

CHAPTER 1

INTRODUCTION

1.1 Background and Rationale

Anesthesia is used in surgery to minimize pain, discomfort, and shock for surgical patients. When anesthesia works as expected, the patient feels no pain during a procedure, and often does not remember the proceedings either. Anesthesia increases patient comfort, which can in turn reduce recovery times and it easier for a medical staff to work.¹

There are three different types of anesthesia; local anesthesia, regional anesthesia and general anesthesia. In local anesthesia a specific location of the body is numbed, such as the hand. Regional anesthesia numbs larger area of the body by administering anesthesia to a cluster of nerves. Two frequently used regional anesthetics are spinal anesthesia and epidural anesthesia. General anesthesia describes unconsciousness and lack of any awareness or sensation.¹

Inhalational anesthetics are gases that possess anesthetic qualities that are administered by breathing through an anesthesia mask or endotracheal tube (ET) connected to an anesthetic machine. The agents of significant contemporary interest include the volatile anesthetics (halothane, isoflurane, sevoflurane and desflurane) and the gases ethylene, nitrous oxide and xenon.²

A patient is intubated and connected to an anesthesia breathing machine. Potent inhaled anesthetics and halothane pass from the anesthesia

machine into the patient by way of the breathing circuit and endotracheal tube.³ When the patient inhales, the inhalation flutter valve opens, allowing oxygen and anesthetic to enter the hoses. These gases travel through the inspiratory hose to the Y-piece and are directed into the endotracheal tube to the patient's lungs.^{4,5}

Halothane is an inhalation anesthetic has been used widely for the induction and maintenance of general anesthesia. Ventilation should be carefully assessed and under control to insure adequate oxygenation and carbon dioxide removal.⁶ The anaesthetist could vary minute volume by setting tidal volume and ventilatory frequency directly or by adjusting inspiratory time, inspiratory flow rate and the ratio of inspiratory to expiratory time.^{7,8}

Halothane has a depressant action on the cardiovascular system and reduces blood pressure by decreasing the cardiac output; signs of over dosage are bradycardia and profound hypotension.⁹⁻¹¹ It also can cause cardiac arrhythmias or cardiac arrest. Its effects on gastrointestinal tract are nausea and vomiting. The usual dosage of halothane are 0.5% to 1.5%.⁴ Halothane will cause dose-dependent respiratory depression resulting in hypoxia.¹⁰ Adverse effects on the liver range from liver dysfunction to hepatitis and necrosis, and are more frequent following repeated use. Defective proteolytic degradation of the immunogenic trifluoroacetylated protein caused hepatotoxicity.¹¹

Hepatic organ is one of the organs mostly affected.¹² Walton *et al.* have found 204 postoperative hepatic dysfunction from 5,000,000 halothane anesthetics cases, 76 classified as unexplained. Ninety five percent of these cases underwent multiple halothane anesthetics, which 55% were took place within 4 weeks.¹³ Hepatic function tests changes resulting from inhalation anesthetics are usually not clinically important.⁶ Halothane hepatitis is probably caused by trifluoroacetyl-containing metabolites binding to protein and subsequently forming anti-trifluoroacetyl antibodies.¹⁴ Halothane hepatotoxicity can occurs 5-7 days following exposure, although it can be delayed by up to 4 weeks. Each haloalkaline anesthetic causes liver injury is related to the amount they are metabolized by hepatic CYP enzymes: 20% to 30% for halothane, 2% for enflurane, 1% for sevoflurane, and 0.2% or less for isoflurane and desflurane.¹⁵ Halothane is associated with highest incidence of hepatic injury with up to 1 case per 35,000 patients developed halothane hepatitis. While lower incidence are associated with isoflurane and desflurane.¹⁶

Recovery time from halothane anesthesia varies with the physiologic state of the patient. Debilitated animals remain affected longer than vigorous patients. In general recovery from halothane are 60-90 minutes, but it may be depressed for several hours.¹⁷ Recovery from halothane anesthesia is slower than with other agent because of its high blood/ gas solubility, and recovery is prolonged with increasing duration of anesthesia.¹⁸

There are preventive measures for halothane exposure, for example engineering controls, personal protective equipment, work practices, and administrative practices.¹⁹ Medical personnel especially anaesthetists, medical technicians, and nurses need the best conditions of the operating room, i.e. there are adsorption tubes or diffusion dosimeters for personal halothane concentration.²⁰

Cytochrome P450 enzymes are a group of about 60 endogenous enzymes that participate in the metabolism of drugs. The CYP450 enzymes also participate in lipid and steroid hormone synthesis. Most of the CYP450 enzymes that are active in drug metabolism are in the liver and the small intestine. The CYP450 enzymes function as catalysts to facilitate the processes by which the drug transforms from its initial chemical structure to the biochemical forms that have action in the body. CYP450 subtype 2E1 and 2A6 play an important role in halothane metabolism. Cytochrome P450 2E1 (CYP2E1) is a major catalyst in the formation of trifluoroacetylated proteins, which have been implicated as target antigens in the mechanism of halothane hepatitis.

Medical personnel within the operating theatre are at risk of being exposed to wasted halothane during surgery. The total dosage of wasted halothane exposure depends on the cumulative duration of the anesthetic time each working day, every week, every month or even every year. Therefore, such medical personnel have a risk of tissue damage; e.g. liver, kidney, cardiovascular system, pulmonary system, renal system, and skeletal muscle system. We

choose halothane in this research since it is still widely used especially in low income countries (e.g Libya and Egypt) as agent anesthetic of choice in pediatric operation and delivery. High incidence of halothane-associated hepatitis is another reason we choose this agent to be studied in this research.

1.2 Research Question

How does the different exposure time of halothane gives the liver cells changes in Balb/C mice either morphologically or by cytochrome P450 expression?

1.3 Research Objectives

1.3.1 General Objectives

To identify and analyze the liver cells changes of halothane exposure to liver cells of Balb/C mice.

1.3.2 Specific Objectives

- a. To analyze the changes of the liver cells' nucleus of the Balb/C mice caused by halothane exposure
- b. To analyze changes of cytochrome P450 2E1 on the liver of the Balb/C mice caused by halothane exposure

1.4 Research originality

Table 1. Research originality

No.	AUTHOR/ /TITLE /PUBLICATION	RESULT	Novelty of the study
1.	M.W. Anders. <i>Formation And Toxicity Of Anesthetic Degradation Products.</i> ²¹ Annual Review of Pharmacology and Toxicology 2005 : 45: 147-176	<ul style="list-style-type: none"> ▪ 2-Bromo-2-chloro-1,1-difluoroethylene is nephrotoxic in mice: Its LC50 is approximately 0.025% (v/v) (36). Animals exposed to 2-bromo-2-chloro-1,1-difluoroethylene show kidney damage characterized by intense renal tubular degeneration.²¹ ▪ Monkeys were anesthetized with halothane, but no abnormalities were found on postmortem examination. In dogs are found in the reservoir bag, but no macroscopic or microscopic changes are observed on postmortem examination.²¹ 	<ul style="list-style-type: none"> ▪ This research reveal a liver toxicity of halothane exposure in concentration 1,1% by the presence of nucleus changes by histopathology. ▪ Animal use : male balb/C mice
2.	B. Prokes*, I. Mikov, M.	▪ Average values of	▪ This research

<p>Glavaski.. <i>The Effect of Occupational Exposure to Wasted Halothane on Liver Functions of Operating Room Personnel.</i>²² Polish Journal of Environmental Studies 2009l. 18, No. 5, 893-899</p>	<p>“biliary retention syndrome” and “hepatic cell necrosis syndrome” parameters in operating room personnel were significantly higher (p<0.001 for all) than in control group.²²</p>	<p>measure changes of liver cells nucleus.</p>
<p>3. Spracklin, D. K., Emery, M. E., Thummel, K. E. and Kharasch, E. D. (2003),</p>	<ul style="list-style-type: none"> ▪ Pseudocholinesterase activity in exposed medical personnel was significantly lower (p<0.001), while average value of total proteins was significantly higher (p<0.001) than in control group. ²² ▪ Statistical analysis has shown that surgeons have significantly higher activities of γGT, AST and ALT compared to anesthesiologists and instrumenting nurses, and only γGT compared to anesthetists.²² ▪ Cytochrome P4502E1(CYP2E1)-mediated oxidation of halothane induction increased both TFA and 	<ul style="list-style-type: none"> ▪ This research measure changes of liver cells nucleus.

*Concordance between trifluoroacetic acid and hepatic protein trifluoroacetylation after disulfiram inhibition of halothane metabolism in rats.*²³ Acta Anaesthesiologica Scandinavica, 47: 765–770. Article first published online: 13 JUN 2003

TFAprotein formation compared with uninduced halothane-treated rats.²³

4. Einar Bjo Rnsson, Pernilla Jerlstad, Annika Bergqvist & Rolf Olsson. 2005. *Fulminant drug-induced hepatic failure leading to death or liver transplantation in Sweden.*²⁴

- Four most common drugs associated with fatalities were halothane, paracetamol, flucloxacillin and sulfamethoxazole/trimethoprim. All drugs reported with at least two cases of fatal outcome. In 17 cases (16%) two drugs were potentially causally related to liver injury.²⁴
- A total of 58 different drugs, taken either alone

- This research measure changes of liver cells nucleus.

or together with another drug, were the presumptive cause of fatal drug-induced liver injury.²⁴

This research is original and different from previous studies regarding the things below:

1. This study measuring the liver cells nucleus changes after different time of halothane exposure.
2. This study evaluating the cytochrome P450 2E1 after different time of halothane exposure.

1.5 Research Benefit

This research will give additional evidence of halothane toxicity especially in the liver organ. If the dosage used in this research proved toxic on the liver of Balb/C mice, further study on halothane exposure to the medical person per year is warranted. Thus, the medical person can take preventive measures to avoid liver injury.