) to be performed immediately before and at the forth day of treatment in all participants.
- Liver function tests also to be performed before and at the end of treatment.

3.7 Operative definitions

- **Feeding intolerance** is defined as gastric residual > 50% of feed volume given over the previous 3 hours for at least 2 occasions during 48-hours period
- **Full enteral feeding** is defined as being able to tolerate enteral feed at least 150 ml/kg/day of milk for at least 3 consecutive days.

3.8 Outcome measurements

Primary outcome variable:-

- Time from enrollment to achieving full enteral feeding (day)

Secondary outcome variables:-

- Incidence of necrotizing enterocolitis
- Incidence of septicemia during study period
- Death rate
- Length of hospital stay
- Possible side effects related to erythromycin :

  Increased liver enzyme, prolonged QT interval, and pyloric stenosis
- Possible complications related to parenteral nutrition:

  Cholestatic jaundice, catheter-related sepsis

3.9 Sample size calculation

Sample size calculation had been carried out, based on the main outcome of the primary research question, which was the time from enrollment to achieving full feeding. Therefore, the survival analysis was chosen as a method to calculate the required numbers of patients for this study.

Estimating the sample size by calculating the required number of events in survival analysis

Assuming no censoring, the required number of events (E = event; full feeding) in a survival study can be obtained from the following formula by George and Desu (49)

$E = 4c(\alpha,\beta) / \theta^2_R$

Where E = number of events required in survival study (full feeding)

$c(\alpha,\beta) = (z_{\alpha/2} + z_\beta)^2$

α = 0.05, Power = 80% (β = 0.20)

Z_α = 1.96 (two tail), Z_β = 0.84

θ_R = ln – hazard ratio

The hazard ratio is the ratio of median survival (time to full feeding) for treatment group to the median survival (time to full feeding) for control group. The estimated hazard ratio in this study was 2.25

Therefore, the estimated number of patients per group = 23 (total = 46).

The calculation described above is based on the assumption that the trial is to be continued until a given number of those entering the study have reached full feeding.

3.10 Data collection

All infants enrolled into the study were included in the analysis. The assigned staff, who did not involve in patient care team, collected and recorded data into a data record form. Baseline data included gestational age, birth weight, sex, Apgar score, respiratory status expressed in fraction inspired oxygen concentration (FiO_2) requirement, history of antenatal steroid, presence of umbilical artery and venous catheter, age at enrollment, volume of enteral feeding at enrollment, type of milk feeds, and the presence of patent ductus arteriosus.

3.11 Data analysis

The results were analyzed in an intention to treat basis. Data were expressed as percentage, mean and standard deviation, and median and interquartile ranges if data were not normally distributed.

Baseline demographic data were analyzed using descriptive and comparative statistics. Continuous data such as gestational age, birth weight, and age at enrollment were analyzed by unpaired t test or Mann-Whitney test. Proportional or categorical data such as incidence of sepsis, necrotizing enterocolitis, or cholestatic jaundice were analyzed by using Chi-square test or Fisher's exact test. P value ≤ 0.05 was considered statistical significance.

Primary research outcome: The time to establish full feeding was analyzed by survival analysis. The Kaplan-Meier survival curves for each treatment group were constructed and compared using Log-rank test.

Definition used in this trial for a survival analysis.

Time origin: time at starting enrollment of the trial of each patient

Event: Full enteral feeding is defined as when a patient could tolerate feeding up to at least 150 ml/kg/day of milk for at least 3 consecutive days.

Censored observation may occur due to:

- Patients die before establishing full feeding
- Patients are withdrawn from the trial due to the causes that are not related to the treatment
- Patients are loss to follow-up; for example, transferring to other hospital/ward, having an operation due to the causes unrelated to the treatment.

Function of survival time: The survival function *S*(t) was employed for analysis.

Statistical package for analysis

SPSS version 11.0 statistical software was used for statistical analysis and STATA version 7 was used for survival curve analysis.

3.12 Ethical consideration

This study was conducted on the basic evidences supported by both animal and human studies that erythromycin would be effective for treatment of feeding intolerance in preterm infants without serious side effects. Cardiac toxicity or arrhythmia has been reported in infants who received antimicrobial dose of intravenous form. Hepatic toxicity or hepatitis is very rare when using erythromycin ethyl succinate or stearate. Transient auditory impairment has been reported only with intravenous high dose of erythromycin lactobionate or gluceptate or high dose oral form of erythromycin estolate. Recently, Hypertrophic pyloric stenosis has been reported in infants who received antimicrobial dose of erythromycin as post-exposure prophylaxis for pertussis. However, the risk of development of hypertrophic pyloric stenosis occurred in infants who have received an antibiotic dose of erythromycin for greater than 10 days. According to all of the above evidences, low dose oral erythromycin given for a short period as in this study would avoid its possible serious side effects and also would be effective as supported by clinical trials already mentioned earlier.

This study had been conducted according to the Declaration of Helsinki 2000 and the study protocol has been approved by the Institutional Review Board, Faculty of Medicine Ramathibodi Hospital. Before enrollment into the study, the parents were informed by the investigators about the detail of the study protocol, any possible risks and the patient's rights, and then the written informed consent had been obtained from all participants. The participants were able to withdraw from the study at any time with an assurance that they still received the standard neonatal care.

There would not be any harmful or painful procedure in this study. The amount of blood drawn for liver function test was only 1.5 ml and was drawn from an umbilical catheter. EKG was performed only in lead II to calculate QT intervals, which should take only a short period of time.