

CHAPTER 2

LITERATURE REVIEW

2.1 Anaesthetics

2.1.1 Definition of anesthetic

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. With the knowledge that they are not inflicting pain, it also makes 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 a larger area of the body by administering anesthesia to a cluster of nerves. Two frequently used regional anesthesia are spinal anesthesia and epidural anesthesia. General anesthesia describes unconsciousness and lack of any awareness or sensation.¹

2.1.2 Mechanism of anesthetic

Although scientists are not sure exactly how anesthesia works, there are many theories that have been proposed. One theory proposes a relationship between the solubility of the anesthetic agent into the fat cells

of the body (lipid solubility) as determining the degree of its potency as an anesthetic agent. Another feature of anesthetic absorption is the way it is passed from the lungs to other cells in the body. At first there is a quick transmission from the lungs to the rest of the body, but as equilibrium is reached, the anesthetic begins to quickly pass out from the lungs. However, fat cells retain the anesthetic longer than other cells. Studies have shown that some inhaled anesthetics are metabolized by the liver (hepatic metabolism).

The mechanism of anesthesia is closely related to the problem of consciousness. Consciousness is caused by many interacting mechanisms, each in itself or in interaction with other mechanisms sufficient to create consciousness. Is it the degeneracy character of the neural mechanisms causing consciousness the reason for the difficulties to define its neuronal correlate.²¹

2.2 Inhalation Anesthetics

2.2.1 Definition of inhalation anesthetic

Inhalation anesthetics are gases that possess anesthetic qualities that are administered by breathing through an anesthesia mask or ET tube connected to an anesthetic machine.²

Inhalation anesthetics are substances that are brought into the body via the lungs and are distributed with the blood into the different

tissues. The main target of inhalation anesthetics (or so-called volatile anesthetics) is the brain.²²

2.2.2 The types of inhalation anesthetics

Inhalational anesthetic agents include nitrous oxide and the volatile agents such as ether, chloroform, halothane, isoflurane, enflurane, methoxyflurane, sevoflurane, desflurane, cyclopropane and trichloroethylene (trilene).

Table 2. Comparison value among inhalation anesthetics agent

Agent	Blood/Gas Coefficient	SVP mmHg(KPa)	MAC
Ether	12	425 (59)	1.9
Halothane	2.4	243 (32)	0.76
Enflurane	1.9	175 (24)	1.68
Isoflurane	1.4	250 (33)	1.3
Sevofurane	0.69	160 (21)	2.4
Nitrous Oxide	0.47	-	105
Desflurane	0.42	673 (88)	6
Trichloroethylene	9	60 (8)	0.17
Methoxyflurane	13	23 (3)	0.2
Cyclopropane	0.45	-	9.2

Notes:

SVP mmHg (Kpa) : Saturated Vapor Pressure

MAC : Minimum alveolar concentration (MAC) is the alveolar anesthetic concentration at one atmosphere (760 mm Hg) preventing movement in 50% of subjects exposed to noxious stimuli.²³

2.3 The Toxic Effects of Inhalation Anesthetics

The three target organs for toxicity of inhalation agents are the liver, the kidney, and the blood cell.

2.3.1 Liver

Fulminant hepatic necrosis following halothane anesthesia (halothane hepatitis) occurs in one of 6,000 to 35,000 cases and is often fatal. Halothane hepatitis is probably caused by trifluoroacetyl-containing metabolites binding to protein and subsequently forming anti-trifluoroacetyl protein antibodies. During re-exposure of the patient with halothane these antibodies may mediate massive hepatic necrosis.

2.3.2 Kidney

Nephrotoxicity in the earlier years was mainly caused by methoxyflurane. It caused high output renal failure with elevation of serum creatinine and BUN. It was related to the metabolism of methoxyflurane possibly releasing inorganic fluorides. Toxicity started to occur at peak fluoride levels of 50 to 80 mM and was evident at levels of 80 to 175 mM. The threshold of 50 mM was subsequently selected to be the magic number for renal toxicity.

2.3.3 Red blood cells

Carbon monoxide production from inhalation anesthetic degradation in anesthesia circuits can cause carbon monoxide toxicity. Carbon monoxide formation occurs when relatively dry baralyme or soda lime degrades inhalation agents such as enflurane, desflurane and

isoflurane. This occurs usually on Monday mornings after the anesthesia machine had been idle with fresh gas flow all weekend resulting in dry CO₂ absorbents.⁶

2.4 Halothane (Field of study)

2.4.1 Definition

Halothane, USP is supplied as a liquid and is vaporized for use as an inhalation anesthetic. It is 2-bromo-2-chloro-1, 1, 1-trifluoro-ethane. C₂HBrClF₃ The molecular weight is 197.38. The drug substance halothane molecule has an asymmetric carbon atom; the commercial product is a racemic mixture. In another name of halothane is fluothane.

Resolution of the mixture has not been reported. Halothane is miscible with alcohol, chloroform, ether, and other fat solvents. The specific gravity is 1.872-1.877 at 20°C, and the boiling point (range) is 49°C-51°C at 760 mm Hg. The vapor pressure is 243 mm Hg at 20°C. The blood/gas coefficient is 2.5 at 37°C, and the olive oil/water coefficient is 220 at 37°C. Vapor concentrations within anesthetic range are nonirritating and have a pleasant odor. Fluothane is nonflammable, and its vapors mixed with oxygen in proportions from 0.5 to 50% (v/v) are not explosive. Fluothane does not decompose in contact with warm soda lime. When moisture is present, the vapor attacks aluminium, brass, and lead, but not copper. Rubber, some plastics, and similar materials are soluble in Fluothane; such materials will deteriorate rapidly in contact with

Fluothane vapor or liquid. Stability of Fluothane is maintained by the addition of 0.01% thymol (w/w), up to 0.00025% ammonia .

According to the Serbian legislation the threshold limit value (TLV) for halothane is 40 mg/m³ (4.9 ppm). Halothane has quite low TLV, ranging from 0.5 ppm (Australia) to 5 ppm (Sweden and Germany). The National Institute for Occupational Safety and Health (NIOSH) has established a recommended exposure limit (REL) of 2 ppm (16.2 mg/m³) for halothane (as a waste anesthetic gas) as a 60-minute ceiling limit that should not be exceeded during any part of the workday. The American Conference of Governmental Industrial Hygienists (ACGIH) has assigned halothane a TLV of 50 ppm (404 mg/m³) as a time-weighted average (TWA) for a normal 8-hour workday and a 40-hour work week.²²

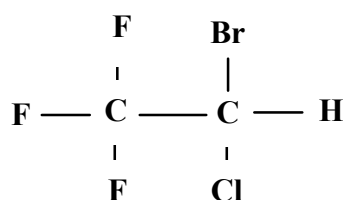


Figure 1. Structure of Halothane

2.4.2 Mechanism of halothane

Halothane may be administered by the nonrebreathing technique, partial rebreathing, or closed technique. The induction dose varies from patient to patient but is usually within the range of 0.5% to 3%. The maintenance dose varies from 0.5% to 1.5%. Fluothane may be administered with either oxygen or a mixture of oxygen and nitrous oxide.

Fluothane should not be kept indefinitely in vaporizer bottles not specifically designed for its use. Thymol does not volatilize along with Fluothane and, therefore, accumulates in the vaporizer and may, in time, impart a yellow color to the remaining liquid or to wicks in vaporizers. The development of such discoloration may be used as an indicator that the vaporizer should be drained and cleaned, and the discolored Fluothane (halothane, USP) discarded. Accumulation of thymol may be removed by washing with diethyl ether. After cleaning a wick or vaporizer, make certain all the diethyl ether has been removed before reusing the equipment to avoid introducing ether into the system.

2.4.3 Advantages and Disadvantages of Halothane

2.4.3.1 Overview:

- a. Halothane causes unconsciousness; however, does not provide adequate analgesia.
- b. Halothane (Fluothane) may not adequately suppress visceral reflexes or provide adequate muscle relaxation for surgery.
- c. Halothane (Fluothane) is associated with reversible reduction in glomerular filtration rates (GFR).

2.4.3.2 Halothane Advantages:

- a. Halothane (Fluothane) is relatively potent and is nonflammable.
- b. This drug's low blood: gas partition coefficient explains both relatively rapid anesthesia induction and recovery from anesthesia

- c. Halothane (Fluothane) can be used to provide controlled hypotension to reduce/manage bleeding.

2.4.3.3 Halothane Disadvantages

- a. Halothane (Fluothane) is associated with unpredictable hepatitis occurrences.
- b. Significant myocardial depression is observed with halothane (Fluothane).¹³

2.4.4 Clinical Indication & System Physiology

Halothane (Fluothane) is effective for general anesthetic maintenance and may be an induction agent of choice in difficult airway

Systems Physiology:

a. CNS:

- 1) Generalized CNS depression
- 2) cerebrovascular dilation causes increased ICP

b. Cardiovascular:

- 1) Halothane (Fluothane) causes a slight decrease in heart rate, a decrease in mean arterial pressure (MAP)-- both associated with its well-documented cardiac depressant property.
- 2) Halothane (Fluothane) may also sensitize the myocardium to circulating catecholamines, explaining the pro-arrhythmogenic property.

c. Pulmonary:

- 1) Halothane (Fluothane) causes decreasing tidal volume with increasing MAC, increasing respiratory rate with increasing MAC
- 2) Halothane (Fluothane) also promote significant bronchodilation

d. Hepatic:

Fulminant hepatic failure (halothane hepatitis): Case history for slides below: "Forty four year old lady had uterine curettage for metrorrhagia. Eight weeks later she had hysterectomy. Three days after hysterectomy she became jaundiced. Seven days later she died in liver failure. Halothane was used as anesthetic in both surgical procedures. The autopsy showed massive liver necrosis. It appears that the liver damage was mediated by an immunoreactive mechanism. Multiple exposures increase the incidence of liver damage.²⁵

e. Mechanism of Hepatotoxicity of halothane

Mechanisms of halothane toxicity are idiosyncratic reaction, immunoallergic type, and the protein adducts formed in the initial toxic reaction provide the haptens for the formation of antibodies which augments damage on re-exposure. Covalent binding of halothane metabolites to tissue proteins has been well described. Up to one-third of inhaled halothane may be oxidatively metabolized by cytochrome P450 2E1 and 2A6. Trifluoroacetyl chloride, which has a strong

ability to acetylate liver proteins, is formed during the synthesis of trifluoroacetic acid. Only a small amount (~%1) of halothane is metabolized through the reductive pathway by cytochrome P450 2A6 and 3A4. This pathway is favored under hypoxic conditions, resulting in the release of bromide and fluoride ions and in the formation of other volatile organic metabolites.²² The release of these ions damage membrane integrity of cells and results in fatty change, acute yellow atrophy and widespread nucleus changes (e.g, enlarge, karyorhexis, karyolysis nucleus) that is indistinguishable from fulminant viral hepatitis.

Several drugs or chemicals were shown to induce hepatitis with autoimmune involvement. Adduct formation of an activated metabolite is believed to act as a trigger and to induce a specific immune response.¹² Two types of halothane –mediated hepatotoxicity have been defined. The first type, type I, is a mild, self-limited postoperative hepatotoxicity, with a mild form of hepatocellular injury that can be observed in about 20% of halothane –treated patients. The mild hepatic injury is assumed to result from the direct action of halothane on the liver cells. Two clinically detectable factors appear to contribute to the mild form of hepatic injury. The first is a transient elevation of liver enzymes and the second is alteration of cellular integrity, which can be detected by electron microscopy. Lesions result from intracellular degradation of halothane via its anaerobic and aerobic pathways in

combination with local hypoxia caused by an alteration of the hepatic oxygen demand and supply relationship. The second type of halothane-mediated hepatotoxicity is type II-halothane hepatitis. The incidence of this type of hepatotoxicity after halothane administration is one case per 10000-30000 adult patients. The probable mechanism is most likely an immune-mediated hepatotoxicity; antibodies are against modified liver microsomal proteins on hepatocyte surfaces.⁶

There is strong evidence that the fulminant form of halothane hepatitis is mediated by the patient's own immune system. Besides signs of mild cellular injury, tissue acetylation is usually found due to the generation of reactive intermediates from halothane metabolism.²⁶ The acetylation of intracellular proteins is considered the first step in the pathogenesis of the severe type of hepatic injury. The second step then involves the formation of antibodies directed towards these acetylated neo-antigens. The consequence of the immune response with regard to hepatic injury is severe and sometimes even fatal, including enlarge, karyorrhexis, and even karyolysis of the nucleus.²² 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.²⁷ Patients with halothane hepatitis or severe hepatic damage to other halogenated anesthetics produce metabolites against several liver trifluoroacetylated microsomal proteins.

There has no data yet showing that these changes are reversible.

Adverse reverse prognostic factors include: serum bilirubin >20 mg/dLb, coagulopathy, older age (> 40 years), obesity, and short duration interval to onset of jaundice.^{21,28}

1. Type I (mild) halothane hepatotoxicity
 - a. Type I occurs within hours of halothane exposure.
 - b. It does not occur after other agents.
 - c. Type I is characterized by mild, transient elevations in serum transaminase and glutathione *S*-transferase concentrations.
 - d. Jaundice is not observed, and no evidence of hepatocellular disease is present.
2. Type II (fulminant) halothane hepatotoxicity
 - a. Type II usually occurs 5-7 days following exposure, although it can be delayed by up to 4 weeks.
 - b. Fever, leukocytosis, and eosinophilia are observed.
 - c. Nonspecific gastrointestinal upset may be noted.
 - d. Nausea and vomiting may occur.
 - e. Patients may report arthralgias.
 - f. Most prominently, the patient looks and feels unwell.
 - g. Fulminant liver failure may ensue.
3. Hepatic dysfunction in the postoperative period has many possible causes. Halothane hepatotoxicity is a diagnosis of exclusion. Other potential causes of liver dysfunction should be considered,

including other hepatotoxic medications, hypotension, hypoxia, and infection.

The phenotype can be distinguished by the actual activity or the amount of the expressed CYP enzyme. The genotype, however, is determined by the individual DNA sequence. Since human has two sets of chromosomes, that mean the same genotype enables different phenotypes. Depending on the metabolic activity (phenotype), three major cathegories of metabolizers are separated: extensive metabolizer (normal), poor metabolizer, and ultra-rapid metabolizer (increased metabolism of xenobiotics). However, until recently there is no published evidence of CYP2E1, CYP2A6, and CYP3A4 genetic polymorfism that correlate with halothane metabolism.²⁹

A genetically determined toxic effect of halothane metabolism is not the only explanation of the fulminant cases of halothane hepatitis.³⁰ The fact that many patients / health worker with severe halothane hepatitis have circulating antibodies directed against other organs in the body strongly suggests an underlying, genetically determined defect in immune regulation. In contrast, some patients with hepatitis from the drug have no evidence of immune involvement, and liver damage in these cases is probably the result of overproduction of a hepatotoxic derivative produced by reductive halothane metabolism.³¹

2.5 Balb/C Mouse

The Balb/c mouse originated in 1923 by McDowell. It is a popular strain and is used in many different research disciplines, but most often in the production of monoclonal antibodies. BALB/c mice are particularly well known for the production of plasmacytomas following injection with mineral oil, forming the basis for the production of monoclonal antibodies.³²

Also classified as an inbred from the production of 20 or more successive brother-sister mating, the Balb/c mouse is albino and small in size.

Strain characteristics related to the Balb/c mouse include:

- Low alcohol preference
- Low incidence of mammary tumors
- Resistance to atherosclerosis development when fed an atherogenic diet.
- High incidence of cardiac calcification, especially in males.
- Overall higher incidence of heart defects and spontaneous myocardial lesions of the right ventricle.
- Generally good breeding performance, with low incidence of barbering.
- Sensitivity to radiation.
- Low incidence of ovarian cysts.
- Moderate hearing loss usually occurs before one year of age.³³

2.6 Personal Protective Equipment

Workers should use appropriate personal protective clothing and equipment that must be carefully selected, used, and maintained to be effective in preventing skin contact with halothane. The selection of the appropriate personal protective equipment (PPE) (e.g., gloves, sleeves, encapsulating suits) should be based on the extent of the worker's potential exposure to halothane. The resistance of a protective material to permeation by halothane is shown below:

Table 3. The resistance of a protective material to permeation by halothane

Material	Breakthrough time (hr)
polyvinyl alcohol	>8
butyl rubber	<1(*)
natural rubber	<1(*)
Neoprene	<1(*)
nitrile rubber	<1(*)
polyvinyl chloride	<1(*)
Viton	<1(*)

Materials with breakthrough times of <1 hr are not recommended for use against halothane.

Any chemical-resistant clothing that is used should be periodically evaluated to determine its effectiveness in preventing dermal contact. Safety showers and eye wash stations should be located close to operations that involve halothane. Splash-proof chemical safety goggles or face shields (20 to

30 cm long, minimum) should be worn during any operation in which a solvent, caustic, or other toxic substance may be splashed into the eyes.

Protective clothing should be kept free of oil and grease and should be inspected and maintained regularly to preserve its effectiveness.³⁴