The Effect of Sevoflurane, Isoflurane and Propofol on Human Cell Apoptosis

Thesis submitted for partial fulfillment of M.D. Degree in Anesthesiology

By

Mohamed Ahmed Maher Mohamed
(M.B; B.Ch, M.Sc Anesthesia)

Supervisors

Prof. Dr. Abdel-Rahman Mahmoud Fathallah
Professor of Anesthesiology
Head of Anesthesiology Department
Faculty of Medicine
Cairo University

Prof. Dr. Ahmed Mounir Shash
Professor of Anesthesiology
Faculty of Medicine
Cairo University

Prof. Dr .Mona Mohamed Hassan
Professor of Clinical &Chemical Pathology
Theodor Bilharz Research Institute

Prof. Dr. Emad Seddik Osman
Professor of Anesthesiology
Theodor Bilharz Research Institute

Faculty of Medicine
Cairo University
2009

Dedication

I would like to dedicate this work to my family specially my ***Father*** and ***Mother*** who supported me through my entire life. I cannot express my gratitude to all they did and still doing to me. Thank you my guarding angels.

Abstract

Apoptosis occurs in various physiological and pathological conditions. It is suggested that surgery and general anesthesia can induce apoptosis. This study compares the effect of isoflurane, sevoflurane and propofol on human cell apoptosis. Sixty patients scheduled for elective open cholecystectomy or recurrent hernia mesh repair were randomly allocated to receive propofol, isoflurane or sevoflurane for maintenance of anesthesia. Evaluating apoptosis was done by measuring caspase-3 activity and TRAIL levels in the three groups. We found that isoflurane is superior to sevoflurane and propofol in protecting against apoptosis.

Key words: Apoptosis, Sevoflurane, Isoflurane, Propofol.

ACKNOWLEDGMENT

“First and Foremost, Thanks are Due to GOD,
The Beneficent and Merciful of All”

I would like to express my deepest gratitude and sincere thanks to ***Prof. Dr. Abd El Rahman Fathallah*** *Professor and Head of Anesthesia Dpt, Faculty of Medicine, Cairo University, for his expertise that he showed in the supervision of both this work and my entire academic work. His continuous encouragement and thoughtfulness were most valuable to me.*

I am greatly indebted to ***Prof. Dr. Ahmed Shash,*** *Professor of Anesthesia, Faculty of Medicine, Cairo University, for his expert guidance, valuable suggestions and excellent supervision.*

I owe much to ***Prof. Dr. Mona Hassan,*** *Professor and Head of Clinical & Chemical Pathology, Theodor Bilharz Research Institute, for planning the design of the work, devoting her intellectual energies and high sense of profession in the entire development of this thesis. offering all help, supervision, patience, guidance and all laboratory facilities throughout the entire work.*

I am truly grateful to ***Prof. Dr. Emad Seddik,*** *Professor of Anesthesia Theodor Bilharz Research Institute, for his valuable support. Without his creative thinking, valuable suggestions and constructive criticism, the performance of this work would have been difficult.*

I would like to express my deepest gratitude and sincere thanks to ***Prof. Dr. Maha Abd El Bar*** *For her support and valuable guidance.*

I am truly grateful to ***Prof. Dr. Gihan El-Fandy,*** *Professor of Anesthesia and President of Theodor Bilharz Research Institute, for her encouragement and kind support.*

Many Thanks to ***Prof. Dr. Hend Kamel,*** *Professor of Anesthesia and Head of Surgical Clinical Board, Theodor Bilharz Research Institute for approving the registration of this thesis and valuable advice.*

I am greatly indebted to ***Prof. Dr. Yasser Samhan,*** *Professor and Head of Anesthesia Dpt, Theodor Bilharz Research Institute for his expert guidance, valuable suggestions and encouragement.*

Many thanks to ***Ass. Prof Dr Hanan Farouk*** *who supported me all the way till this work has been accomplished.*

Many thanks to all staff members of Anesthesia at Theodor Bilharz Research Institute who bore with me the brunt of this work.

I would like to really thank all my family for their infinite support.

Finally, I cannot miss thanking the patients that were the mainstay of the study.

Chapter 1

Apoptosis

Chapter 2

Anesthesia & Apoptosis

List of Tables

List of Figures

List of Abbreviations

- **PCD:** Programmed Cell Death.
- **DNA:** Deoxyribonucleic Acid.
- **FAS:** Apoptosis Stimulating Fragment.
- **TNF:** Tumor Necrosis Factor.
- **Bcl-2:** B cell lymphoma 2.
- **DR5:** Death Receptor 5.
- **TRAIL:** TNF-Related Apoptosis Inducing Ligand.
- **CAD:** Caspase Activated Dnase.
- **ICAD:** Inhibitor of CAD.
- **Bax:** Bcl-2 associated X protien.
- **Bad:** Bcl-2 associated death promotor.
- **B cell:** Bone cell. (as it is formed in bones)
- **T cell:** Thymus cell.
- **ROS:** Reactive Oxygen Species.
- **A-V node:** Atrioventricular node.
- **MODS:** Multiple Organ Dysfunction Syndrome.
- **IL 1b:** Interleukin 1b.
- **LPS:** Lipopolysacharide.
- **ARDS:** Adult Respiratory Distress Syndrome.
- **IGF-1** Insulin-like Growth Factor 1.
- **APC:** Anesthetic Preconditioning.
- **Cox-2:** Cyclooxygenase-2.
- **GABA:** Gamma Amino Butyric Acid.
- **Vd:** Volume of distribution.

- **Compound A:** fluoromethyl-2,2-difluoro-1-[trifluoromethyl]vinyl ether
- **CYP**: Cytochrome P-450.
- **CBF:** Cerebral Blood Flow.
- **BP**: Blood Pressure.
- **HR:** Heart Rate.
- **SVR:** Systemic Vascular Resistance.
- **CO:** Cardiac Output.
- **Vt:** Tidal Volume.
- **RR:** Respiratory Rate.
- **CMRO2:** Cerebral Metabolic Rate Oxygen Consumption.
- **ICP:** Intra Cranial Pressure.
- **RBF:** Renal Blood Flow.
- **HBF**: Hepatic Blood Flow.
- **MAC:** Minimum Alveolar Concentration.
- **Ppm:** Part per million.
- **(CO):** Carbon Monoxide.
- **(CO2):** Carbon Dioxide.
- **ASA:** American Society of Anesthesiologists.
- **BMI:** Body Mass Index.
- **ECG:** Electrocardiograph.
- **CBC:** Complete Blood Picture.
- **FBS:** Fasting Blood Sugar.
- **CNS:** Central Nervous System.
- **NIBP:** Non Invasive Blood Pressure.
- **EtCO2:** End Tidal Capnography.
- **SPO2:** Oxygen Saturation.
- **TOF:** Train Of Four.

- **ELISA:** Enzyme Linked-Immuno-Sorbent Assay.
- **ALT:** Alanin Aminotransferase.
- **AST:** Aspartate Aminotransferase.
- **HB:** Hemoglobin.
- **HCT:** Hematocrite.
- **MAP:** Mean Arterial Blood Pressure.
- **BUN:** Blood Urea Nitrogen.
- **MAP** kinase: Mitogen Activated Protien Kinase.
- **FCM:** Flow Cytometry.
- **mRNA:** Messenger Ribonucleic Acid.
- **ICE:** Interleukin Converting Enzyme.
- **LDH:** Lactate Dehydrgenase.

History

In the 1880s Weigert & Cohnheim described the microscopic appearance of cell death in necrotic tissue as coagulation necrosis **[1]**. In 1885, Flemming described the process of `chromatolysis' in which the nuclei of mammalian ovarian follicles broke up and ultimately disappeared in spontaneous cell death **[2]**. In the early 1970s, Kerr described the electron microscopic appearance of singe-cell death in the livers of animals **[3]** treated with toxins (heliotrine and albitocin) and ischaemia (by ligation of a large branch of the portal vein) and called it `shrinkage necrosis' **[4]**.

A landmark paper in 1972 described the characteristic sequential changes occurring in cell structure during the death process in healthy tissues, normal development, tumors regression, atrophy and involution **[5]**. The term `apoptosis' (derived from the Greek word for `falling off ', a reference to the falling of leaves from trees in autumn in response to the impending threat of freezing and damage in winter) was coined **[5]**. During the 1990s, the molecular mechanisms that keep apoptosis in check and the molecular events involved in disordered apoptosis were unravelled. **[6]**

Definitions

Apoptosis specifically refers to an energy-dependent, asynchronous, genetically controlled process by which unnecessary or damaged single cells self-destruct when the apoptosis genes are activated **[7]**. Briefly, the cell shrinks and detaches from neighbouring cells and the nucleus is broken down. The nuclear fragments and organelles condense and are ultimately packaged in membrane-bound vesicles, exocytosed and ingested by surrounding cells. The absence of inflammation differentiates apoptosis from

necrosis. The differences between apoptotic and necrotic cell deaths are summarised in Table 1. **[8]**

Ischaemic and accidental (caused by toxins) cell death are characterised by cell swelling or `oncosis' resulting in cytoplasmic and nuclear swelling and karyolysis (loss of affinity for basic dyes). After either oncosis or apoptosis, cells reach the stage of necrosis where phagocytosis occurs, and in the case of oncosis, this is accompanied by inflammation. Apoptosis and oncosis/necrosis are part of an overlapping spectrum. Cells exposed to an overwhelming noxious stimulus, such as ischaemia or toxins, undergo oncosis and necrosis **[9]**. Programmed cell death (PCD) refers to cell suicide occurring during normal embryological development of an immature organism and the maturation of tissues or organs and does not require de novo gene expression **[10]**.

Table (1) Differences between Apoptosis and Necrosis [7]

Apoptosis	***Necrosis***
Physiological or pathological	Always pathological
Asynchronous process in single cell	Occurs synchronously in multiple cells
Genetically controlled	Caused by overwhelming noxious stimuli
Late loss of membrane integrity	Early loss of membrane integrity
Cell shrinkage	Generalized cell and nucleus swelling
Condensation of nuclear contents	Nuclear chromatin disintegration
No inflammatory reaction	Inflammatory reaction

Mechanism of Apoptosis

Following an appropriate stimulus, the first stage or *`decision phase'* of apoptosis is the genetic control point of cell death. This is followed by the second stage or *`execution phase'*, which is responsible for the morphological changes of apoptosis. **[1]**

A. Stimuli for Apoptosis

- DNA (genome) damage by ionizing radiation and anti-cancer drugs.
- Activation of death receptors (Fas receptor, TNF receptor) e.g. drugs and glucocorticoids acting on thymus.
- Direct physical cell damage by heat, ultraviolet light, oxygen free radicals, hydrogen peroxide.
- Biochemical agents that enhance the downstream components of the apoptotic pathway e.g. phosphatases and kinase inhibitors (Calphostin C, Staurosporine). **[11]**

N.B. Many of these stimuli cause necrosis in large doses. **[11]**