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Intra-abdominal Hypertension and Abdominal Compartment Syndrome

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ABSTRACT

Intra-abdominal hypertension (IAH) and abdominal compartment syndrome (ACS) are increasingly recognized in critically ill patients; no non-operative treatments exist, and mortality remains high.

The purpose of this thesis was to prospectively characterize the incidence of IAH in a mixed medical-surgical intensive care unit, and to test the potential therapeutic benefit of carbon monoxide (CO) and hydrogen sulphide (H_2S) using an animal model of ACS. IAH was diagnosed in 30% of patients on admission; further 15% developed IAH during ICU stay. Incidence of ACS was 3%, with obesity, sepsis, mechanical ventilation and 24-hour fluid balance as independent predictors, also predicting ICU mortality. In a rat model of ACS, CO and H_2S were found to improve ACS-induced microcirculatory dysfunction, inflammation, cell death and overall organ dysfunction.

IAH incidence in the critically ill is high, with its correction possibly reducing mortality. CO and H_2S show promise in animal model of ACS, as potential therapeutics.

Keywords: *intra-abdominal hypertension, abdominal compartment syndrome, carbon monoxide, hydrogen sulphide, CORM-3, GYY4137, hepatic microcirculation*

THE CO-AUTHORSHIP

While each of the co-authors listed below made important contributions to this work, I am the principal author who designed all the projects, performed all of the experimental design, data collection and analysis. All manuscripts presented in this thesis were prepared by me, with the consultation and critical review by the co-authors.

Kelly Vogt, MD, MSc, FRCSC; **Neil Parry, MD, FACS, FRCSC** in their role as my research co-supervisors, provided direction and guidance on data interpretation, relevant literature and manuscript revisions. Both were instrumental in the review and publication of each chapter.

Ian Ball, MD, MSc, FRCSC in his role as committee member provided exceptional feedback and direction as well as critical analysis of data and content in each chapter.

Abdel-Rahman Lawendy, MD, PhD, FRCSC, provided leadership and mentoring on the project, offering direction and guidance on data interpretation for Chapter 3.

Aurelia Bihari, MSc taught me all of the experimental techniques used in Chapter 3 and assisted with model development, animal setup, data collection, analysis and much needed technical support, manuscript editing and publishing. She has been paramount to the success of the presented work.

Nathalie Sela, MD assisted with data collection for Chapter 2.

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LIST OF ABBREVIATIONS

AAA, abdominal aortic aneurism

ACS, abdominal compartment syndrome

ALT, alanine transaminase

AST, aspartate aminotransferase

AlkPhos, alkaline phosphatase

BMI, body mass index

BUN, blood urea nitrogen

CCTC, critical care trauma centre

cGMP, cyclic guanosine monophosphate

CI, confidence interval

CLP, caecal ligation and puncuture

CO, carbon monoxide

CO_2, carbon dioxide

COHb, carboxyhemoglobin

CO-RMs, carbon monoxide-releasing molecules

CORM-3, carbon monoxide-releasing molecule-3

CPS, continuously perfused sinusoids

ERK, extracellular signal regulated protein kinase

GC, guanylate cyclase

HO, heme oxygenase

HO-1/2/3, heme oxygenase type-1/-2/-3

HSREB, health sciences research ethics board

H_2S, hydrogen sulphide

IAH, intra-abdominal hypertension

IAP, intra-abdominal pressure

ICP, intra-cranial pressure

ICU, intensive care unit

IL-1β, interleukin-1 beta

IL-6, interleukin-6

IL-10, interleukin-10

IPS, intermittently perfused sinusoids

IQR, inter-quartile range

I/R, ischemia-reperfusion

JNK, c-Jun NH_2-terminal protein kinase

MAP, mean arterial pressure

MAPK, mitogen-activated protein kinases

MOD, multi-organ dysfunction

MODS, multi-organ dysfunction syndrome

MPO, myeloperoxidase

NADPH, nicotinamide adenine dinucleotide phosphate (reduced)

NF-κB, nuclear factor kappa B

NO, nitric oxide

NOS, nitric oxide synthase

NPS, non-perfused sinusoids

PBS, phosphate-buffered saline

PEEP, positive end-expiratory pressure

ROS, reactive oxygen species

SAPS, simplified acute physiology score

SD, standard deviation

sGC, soluble guanylate cyclase

SOFA, sequential organ failure assessment

STROBE, strengthening of reporting of observational studies in epidemiology

SVR, systemic vascular resistance

TNF- α, tumor necrosis factor-alpha

WSACS, World Society of Abdominal Compartment Syndrome

CHAPTER 1

INTRODUCTION AND HISTORICAL REVIEW

CHAPTER 1: INTRODUCTION AND HISTORICAL REVIEW

1.1 INTRA-ABDOMINAL HYPERTENSION AND ABDOMINAL COMPARTMENT SYNDROME

Intra-abdominal hypertension (IAH) and abdominal compartment syndrome (ACS) are increasingly recognized clinical entities in the critically ill patient. IAH is likely more common than previously established and it is unknown whether IAH is a sign of illness severity or rather a disease that, when treated, will improve patient outcomes.

1.1.1 Overview and History

The history of IAH and ACS is one typical of many topics in medicine. An ebb and flow of use, development, and perception, falling in and out of favour with eventual permanence in practise or a complete fall from favour as the final value of a measurement, technique or therapeutic is decided upon (Figure 1.1) (1).

The first description of the effects of IAP dates back to at least the mid- to late 1800s. Commenting on respiration, Marey described the inverse relationship between thoracic excursion and abdominal movement, although no actual measurements were made (2). Dr. Matthews Duncan, an obstetrician, described the influence of the abdominal cavity and “the retentive power of the abdomen,” acknowledging the abdomen as a compartment that when violated loses influence over abdominal organs (3). In Germany, Braune successfully measured

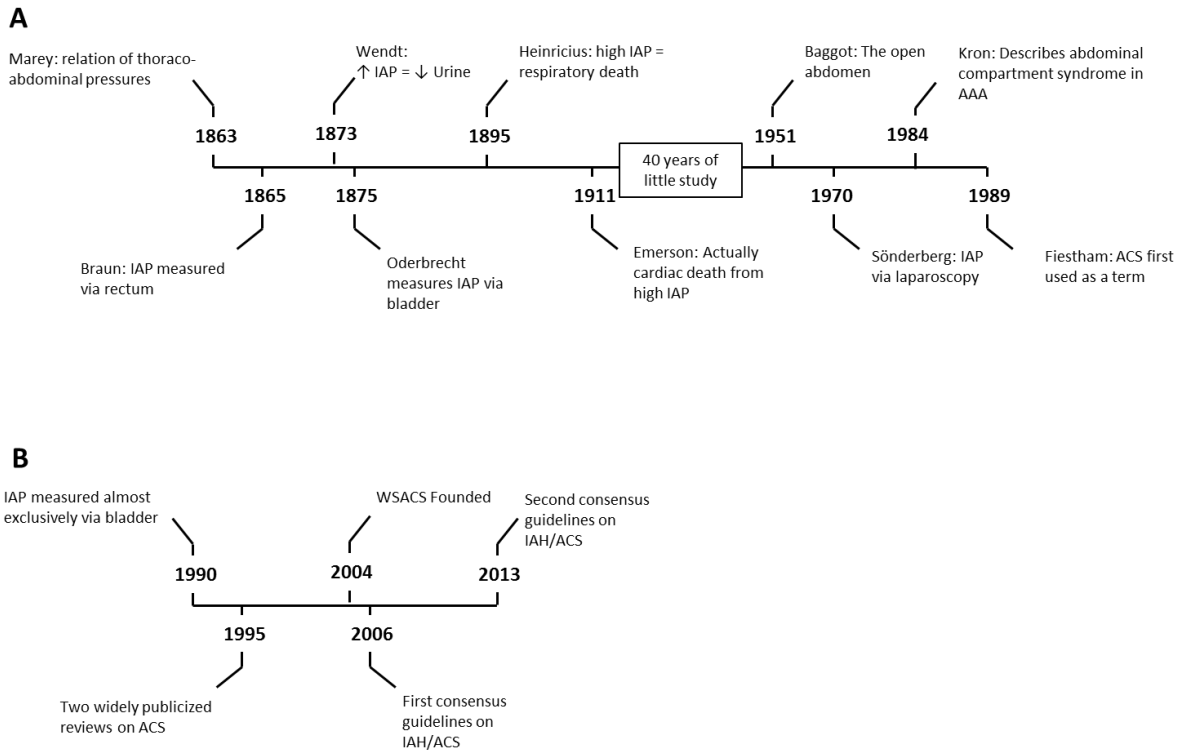

Figure 1.1. Historical timeline of IAH/ACS diagnosis.

IAP via the rectum, by inserting a glass tube connected to a graduated cylinder. After injecting water into the rectum, he measured the column of water at 40cm, and found minimal variation between individuals regardless of the amount of water injected. However, the amount of pressure was increased when participants performed a Valsalva manoeuvre, and was reduced by supine positioning (4). Rapid recognition of the IAP and advancement of measurement techniques and improved understanding of the effects of altered IAP on normal physiology followed. Before the turn of the 19th century, the IAP could be measured via the bladder (5); it was recognized that high pressures resulted in reduced urine output and decreased venous return. In animals, pressures of 27-46cm H_2O were found to be fatal, due to impaired respiration, decreased cardiac distension and low blood pressure (6). In 1911, Haven Emerson (7) re-affirmed Marey's original observations: using a canine model, Emerson demonstrated that contraction of the diaphragm resulted in an elevation of IAP, and that anaesthesia and neuromuscular blockade decreased IAP. Furthermore, he demonstrated a cardiac aetiology (rather than respiratory a one) in the setting of significant IAP which ultimately led to death (7).

For the following 40 years, IAP and IAH were not well studied. An anaesthetist, Dr. Baggot (8), attributed elevated IAP to the high mortality following emergency surgery for wound dehiscence. Based on his observations, he hypothesized that it was the closure under tension, rather than the dehiscence, that led to increased IAP and, ultimately, death. Additionally, he

suggested leaving the abdomen open; a technique developed by Ogilvie during World War II (8,9).

Through the 1960s and 70s, much of the work related to IAP and IAH was related to ascites and laparoscopy (10–14). It was during this time that non-surgical management of elevated IAP, through paracentesis, was demonstrated (11). While the term 'abdominal compartment syndrome' was not formally used to describe a clinically evident elevation in IAP until 1989, already in 1984 Kron *et al* (15) described the "landmark" clinical and animal study around what is now called ACS. The authors described four patients undergoing abdominal aortic aneurysmal repair who progressed to medically refractory anuria (15). Upon decompression, an immediate output of urine was noted, even without identification of any intra-abdominal pathology. The single patient who did not undergo decompression died, while the other three survived to discharge. Early experiments on canines by the same authors suggested that an IAP of 20mmHg reduced renal function. This process was unresponsive to cardiac output augmentation (16,17). Kron *et al* (15) measured IAP as high as 40 and 77 mmHg in 10 post-operative patients. Four of the seven anuric patients with IAP > 25mmHg received decompression and diuresied immediately. The conclusions of the study form the underpinning of ACS.

Interestingly, around the same time, Smith *et al* (18) reported the case of a woman who received a decompressive laparotomy for anuria post duodenal ulcer repair and diuresed immediately following decompression. The authors go insofar as to compare the decompression to limb compartment syndrome and

fasciotomy. The authors did not believe that IAP measurements, if available, would have been helpful. Instead the diagnosis was made on the basis of relatively normal hemodynamics and anuria (18).

At the end of the 1980s, IAP measurements continued to be described in the intensive care unit population. At the time, measurements were done using the bladder exclusively, using higher instillation volumes and the pubic symphysis as a zero reference point, resulting in pressures much higher than those seen today (19). Fietsam *et al* (20) coined the term 'abdominal compartment syndrome', after describing bedside laparotomy for decompression due to ruptured AAA repair in four patients. All of the patients had received >25L of blood and fluid, and a Marlex mesh had been placed at the time of decompression. In the mid-1990s, two reviews were published on IAH/ACS, followed by an ever-growing body of literature describing the causes, risk factors and pathophysiology of IAH/ACS (21,22). IAH and ACS have expanded beyond the post-operative patient population to include virtually all critically ill medical, trauma and surgical patients.

1.1.1.1 Modern History – The World Congress

The key developments in the 2000s arose largely due to increasing recognition of IAH/ACS as a common, yet under-recognized, phenomenon and physiologic sequelae of the pneumoperitoneum of laparoscopy. Further, "damage control" surgery, as well as the concept of the open abdomen and abdominal decompression became more popular. The creation of the World

Society of the Abdominal Compartment Syndrome (WSACS) in 2004 improved recognition of IAH/ACS by intensivists. The WSACS is an organization headed by a group of international physicians and surgeons, with a mandate to promote IAH/ACS research and education. The society accomplishes these goals by hosting an international clinical congress every 2-3 years. The Society also produces evidence-based algorithms defining patient populations that benefit most from regular IAP measurements, thereby providing a standardized approach to prevention and treatment.

The first consensus guidelines from the WSACS were released in 2006 (23), followed by a 2013 update (24). Additionally, clinical practice guidelines and recommendations for research were released in 2007 (25) and 2009 (26), respectively . Perhaps the most important results were the guidelines that recommend a common technique for IAP measurement. The expert panel established the modified Kron technique as the preferred method for measuring IAP. Prior studies may have had falsely elevated IAP measurements due to a higher instillation volume (50ml versus the current practice of 25ml), and a zero-point reference of the symphysis pubis rather than the mid-axillary line.

The consensus guidelines suggested screening for IAH/ACS in an intensive care unit population if risk factors were present on admission. Any patient admitted to the intensive care unit with two or more of the risk factors listed in Table 1.1 should have a baseline IAP measurement. However, upon

Table 1.1. Risk factors for the development of IAH.

Adapted from Holodinsky *et al* (27) and WCACS consensus guidelines (24).

Category	Risk Factor	Odds Ratio
Demographics	Obesity	5.1
	Age (per year)	2.8
Diagnosis	Sepsis	2.4
	Intra-abdominal infection	2.5
	Abdominal surgery	1.9
	Pancreatitis	4.7
	Liver failure	2.1
	Gastro-intestinal bleed	3.4
	Ileus	2.1
	Respiratory failure	1.9
	ARDS	3.6
Disease Severity	APACHE II Score (per point)	1.7
	Base deficit	1.2
	Acidosis	1.9
	Shock	4.7
	Hypotension	2.1
	Massive resuscitation	2.2
	Positive fluid balance (per litre increase)	5.2
Other	Mechanical ventilation	6.7
	PEEP >10cm H_2O	2.4

examination of the risk factors present, virtually every intensive care unit patient meets at least two criteria. Additionally, the criteria themselves are poorly defined. The lack of high-quality and rigorous literature on which to generate evidence-based guidelines for the measurement and management of IAH/ACS is one of the recognized drawbacks. This is further highlighted by recent meta-analysis and systematic reviews (27–29) attempting to characterize the prevalence and risk factors for IAH/ACS: significant heterogeneity exists, with respect to definitions of IAH, measurement techniques and patient populations studied.

1.1.2 Definition of IAH

IAH is the sustained or repeated pathological elevation of intra-abdominal pressure (IAP) to ≥12mmHg, as defined by the WSACS consensus guidelines (24,25). An acknowledged limit of this definition is establishing the definition of "sustained". A single measurement over 12mmHg may not be clinically relevant if all other measurements are less than 12mmHg. Similarly, it is unknown whether maximal, mean or median IAP should be used to determine the presence or absence of IAH. Total time spent above a certain IAP may have the most significance clinically, but continuous IAP measurements have not been well studied, or likely to be practical. This has led to significant heterogeneity in the published literature: many definitions of IAH are used and this is compounded with evolving IAP measurement techniques. This is particularly evident prior to

consensus guidelines in 2006 (when the new instillation volume and reference point were adopted).

1.1.3 Epidemiology of IAH

The incidence and prevalence of IAH are challenging to determine, for a number of reasons. First, IAH is an acute and usually transient disease, applicable to a very specific population of patients who are critically ill. Second, the incidence (the number of new cases of IAH during the *time* period of the study) is not to be confused with incidence rate (the number of new cases of IAH in person-years). Incidence assumes each patient is at risk for the same period of time.

Prevalence is typically used to describe point prevalence. For example, the point prevalence would be the number of patients who had IAH at a specific date and time of the year, not to be confused with period prevalence (the number of patients who had IAH at any time during the study period). Prevalence is the sum of the point prevalence at the beginning of the interval, plus the incidence over the time interval. Thus, the best measure is the number of new cases of IAH, with the assumption that no patients had IAH at the beginning of the study period. This measure is equivalent to incidence or period prevalence.

Finally, the definition of IAH used can greatly impact the reported incidence. Videl *et al* (30) adhered to reporting guidelines established by the WSACS and found 31% of patients had an IAP ≥12mmHg on admission, 54%

had IAP_{mean} ≥12mmHg over the study period and 61% had IAP_{max} ≥12mmHg over the study period.

The incidence of IAH has been described in a wide subset of discrete patient populations, with ranges from 20 to 80% depending on the study population in question, and technique for measuring IAP (31–33). When considering only studies in consecutive mixed-medical intensive care unit patients using the modified Kron technique, the incidence of IAH ranges from 30 to 61% (Table 1.2).

Although most studies do not include a measurement of error, standard error can be calculated. Combining the results of the four published studies using appropriate methods (34), the incidence of IAH is 40% (95% CI: 33%-47%). This is much higher than that cited in a more recent literature, in populations deemed high-risk (31). In a retrospective study Blaser *et al* (31) demonstrated that increased screening for IAH was not associated with increased detection of IAH (20%). The largest prospective study in a mixed medical-surgical ICU to-date is out of Australia. Iyer *et al* (35) included 403 consecutive admissions, with a reported incidence of 39%; IAH was defined as two consecutive readings of ≥12mmHg. The results however may lack generalizability since the majority of admissions were cardiothoracic surgery patients and overall mortality was 11%.

Risk factors for the development of IAH have largely been established based on retrospective studies, a lack of clear definitions, or the use of heterogeneous measurement techniques (27). Additionally, both the size and characteristics of the populations have been variable. The majority of studies

Table 1.2. Prevalence of IAH in modern mixed ICU populations.

Study	Population	Type	Size	IAH Definition	Incidence (±2 SE)
Iyer *et al* (2014)	Mixed Medical/Surgical	Prospective	403	IAP≥12mmHg in 2 consecutive measurements	39±5%
Kim *et al* (2012)	Mixed Medical/Surgical	Prospective	100	IAP_{max}≥12mmHg	42±10%
Dalfino *et al* (2008)	Mixed Medical/Surgical	Prospective	123	IAP≥12mmHg in 2 consecutive measurements	30±8%
Videl *et al* (2008)	Mixed Medical/Surgical	Prospective	83	IAP_{max}≥12mmHg IAP_{mean}≥12mmHg IAP_{max}≥12mmHg (Admission)	64±10% 54±10% 31±10%
		Total	709	–	40±7%†

IAP, intra-abdominal pressure; *SE*, standard error

† Calculated using random-effects modeling (I^2=71%); SE = $\sqrt{\frac{p \times (1-p)}{n}}$: not reported but calculated for this table

report on a specific patient population, such as trauma, mechanically ventilated patients, and those deemed high risk of developing IAH (27,28,31). Few studies include a sample size or power calculations.

Whether or not IAH is an independent risk factor for mortality is controversial. Some literature supports the notion of IAH as an independent predictor (30,36–38), while some studies show no association (35,39,40). In a meta-analysis of individual patient data from 1669 patients, Malbrain *et al* (28) found an IAH incidence of 27%, using the mean of daily IAP and a cutoff of 12mmHg. Furthermore, the results suggest that IAH was an independent predictor of mortality, in addition to elevated sequential organ failure assessment (SOFA) score, simplified acute physiology score (SAPS) II score, and a surgical admission.

The current body of evidence suggests IAH is common in critically ill patients, particularly trauma, as well as post-operative patients. Other than data from a single modern study, the incidence of IAH in a mixed-medical ICU is not known. Further, methodologically rigorous study is required to establish both the true incidence of IAH, and clinically important and measurable risk factors for the development of IAH.

1.1.4 Risk Factors for the Development of IAH

The risk factors for the development of IAH have been reviewed systematically and are numerous (Table 1.1) (27). The WSACS describes a lengthy list of risk factors, broadly categorized as those relating to diminished

abdominal wall compliance, increased intra-luminal contents, increased intra-abdominal contents, capillary leak/fluid resuscitation and others (24). Many of these are limited, however, by the amount and quality of evidence available. The majority of risk factors come from studies of a single small study with less than 150 patients (30,41). The definitions of the described risk factors also vary substantially.

The larger studies investigating risk factors have been performed in specific patients cohorts such as trauma patients, patient with pancreatitis, or only those who were mechanically ventilated (24,27). This limits external validity of the data (42). When considering consecutive mixed medical-surgical ICU patients, the best predictors for the development of IAH have been established by Iyer *et al* (35). Multivariable regression analysis showed that elevated lactate, high SOFA score, massive fluid resuscitation, obesity, hemoperitoneum and abdominal distension were all predictive of developing IAH on multivariate regression. The finding of abdominal distension as a predictor is relevant, given that physical exam is often quoted as unreliable as a predictor of IAH (43,44). While the WCACS recommends measuring IAP in patients with at least two risk factors, Iyer *et al* found this to be only 45% specific. In patients with three or more risk factors, sensitivity was 75% and specificity was 76%. If applied to every ICU admission, almost all patients would meet criteria for the routine measurement of IAP; however it is clearly not important in all patients (31).

Risk factors for the development of ACS have been poorly studied outside of the trauma population (27,45,46). The commonly cited risk factors include

severe organ derangements including oliguria, massive blood transfusion, hypothermia and elevated APACHE II score (27).

Given the number of risk factors for the development of IAH, some physicians advocate surveillance in nearly all patients in an ICU (47). While the 2013 consensus guidelines suggest patients with two or more risk factors in the intensive care unit undergo surveillance for IAH with regular IAP monitoring every 4 hours, the frequency of measurement is not evidence-based (24). The recommendation is made, in part, due to the inexpensive nature of IAP monitoring and the limited harm to the patient. Although there is a theoretical risk of higher urinary tract infections, this has not been shown even in large cohorts (48). It still remains to be established, however, whether increased surveillance leads to a decrease in mortality (31,42,47).

1.1.5 Clinical Features and Diagnosis

The clinical features of IAH are subtle and this has led to the recommendation of surveillance for patients deemed at high risk of developing IAH (24). Indeed, given the breadth of patient cohorts in which IAH/ACS has been described, including both surgical and non-surgical, it is understandable why high suspicion is required to accurately detect IAH. The IAP that causes end-organ dysfunction depends on the individual patient, abdominal wall compliance and disease process (49). Similar to cerebral perfusion pressure, the abdominal perfusion pressure [Mean Arterial Pressure (MAP) – IAP] has been suggested as a method to assess the adequacy of blood flow and the severity of

IAP and may be a relevant end-point for resuscitation (50). An IAP of 15mmHg has been shown to have negative influence on perfusion and organ dysfunction, and reflects laparoscopy settings during surgery (51). Table 1.3 outlines the grading scheme of IAP, where normal is defined at less than 12mmHg. In a non-critically ill patients, normal values are as high as 7 mmHg (49).

The current practices of most ICUs are quite variable (31,52). Compared to IAP measurements, the clinical exam has unreliable sensitivity and specificity (43,44), and, therefore, unless clinicians are aware of IAH/ACS as a common entity in critically ill patients, it may be missed.

The modified Kron technique is the most accepted method of measuring IAP, and is recommended by the consensus guidelines (24). The technique uses an existing indwelling urinary catheter to transduce the pressure of the bladder, which accurately measures IAP. The measurement is very reproducible and requires minimal training to perform. Briefly, the urinary catheter is completely drained, and then cross-clamped. A pressure transducer is attached proximally and placed at the mid-axillary line with the patient supine. Twenty-five millilitres of sterile normal saline is then injected. The IAP measurement is taken at end-expiration to ensure relaxation of the abdominal wall; no routine paralysis is required (53).

When diagnosing elevated IAP, it is important to reflect on factors other than relevant pathology that may cause abnormal elevation. Pain, agitation and accessory muscle breathing may increase thoraco-abdominal tone and produce high, non-pathologic elevated IAP measurements. Neuromuscular blockade is, in

Table 1.3. Grades of intra-abdominal hypertension.

Grade	IAP (mmHg)
I	12-15
II	16-20
III	21-25
IV	>25

fact, a recommended treatment for elevated IAP (54). Body positioning, including elevation of the head of the bed above 20 degrees, can increase IAP. This is an important consideration in patients exhibiting elevated IAP, who are being treated for head injuries or severe lung disease.

Questions still remain with regards to exactly which patients would benefit from routine surveillance for IAH with regular IAP. In addition, the frequency of measurements still remains to be agreed upon (31,47).

1.2 PAHTOPHYSIOLOGY OF IAH

The abdomen is a closed compartment, contained by fixed and flexible boundaries. The spine, pelvis and costal margin are relatively inflexible, while the abdominal wall and diaphragm are comparatively elastic.

1.2.1 Intra-Abdominal Pressure

Given the primarily fluid nature of abdominal contents (49), the abdominal compartment generally conforms to Pascal's established principles. The pressure exerted on the abdominal boundaries will be directly related to the intra-abdominal volume (IAV) and the compliance of the abdominal wall. Similar to other compartments, the pressure increases exponentially at a critical volume (55). Further, the pressures, but not the curve shape, are influenced by abdominal wall compliance, with low abdominal wall compliance leading to higher pressures at the same volume (56).

While the abdominal wall is the most compliant boundary, the diaphragm does have elasticity and is dynamic. During the respiratory cycle, the diaphragm relaxes and contracts, generating IAP variability. The nadir of pressure during this cycle is at end-expiration, making it important to consider when IAP is measured (55).

Much of the research surrounding the effects of elevated IAP on human pathophysiology has been gained from studies of laparoscopy (51,57,58), or hypothesized from more controlled and rigorous animal studies (59–62). Laparoscopic abdominal surgery requires a pneumoperitonium. In adults, the pneumoperitoneum is established with CO_2 gas at a pressure of 15mmHg. At this value, there is an increase in systemic vascular resistance (SVR) and a decrease in cardiac output proportional to the IAP (51). The mean arterial pressure is increased as the SVR increase overcomes the decrease in cardiac output. Pneumoperitoneum is generally well tolerated in healthy patients, and the rise in IAP occurs only for the duration of surgery. This is significantly different from elevated IAP in a critically ill patient with other physiologic derangements, particularly hypovolemia and elevated intrathoracic pressures from positive pressure ventilation (58,63,64). The effects of elevated IAP on different organ systems are outlined in Table 1.4, and are further discussed in Appendix II.

Table 1.4. Organ effects of elevated IAP.

System	Effects
Cardiac	Decreased ventricular end-diastolic volume
	Increased afterload
	Reduced preload through compression of IVC, SVC and portal vein
	Reduced CO
Respiratory	Impaired diaphragmatic function
	Reduced pulmonary compliance
	Pulmonary vasoconstriction
	Increased intra-thoracic pressure
Gastrointestinal	Splanchnic hypoperfusion
	Bacterial translocation
	Bowel ischemia
Liver	Reduced arterial and venous flow
	Cellular dysfunction
Renal	Reduced arterial flow
	Reduced glomerular filtration
	Oliguria
Neurologic	Elevated intra-cranial pressure

1.2.2 Ischemia-Reperfusion (I/R) and Inflammatory Injury in IAH/ACS

Elevation of IAP reduces blood flow to abdominal organs, producing tissue hypoxia (51). The reduced oxygenation leads to an increase of intracellular calcium, which is responsible for upregulation of many downstream pathways, including those related to cellular necrosis (65). Necrotic cells release their contents; once they enter systemic circulation, activation of the transcription factors responsible for the upregulation of pro-inflammatory cytokines and chemokines results (66). This triggers leukocyte activation cascade, characterised by rolling, eventually progressing to firm adhesion and extravasation from the circulation into the affected tissue (67). Each step is mediated by the differential expression of adhesion molecules, both on the leukocyte and endothelium. As a result, the endothelial barrier becomes leaky, leading to the formation of edema, which itself contributes to further tissue damage. In parallel, hypoxia also triggers activation of apoptotic pathways (a process mediated by various caspases), further contributing to an increase in overall cell death.

While the initial ischemic insult leads to deleterious cellular effects, the sudden reperfusion of tissue also carries harm at the cellular level, via the production of reactive oxygen species (ROS). The lipids in cellular membranes are vulnerable to peroxidation by these highly-reactive entities, leading to microvascular changes, which can precede macroscopic organ damage (68). Morris *et al* had suggested that the reperfusion injury following abdominal decompression is a significant contributor to mortality in ACS patients (69).

ROS are free radicals that trigger leukocyte recruitment, and can lead to tissue injury through neutrophil activation (70). Neutrophils are polymorphonuclear granulocytes, containing various proteolytic and ROS-producing enzymes (myeloperoxidase (MPO), elastase, proteases, peroxynitrites). Activated neutrophils release the granules during oxidative burst, which can further compound ischemia-induced tissue damage. Different organ systems have varying susceptibilities to I/R injury; for example, the small bowel seems to be more susceptible to I/R than the large bowel (71).

The induction and deflation of pneumoperitoneum appears to represent an I/R injury model in humans. It has been demonstrated that post-operative laparoscopic cholecystectomy patients have elevated liver enzymes. Additionally, there is evidence of ROS-induced lipid peroxidation after deflation of the pneumoperitoneum (72). In a systematic review of the oxidative stress, as it pertains to pneumoperitoneum, there was agreement that pneumoperitoneum decreased splanchnic blood flow in a pressure- and time-dependent manner (73). It is unlikely, however, that this effect is entirely due to the pneumoperitoneum, since open operations also induce oxidative stress. Further, the pneumoperitoneum associated with surgery is considerably different from that of the pathologic rise in IAP leading to IAH/ACS; these are less controlled, and in generally unwell patients, coupled with many pathophysiologic derangements (such as hypotension and mechanical ventilation) (58,63,64).

1.2.3 From IAH to ACS

The aforementioned pathophysiology is evident and exacerbated in abdominal compartment syndrome. The first typical clinical sign is oliguria in an adequately resuscitated patient, progressing to additional intra-abdominal and systemic effects (15). As a result, the patient will become more unstable, and if untreated, will die. The incidence of ACS is quite variable depending on the patient cohort: in a mixed medical and surgical population, ACS affects at least 2-3% of patients and has a mortality of approximately 80%, even with surgical decompression (23). Beyond prevention, the only current treatment option available is decompressive laparotomy to release the abdominal pressure and allow organ perfusion, often performed at the bedside. Urine output will increase almost immediately after decompression (74). Unfortunately, the ensuing I/R injury is significant, and may be responsible for the observed high mortality rate (69).

Intra-abdominal organs, including the liver, kidney and bowel, will have experienced a significant reduction in the blood flow for a period of time. The sudden increase to nearly normal flow may result in significant organ dysfunction (5,62,75–77). This pattern has been observed in several other models of compartment syndrome (CS), particularly that in the extremities. In acute limb CS, fasciotomy to release the compartment pressure often results in a severe reperfusion injury (78) which can be more substantial than the ischemic insult (69).

While it is likely that a patient cohort may be at particularly high risk of progression to ACS, the incidence is relatively lower than that of IAH, and thus has been less studied (27). In the absence of treatment, not all patients who develop IAH progress to ACS; it remains a challenge to determine who is at the highest risk and who should undergo regular surveillance, since this is the most reliable approach to early detection of ACS. Some researchers advocate for early abdominal decompression, as the management of the open abdomen has progressed significantly in recent years (79).

1.3 THERAPEUTIC APPROACHES TO IAH AND ACS

As previously mentioned, there is disagreement in the literature on whether IAH is an independent predictor of mortality (30,35–40). The 2013 consensus guidelines recommend efforts or protocols to avoid sustained IAH, compared to inattention to IAP (24). Non-operative management strategies are both preventative and therapeutic. While a number of measures have been suggested, there have been no studies comparing treatments or demonstrating a reduction in mortality, with all recommendations based on low-grade evidence (24). Preventative measures include increased awareness, surveillance of those at risk, and using protocols for management of IAH/ACS. Cheatham *et al* prospectively studied nearly 500 patients with an open abdomen, managed with a protocoled approach based on the 2006 consensus guidelines (23). This resulted in an increase in, adjusted, survival to discharge compared to a historical control (79).

Other treatment options are outlined in Table 1.5, with the goal of any treatment for IAH/ACS being a reduction of the IAP, in order to restore optimal perfusion to organs. Given the nature of critically ill patients, this may represent a challenging goal, particularly with respect to fluid management and the appropriate response to the pathology accountable for the patient's condition (80).

1.3.1 Surgical Decompression

If end-organ failure occurs in the setting of elevated IAP, prompt surgical decompression is warranted (24). While the procedure has the effect of restoring organ blood flow, it is not without significant morbidity. Further, mortality remains high even with decompression. Definite abdominal closure can be challenging, and is associated with significant long-term complications (80).

1.3.2 Pharmacological Approaches

Although no study has compared potential therapeutics for IAH/ACS in humans, a number of pharmacological therapies have been tested in animal models of IAH/ACS. Some have shown to be effective, including doxycycline (81), minocycline (82), dopamine (76) and octreotide (83). In animal models of limb CS and I/R, gas-releasing molecules have shown therapeutic potential, by reducing the levels of tissue injury and microvascular dysfunction associated with these conditions (84,85).

Table 1.5. Treatment of IAH/ACS.

Intra-Luminal Contents	Space Occupying Lesions	Abdominal Wall Compliance	Fluid/Organ Perfusion
Naso/oro-gastric tube and/or rectal tube	Ultrasound or CT to identify lesions	Adequate sedation and pain control	Goal directed resuscitation
Prokinetic agents	Percutaneous drainage	Remove constrictive dressings	Hemodynamic monitoring
Limit enteral nutrition enemas	Surgical evacuation of lesions	Reverse Trendelenberg neuromuscular blockade	Consider hemodialysis
Colonoscopic decompression			

1.3.3 Gasotransmitters and Gas Releasing Molecules

Gaseous signalling molecules, or gasotransmitters, are a relatively new family of molecules. The term gasotransmitter was first introduced by Wang in 2002, to describe the cellular actions of nitrous oxide (NO) and carbon monoxide (CO) (86). Wang further suggested the candidacy of hydrogen sulphide as a gasotransmitter, which has since been confirmed (86,87). The concept of gases as signalling molecules was demonstrated in the 1980s in vascular endothelium (88), with the discovery that endothelium-derived relaxing factor was nitric oxide, resulting in a Nobel Prize (89). At the same time, NO was shown to have analgesic effect similar to morphine (90), and acted as an agonist for membrane opioid receptor (91,92).

Gasotransmitters differ significantly in size, compared to normal cell signalling molecules, and easily diffuse through the cell membranes. There are many distinct differences between classic neurotransmitters and gasotransmitters: the latter are released in the cytoplasm rather than via an exocytosic vessel, there is no re-uptake mechanism or enzymatic removal – rather concentrations decrease via oxidation, radical scavenging and methylation; in addition, membrane receptors are not required for action. Criteria required to meet the definition of a gasotransmitter are outlined in Table 1.6.

The three best-described gasotransmitters are nitric oxide (NO), carbon monoxide (CO) and hydrogen sulphide (H_2S), all of which are endogenously produced. They have been shown to function as vasodilators, anti-inflammatory and cytoprotective agents at physiologic concentrations (Table 1.7) (87,93–95).

While the mechanisms of action of each continue to evolve and are in some ways unique, there is significant overlap and even inter-play among one another (96). A number of current therapeutics act through mechanisms that rely on gasotransmitters (97). Perhaps the most well-known and commonly used clinically are nitroglycerin and the phosphodiesterase-5 inhibitors. All of the gasotransmitters are present at basal concentrations in many cells, and have been shown to increase or decrease in various disease states. A significant body of literature exists around the idea of gasotransmitters as potential therapeutics; various novel releasing molecules have been synthesised, and significant results have been demonstrated in a number of animal models of disease (87,94,98).

1.3.3.1 Carbon Monoxide

CO is a highly publicized toxic molecule, as a cause of sudden death at home from leaky furnaces and as a toxin in cigarettes; general avoidance of CO is mandated to remain healthy, and alive. As a colourless, odourless, tasteless and initially non-irritating gas, CO monitors are recommended for households, while warnings are placed on cigarette packages. The mechanism of acute toxicity, and eventual death if untreated, is the formation of a fairly stable complex, carboxyhemoglobin (COHb). COHb interferes with the normal oxygen-carrying capacity of hemoglobin, resulting in hypoxia. Concentrations of 100ppm have been shown to ellicit neurologic symptoms; concentrations of 1600ppm cause death within 2 hours. The 20% level of COHb in humans will elicit

Table 1.6. Criteria for gasotransmitters.

1) Small gaseous molecules
2) Membrane permeable and do not rely on cell repectors or exocytosis for cell-signalling
3) Endogenously generated in a controlled manner
4) Specific function at physiologic concentration that is mimicked with exogenous administration
5) Specific cellular and molecular targets which many or may not be mediated by second messengers

Table 1.7. Characteristics of gasotransmitters nitric oxide, carbon monoxide and hydrogen sulphide.

	Nitric Oxide	Carbon Monoxide	Hydrogen Sulphide
Molar Mass (g/mol)	30	28	34
Substrate	L-arginine	Heme	L-cysteine
Endogenous Production	NOS	HO	CBS, CSE
Breakdown Products	Nitrite/Nitrate	None	Thiosulphate, Sulphate, Sulphite
Identified Targets	sGC, K_{Ca}, Heme Proteins Cytochrome C Oxidase	sGC, K_{Ca}, Heme Proteins Cytochrome C Oxidase	Ca, K_{ATP}, Heme Proteins, Cytochrome C Oxidase, PDE
Singalling Pathways	cGMP, p38 MAPK, ERK, PI3K/PKB	cGMP, p38 MAPK, ERK, PI3K/PKB	cGMP, p38 MAPK, ERK, PI3K/PKB
	NOS, Ras	PERK, p21	JNK
Free Radical	Yes	No	No
Direct Oxidant Scavenger	No	No	Yes
Donors	Organic Nitrates Sodium Nitroprusside	CO-RMs	Sodium Salts Synthetic Donors

symptoms, and death will occur at 50-80% saturation. Smokers have been demonstrated to have slightly elevated levels of 10-15% COHb, as opposed to non-smokers whose basal levels of COHb are 1-3% (99). The exact cellular mechanism of death due to CO overdose is still debated, but is most likely due to COHb-associated hypoxia, with minor extra-hemoglobin influence (100).

Despite its toxicity, nearly every organism contains CO, the output of which has been demonstrated to increase during the stress response (101). Under homeostatic conditions, CO is produced as a metabolite of heme degradation pathway. Heme is a vital cofactor and regulator of oxygen transport protein (hemoglobin), respiration and inflammation (102). In the liver, the quantity of heme is comparatively high, as it provides the catalytic domain for mono-oxygenase enzymes, such as cytochrome P-450(103). Heme is also a functional moiety in a number of signal transduction proteins, including NO synthases (NOS) and guanylate cyclase (GC), with generate cGMP (104,105).

Heme oxygenase (HO), the enzyme responsible for heme catabolism, has been identified in nearly every species ever studied. Heme is broken down by HO into biliverdin (which immediately gets converted to bilirubin) and CO gas, a reaction facilitated by NADPH (ref). Three genetically distinct forms of HO have been characterized: the inducible heme oxygenase-1 (HO-1), and the constitutively expressed heme oxygenase-2 (HO-2) and heme oxygenase-3 (HO-3). HO-1 has recently been identified as the 32kDa heat-shock protein (HSP32) that can be induced by a number of stimuli (106), particularly oxidative stress, thus suggesting a protective role (103).

Originally described as a metabolic enzyme to aid in turnover of heme proteins, HO-1 has more recently been identified as paramount for a number of agents to impart effect: statins, acetaminophen and prostaglandins (107). Induction of HO-1 prior to a stress results in significant cell and tissue protection. While the mechanism of effect has not been clearly elucidated, increasing evidence suggests the generation of endogenous CO is responsible for the cell and tissue protection.

Thus, the physiological role of CO is clearly beyond that of a waste product. The mechanisms of action for CO are largely mediated through metal-binding, particularly with heme-containing proteins: soluble GC, cytochrome c oxidase and cytochrome P450 (98). Further, a clear interaction with NO has been described, via the effect on inducible NOS (iNOS) (98). The rapid upregulation of iNOS by CO is complemented by an increase in HO-1, which leads to more CO (108,109), as well as the production of biliverdin, a molecule that has also been shown to have cytoprotective effects (110). The reciprocal is also true; NO may be one of the strongest inducers of HO-1 (111).

1.3.3.1.1 CO Donors

Carbon monoxide-releasing molecules (CORMs) were designed to overcome a number of drawbacks related to CO delivery, particularly with respect to controlled, measurable and safe delivery (112,113). Gas mixtures offer a number of constraints, especially related to the rapid increase of blood COHb levels, leading to toxicity and interference with oxygen transport.

Transition metal carbonyls, traditionally used as industrial catalysts during purification, offer a safer, stable chemical form for CO delivery (112). The first synthesised molecule, CORM-1, required activation with light to achieve dissociative loss of CO. This drawback was soon overcome through the use of ruthenium-based carbonyl complexes, CORM-2 and CORM-3. CORM-2 still had limited solubility in water; this would pose a problem for use in pharmaceuticals, but not in animal model. Water solubility of CORM-3 was achieved by glycination; the substance has been shown to be safe in regards to cell viability (114). The effects of CO have been clearly demonstrated in experiments where comparisons in responses were made to those with inactivated CORMs (those that were intentionally depleted of CO through dissolution) (112). CORM-3 and CORM-A1 have been paramount in developing hypotheses related to the dose-related effects of CO. CORM-3 has a very short half-life (less than one minute), while CORM-A1 releases CO at a much slower rate (half-time of 21 minutes). The vasorelaxation due to CORM-3 is quite rapid and profound and likely works through different mechanisms than the more prolonged but milder effects of CORM-A1 (115).

CO delivery of CORM-3 mimics the effects of endogenously derived CO, and has since been shown as an effective protective agent in a variety of animal models of disease, ranging from sepsis, acute limb CS, transplantation, cardiac disease and I/R injury (113,115). For example, in a rat model of cardiac I/R injury, CORM-3 attenuated cell death during hypoxia and re-oxygenation, clearly reducing the infarct size – an effect completely inhibited by a K_{ATP} channel

blocker (116). In another study, cardiac allograft time to rejection was increased from 9 days to over 25 days post-transplant in rats treated with CORM-3 (116). In a rat model of surgical ileus of the small bowel, CORM-3 reduced the development of paralytic ileus, and limited leukocyte infiltration. Finally, In a caecal ligation and puncture (CLP) animal model of sepsis, CORM-2 was able to inhibit upstream molecules implicated in severe sepsis, preventing downstream sequelae while improving animal survival (117).

1.3.3.2 Hydrogen Sulphide

Hydrogen sulphide (H_2S) has only recently been recognized as an important endogenous mediator of cellular activity, along with NO and CO (87). H_2S is a colourless gas with a distinct foul odour of rotten eggs, attributed to the bacterial breakdown of organic matter without oxygen. Similar to CO, in appropriately high doses, H_2S is quite poisonous, particularly to the nervous system, by forming a complex with iron and cytochrome enzymes, limiting cellular respiration (118). While the biological role of H_2S continues to be elucidated, it has been implicated as an endogenous regulatory agent in inflammation, oxidative stress and a control of vascular tone (87,119,120).

The role of H_2S has largely been elucidated through inhibition of H_2S synthesis. Cellular production of H_2S is cysteine-based and relies on two enzymes, cystathionine γ-lyase (CSE) and cystathionine β-synthetase (CBS) (Figure 1.2) (87). Both CBS and CSE are expressed in many tissues, including the liver, kidney and brain (121,122). Recently, it has been hypothesized that

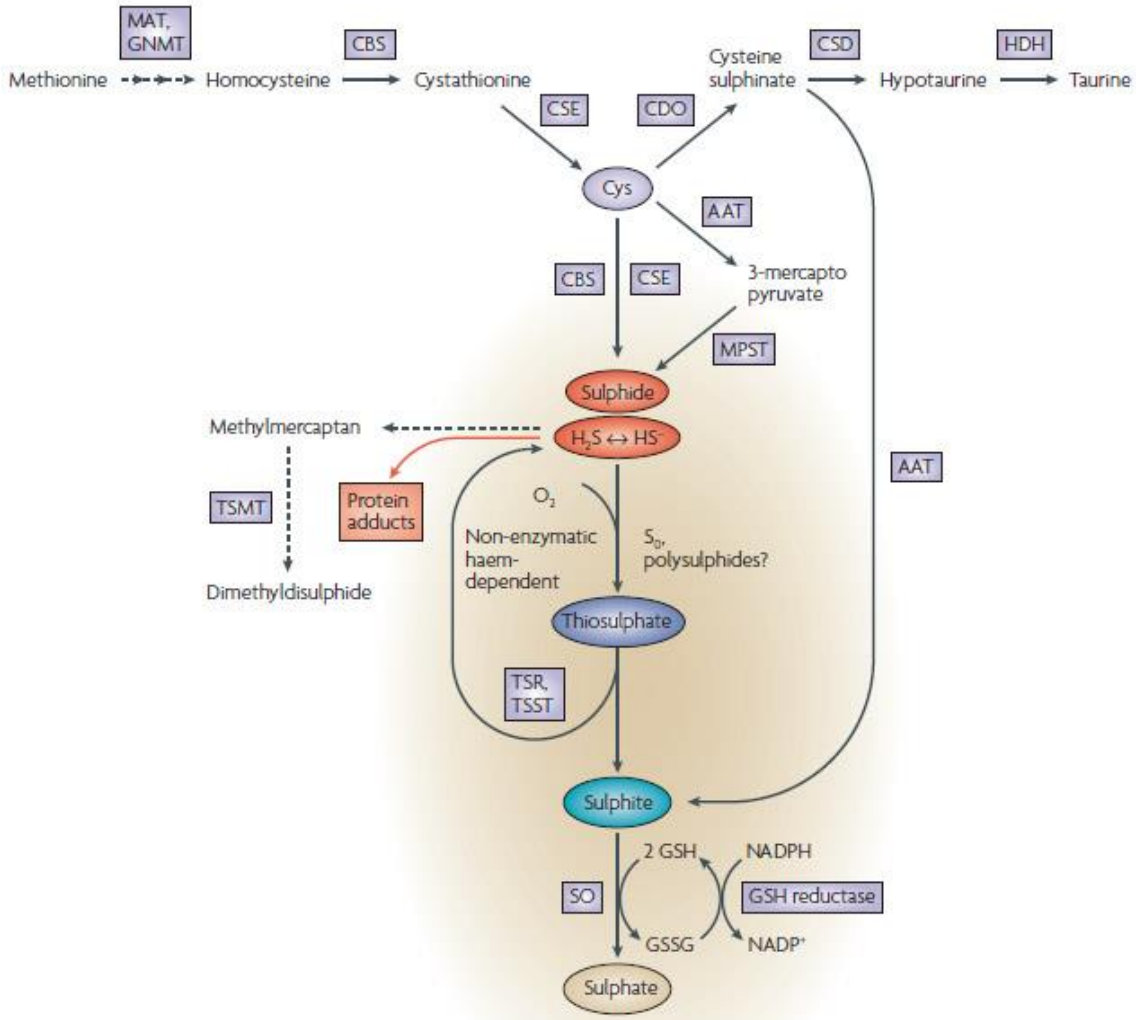

Figure 1.2. Endogenous formation of hydrogen sulphide.

Reproduced from Szabó (2007), with permission.

CSE depletion may contribute to the development of Hungtinton's disease, suggesting a potential role of H_2S in the neurodegeneration and loss of cellular protection; cysteine supplementation appears to reverse the cellular changes (123). Beyond human cells, bacteria, including those of the intestinal flora, produce H_2S. Enteric H_2S absorption is limited by the epithelial enzymes within the intestine; these metabolize sulphide to thiosulphate and sulphate. As as result, local increases in concentrations are prevented, and systemic absorption of this gas is limited (124,125). Detoxification appears to be highly localized to the caecum and right colon, leading to the postulation of the role of H_2S in some forms of colitis (124).

Outside of the brain, CSE inhibitors have been used to block the production of H_2S in the aorta, portal vein and ileum. Exogenously applied H_2S has been demonstrated to produce vasorelaxation, and, at least in part, appears to work in synergy with NO (particularly in the aorta, where H_2S may potentiate the effects of NO) (126). The vasorelaxation may also be mediated through the endothelial calcium-dependent potassium channels and ATP-sensitive potassium channels (127,128). Similar to NO and CO, the vascular relaxation most likely involves cGMP pathways, either through increased synthesis, reduced degradation or combination of both, although cGMP-independent pathways also exist (129). The effect of H_2S depends on its administered dose. Rapidly-releasing molecules are more likely to have an effect through cGMP compared to slow-releasing donors. The exact mechanism of cGMP increase is likely mediated through phosphodiesterase (PDE) inhibition (130–132).

1.3.3.2.1 H_2S Donors

The original H_2S-releasing compounds were hydrogen salts, most commonly in the form of sodium sulphide (NaHS). Aqueous salts completely dissolve in solution, and, in this case, release all of the cations and anions (i.e. hydrogen sulphide) instantaneously. This is not what happens in the biological systems, and is responsible for much of the confusion regarding the actions of H_2S when tested *in vitro* and *in vivo*. In order to better mimic biological rates of release and tissue concentrations, other H_2S-releasing organic molecules were pursued. This led to the development of morpholin-4-ium 4-methoxyphenyl (morpholino) phosphinodithioate, also commonly known as GYY4137 (133).

GYY41317 has traditionally been used in the industry for the vulcanization of rubber, and was “re-discovered” as a potentially improved agent for H_2S delivery. One of the first descriptions of the use of GYY4137 in a biological system compared it against NaHS (133). Using anesthetised rats, GYY4137 demonstrated a much different profile of H_2S release from that of NaHS: a sustained increased in plasma concentration of H_2S is seen with GYY4137, versus the near instantaneous peak of H_2S with NaHS (133). NaHS-induced apoptosis has been of concern; the slow release of H_2S from GYY4137 resulted in no apoptotic effect, as well as no effect on p53 (which was the reported mechanism of cell death from NaHS) (134). The difference in effects on cardiovascular function between the two compounds was also notable: NaHS had significant negative ionotropic and chronotropic effects on rat heart, while

GYY4137 had displayed none. The transient vasodilation and blood pressure reduction was slower in onset, and longer lasting with GYY4137, and appears to be mediated by the opening of vascular K_{ATP} channels. In the rat model of lipopolysaccharide-induced endotoxic shock, GYY4137 was shown to relax vasculature and normalize blood pressure (135). While the mechanism of action of GYY4137 is unclear, it has been hypothesized to be related to modulation of NF-κB, resulting in a release of nitric oxide and vasodilation (136). Additionally, GYY4137 demonstrated hepatic and renal protection, as measured by alanine transaminase (ALT) and creatinine, respectively. The anti-inflammatory effects of GYY4137 has been demonstrated by the reduction in lung MPO and pro-inflammatory cytokines, coupled with an increase in anti-inflammatory cytokine, IL-10 (135). One of the most interesting findings from this work was the importance of timing: administration of GYY4137 one hour prior to the induction of shock had little, or no anti-inflammatory effect (135).

1.4 THE PURPOSE OF THE THESIS

The goal of this work was two-fold. The first objective was to characterize the incidence of IAH in a mixed medical-surgical ICU population using modern definitions of IAH and modern measurement techniques, according to consensus guidelines (24). While a single contemporary study (35) has attempted a similar goal, it is our hope that the results generated will be more generalizable. We hypothesize that IAH will be more prevalent when screening consecutive patients compared to only measuring IAP in those deemed high risk. Finally, our results

were hypothesis-generating, with respect to factors which influence the development of IAH, as well as the impact of IAH on patient mortality.

The second goal of this thesis was to investigate a possible therapeutic effect of CO and H_2S on the physiological sequelae of ACS, using an animal model (rat). The mainstay of treatment of ACS is prompt recognition and early decompressive laparotomy; however, even with maximal therapy, mortality remains high (80). Both CORM-3 (CO donor) and GYY4137 (H_2S donor) have demonstrated potent protective effects in ischemia-reperfusion injury in other non-abdominal compartment syndromes (84,85,112), as well as various models of systemic inflammation and shock (87,98).

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CHAPTER 2

INCIDENCE OF INTRA-ABDOMINAL HYPERTENSION IN A MIXED MEDICAL-SURGICAL INTENSIVE CARE UNIT

CHAPTER 2: INCIDENCE OF INTRA-ABDOMINAL HYPERTENSION IN A MIXED MEDICAL-SURGICAL INTENSIVE CARE UNIT

2.1 INTRODUCTION

Intra-abdominal hypertension (IAH) and abdominal compartment syndrome (ACS) are increasingly recognized in critically ill patients and have been shown to contribute significantly to both morbidity and mortality (1–3). Equipoise remains regarding the clinical importance of IAH. For instance, IAH has been shown to be an independent predictor of mortality in a number of studies (1,4–6), while having no association in others (7–9). Further, published risk factors for IAH are based on isolated patient cohort data; the studies are mostly retrospective and use inconsistent definitions and measurement techniques for intra-abdominal pressure (IAP). The established risk factors are broad, and apply to the majority of critically ill (10,11). Indeed, based on the seminal papers on the epidemiology of IAH (1,2), consensus guidelines by the World Society of the Abdominal Compartment Syndrome (WSACS) have advocated for standardized measurement of IAP, standardized definitions and reporting (11).

There is a lack of high quality studies evaluating IAH in ICU patients, making determination of the true incidence difficult. These concerns have been highlighted in a recent review (12), and at least one recent study has attempted to generate higher quality data (9). The majority of incidence studies measure IAP in patients

thought to be at high risk, despite no definitive indications regarding the subset of patients at highest risk of developing IAH (13).

This study aimed to apply the WSACS definitions to consecutively admitted patients in a mixed medical-surgical ICU to determine the incidence of IAH. Secondarily, risk factors for IAH and ICU mortality were investigated in a descriptive model.

2.1.1 Outcomes

The primary outcome was the incidence (period-prevalence) of IAH in a mixed medical-surgical intensive care unit population, defined by the event rate of IAH during patients' ICU stay. The WSACS recognizes the limitations of a single IAP pressure measurement, and formally define IAH as "sustained or repeated pathological elevation in IAP ≥12mmHg." In the consensus guidelines, maximal IAP measurement was suggested, although consideration is given to using mean or median values of consecutive measurements (11). Given this, two consecutive measurements of IAP ≥12 mmHg during admission represented an event of IAH, and were used to calculate incidence, as well as to perform the analysis. This definition is consistent with the most recent IAH epidemiology literature (4,9).

Secondary outcomes included the point prevalence of IAH on admission (defined by the mean of the first two IAP measurements), the identification of IAH risk factors (at the time of admission) and ICU mortality. The diagnosis of ACS was made at the discretion of the treating physician and invariably involved the general surgical team.

2.1.2 Hypothesis

We hypothesized that the incidence of IAH would be lower than that reported in the literature.

2.2 METHODS

A prospective, observational cohort study at the Critical Care Trauma Centre (CCTC), Victoria Hospital in London, Ontario was conducted. Victoria Hospital is a tertiary care teaching hospital and level one trauma centre with a catchment area of two million people. The CCTC is a 28-bed unit, with approximately 50% surgical and 50% medical patients at any given time. We adhered to the strengthening of reporting of observational studies in epidemiology (STROBE) guidelines (14). Recruitment began September 9th, 2015 and continued until Dec 31, 2015; ICU follow-up concluded on Jan 18th, 2016 for all patients. Ethics approval was obtained from the Health Sciences Research Ethics Board at the University of Western Ontario (HSREB#106031). Patient consent for bladder pressure measurement and data collection was required. The trial was registered at ClinicalTrials.gov (NCT02514135).

2.2.1 Sample Size

A sample size calculation was performed to determine the number of patients required given an incidence of 25% (2) and an error of 5% and calculated a sample size of 289 patients (Table 2.1).

2.2.2 Eligibility Criteria

All adult (>18 years of age) patients admitted to the CCTC with a bladder catheter in-situ were considered for inclusion. Exclusion criteria included death prior to IAP measurement, lack of informed consent, pregnancy, discharge prior to IAP measurement, no IAP measurement within 24 hours of admission, organ donors, and cases where the care team declined to enroll the patient.

2.2.3 Bladder Pressure Measurement

Bladder pressure was measured using the modified Kron technique, the method suggested by consensus guidelines (11). Briefly, residual urine from the bladder was completely drained, and the bladder catheter clamped. Twenty-five millilitres of sterile saline was injected into the bladder via a port on the Foley catheter. IAP was measured via a pressure transducer at end-expiration and was expressed in mmHg. To ensure the study was pragmatic and represented normal practise, the ICU nurse assigned to each patient performed the measurement. All the nurses had the opportunity to ask questions about IAP measurement prior to the study commencement. The ICU nurses have previous clinical experience measuring IAP but for study purposes detailed instructions were provided in person and via the hospital website. An IAP measurement was performed at each 12-hour shift, and recorded in the patient care record. Study personnel collected data on a daily basis and were available to answer study related questions.

Table 2.1. Sample size calculation.

	Confidence limits at % of 100		
Prevalence	±2.5%	**±5%**	±10%
10%	553	139	35
15%	784	196	49
20%	983	246	62
25%	1152	**289**	73
30%	1290	323	81
35%	1397	350	88
40%	1473	369	93
45%	1519	381	96
50%	1535	384	97

$N = p(1-p)(Z/E)^2 = 0.25(1-0.25)(1.96/0.05)^2 = 289$

2.2.4 Data and Statistical Analysis

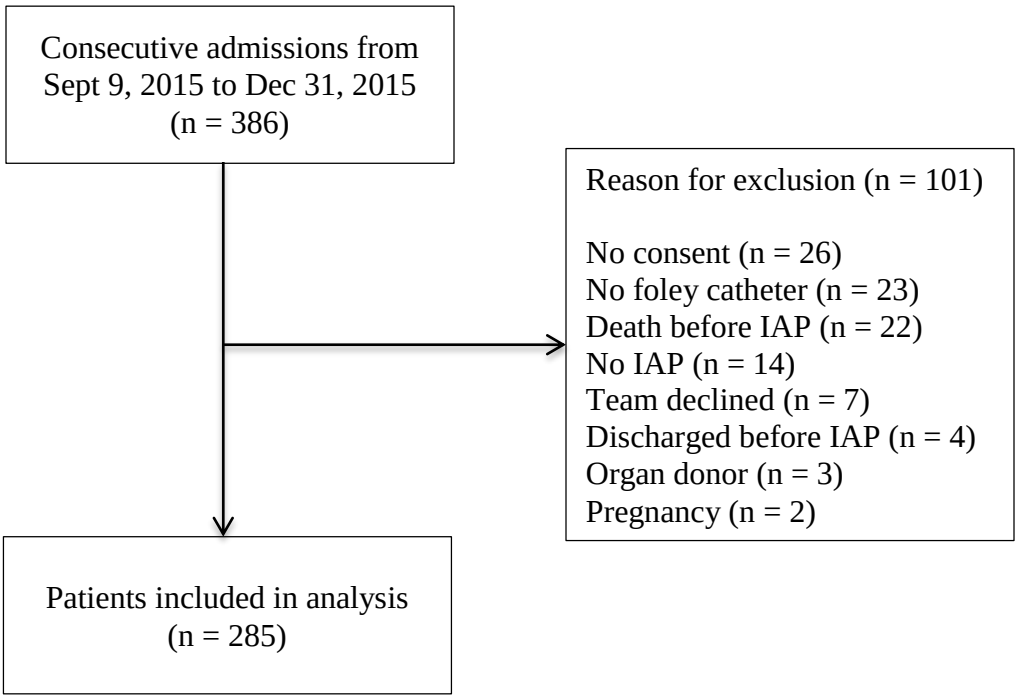
Baseline characteristics (age, sex, body mass index (BMI), admitting diagnosis, service, operative urgency, co-morbidities, Acute Physiology and Chronic Health Evaluation (APACHE) II score and Multiple Organ Dysfunction Scores (MODS)) were recorded. The incidence was calculated as the primary outcome, using two consecutive IAP measurements of ≥12 mmHg as the event rate. The results were used to divide the population into two groups: “IAH” or “no IAH.” Data were expressed as percentages, mean and standard deviation (SD) or median and 25-75% inter-quartile range (IQR) as appropriate. The Student t-test was used to analyse normally-distributed continuous data, while non-parametric testing was used for non-normally-distributed data. Pearson’s χ^2 test was used to determine the relationship between categorical variables. The trial type and design were not powered for analyses of risk factors; however, an explanatory model was developed as a secondary outcome, by performing multivariate logistic regression, with IAH as the dependent variable and admission characteristics as independent variables. An adjusted odds ratio for ICU mortality was calculated using multivariate logistic regression, including age, sex (female as reference), IAH (categorical), ventilation status (categorical) APACHE II score (continuous), sepsis, obesity (BMI>30kg/m^2), lactate (>2.4mmol/L) and high fluid balance (>3L in 24 hr). All analysis was performed using SPSS v. 22 (IBM, Armonk, NY) and STATA 14.1 (StataCorp, Texas, USA).

2.3 RESULTS

Three hundred and eighty six patients were admitted to the intensive care unit between September 9, 2015 and December 31, 2015. A total of 285 patients were included in the final analysis (reasons for exclusion are outlined in Figure 2.1). The demographics and clinical characteristics are outlined in Table 2.2. The prevalence of IAH on admission was 30%, and a further 15% developed IAH during their ICU stay. The overall period prevalence was 45±6%.

The majority (55%) of patients were diagnosed with Grade I IAH. Increasing grade was associated with increasing morality (Figure 2.2). Of the eight patients who developed ACS, seven were medical admissions, while one was surgical. Four patients underwent abdominal decompression, although only one patient (12.5%) survived. Out of a possible of 2993 possible bladder pressure measurements, 2250 (75%) were performed. The average IAP throughout admission was 9.3±3.7mmHg. In patients who were diagnosed with IAH, the average IAP was 12.±3.1mmHg. In the patients who did not have IAH, the average IAP was 7.1±2.5mmHg. Predictors of IAH on multi variable logistic regression are shown in Table 2.3.

Overall ICU mortality was found to be 20%, and was significantly higher in patients diagnosed with IAH (Table 2.4). In a multivariable model attempting to predict ICU mortality, IAH was an independent predictor of ICU mortality (OR 2.95, 95% CI 1.27-6.84, p=0.01, Table 2.5). IAH on admission (Mean of first two IAP ≥ 12mmHg) was an independent predictor of mortality in the same model



IAP = Intra-abdominal Pressure

Figure 2.1. Included patients and reasons for exclusion from analysis.

Table 2.2. Baseline characteristics and physiologic measures of patients with and without IAH diagnosed during admission.

	All	No IAH	IAH	p-value
n, (%)	285	157 (55%)	128 (45%)	N/A
Age, y, mean (SD)	60 (18)	57 (19)	63 (16)	**0.01**
Male, n (%)	182 (64%)	98 (62%)	84 (66%)	0.7
BMI, kg/m^2, median (IQR)	26 (8)	25 (7)	28 (9)	**0.001**
Admission Type				
Medical, n (%)	150 (52%)	84 (54%)	65 (51%)	0.5
Surgical, n (%)	91 (31%)	52 (33%)	39 (30%)	
Trauma, n (%)	46 (16%)	21 (14%)	24 (19%)	
Post-Operative, n (%)	93 (32%)	52 (33%)	41 (31%)	0.9
Elective, n (%)	41 (44%)	27 (52%)	14 (34%)	0.09
Emergent, n (%)	52 (56%)	25 (48%)	27 (66%)	
Comorbidities				
Diabetes, n (%)	79 (28%)	36 (23%)	42 (33%)	0.08
Insulin, n (%)	33 (12%)	16 (10%)	17 (13%)	0.4
CAD, n (%)	56 (20%)	24 (15%)	32 (25%)	**0.04**
Steroid Use, n (%)	11 (4%)	5 (3%)	6 (5%)	0.6
Metastatic Cancer, n (%)	16 (6%)	7 (4%0	9 (7%)	0.4
Dialysis, n (%)	13 (5%)	5 (3%)	8 (6%)	0.3
AIDS, n (%)	2 (1%)	2 (1%)	0	0.2
Cirrhosis, n (%)	12 (4%)	6 (4%)	6 (5%)	0.7
COPD, n (%)	55 (19%)	35 (22%)	20 (16%)	0.2
CHF, n (%)	28 (10%)	16 (10%0	12 (9%)	0.8
Hypertension, n (%)	144 (50%)	67 (43%)	76 (60%)	**0.006**
Hematologic Cancer, n (%)	15 (5%)	7 (4%)	8 (6%)	0.5
Current Smoker, n (%)	93 (33%)	54 (35%)	38 (31%)	0.4
Ex-Smoker, n (%)	73 (25%)	43 (28%)	30 (24%)	0.5
Physiology				
APACHE II, mean (SD)	28 (9)	26 (8)	31 (9)	**<0.001**
MODS, mean (SD)	6 (3)	5 (3)	7 (3)	**< 0.001**
Mechanical Ventilation‡	227 (79%)	114 (72%)	113 (88%)	**0.001**
Septic†, n (%)	82 (29%)	27 (17%)	55 (43%)	**<0.001**
Lactate, mmol/L, mean (SD)	2.4 (2.4)	2.3 (2.4)	2.6 (2.4)	0.27
Fluid Balance*, mL, median (IQR)	1640 (2749)	1135 (2540)	2019 (3284)	**< 0.001**
Total pRBC, units, mean (SD)	1.4 (3.5)	0.7 (1.3)	2.4 (4.9)	**< 0.001**

IAH was defined as two consecutive measurements of IAP≥12mmHg; Body Mass Index, BMI; Coronary Artery Disease, CAD; Acquired Immune Deficiency Syndrome, AIDS; Chronic Obstructive Pulmonary Disease, COPD; Congestive Heart Failure, CHF; Acute Physiology and Chronic Health Evaluation, APACHE; Multiple Organ Dysfunction Score, MODS; pRBC, packed red blood cells;
†Defined according to international guidelines (11)
‡At least one ventilator day
*At 24 hours

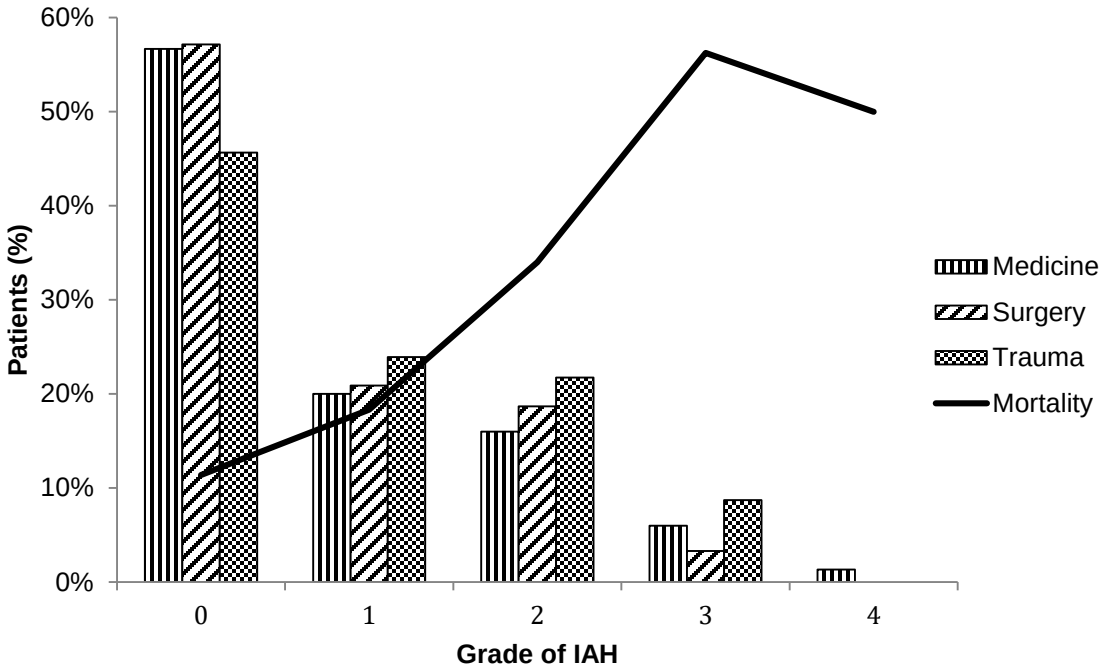


Figure 2.2. Incidence and mortality stratified by grade of IAH.

(OR 2.90, 95% CI 1.37-6.09, p=0.005). The most common cause of death for all patients was withdrawal of life-sustaining therapy (73%), followed by cardio-respiratory death (23%) and brain death (2%).

2.4 DISCUSSION

The incidence of IAH in our missed medical-surgical ICU population was 45±6%. This finding is consistent with the most recent and largest prospective study on mixed medical-surgical ICU admissions that demonstrated an incidence of 39% (9). Furthermore, IAH was present in all admission types (medical, surgical, trauma) and in those not traditionally screened, such as non-ventilated patients. This also represents the first prospective study adhering to consensus guidelines (11) to show an association between IAH and ICU mortality. Patients diagnosed with IAH were close to three times more likely to die in the ICU, compared to those without IAH, independent of illness severity and other traditional predictors.

Since the first 2006 WSACS IAH and ACS (15,16), (with an update in 2013 (11)), there have been at least four prospective observational studies on the epidemiology of IAH in mixed ICU populations (Table 1.2) (4,9,17,18). Unfortunately, all but one (9) have been under-powered, recruiting less than half of the patients required to accurately report the incidence of IAH within an appropriate margin of error (4,17,18). The Australian, prospective observational cohort which represents the best study to-date regarding the incidence of IAH/ACS using modern definitions (9). Regrettably, the generalizability of their work may be limited, as the patient population appeared to be dominated by those undergoing

Table 2.3. Patient outcomes by presence or absence of IAH.

Outcomes	All	No IAH	IAH	p-value
Ventilation Days†, median (IQR)	6 (7)	3 (4)	9 (9)	**< 0.001**
Vasoactive Medication Days†, median (IQR)	1 (4)	0 (2)	3 (5)	**< 0.001**
Death in ICU, n (%)	56 (20%)	18 (11%)	38 (30%)	**< 0.001**
Death in hospital‡, n (%)	64 (22%)	25 (16%)	39 (31%)	**0.004**
ICU length of stay, d, median (IQR)	5 (6)	4 (4)	7 (12)	**< 0.001**

†A day is counted if patient had vasoactive drug or ventilation at any point of any duration
‡Censored at 30-days

Table 2.4. Multivariable logistic regression for IAH.

Variable	Odds Ratio	95% CI	p-value
APACHE II	1.01	0.97-1.06	0.6
Sepsis	2.57	1.31-5.05	**0.006**
Obesity	2.94	1.52-5.70	**0.001**
Age	1.02	0.98-1.03	0.09
Male Gender	1.47	0.80-2.68	0.2
Mechanical Ventilation	2.51	1.09-5.81	**0.03**
Fluid Balance > 3L*	2.02	1.07-3.80	**0.03**
Lactate > 2.4mmol/L*	0.97	0.52-1.8	0.9

Obesity was defined as Body Mass Index >30 kg/m^2; Acute Physiology and Chronic Health Evaluation, APACHE; Age and APACHE II are continuous variables; *At 24 hours

Table 2.5. Multivariable logistic regression for ICU mortality.

Variable	Odds Ratio	95% CI	p-value
IAH	2.95	1.27-6.84	**0.01**
APACHE II	1.10	1.04-1.16	**0.001**
Sepsis	0.86	0.37-2.02	0.7
Obesity	1.85	0.83-4.1	0.1
Age	0.99	0.97-1.02	0.5
Male Gender	1.37	0.62-3.04	0.4
Mechanical Ventilation	0.47	0.15-1.46	0.2
Fluid Balance >3L*	2.67	1.26-5.7	**0.01**
Lactate >2.4mmol/L*	0.64	0.28-1.45	0.3

IAH was defined as two consecutive measurements of IAP≥12mmHg; Obesity was defined as Body Mass Index >30 kg/m^2; Acute Physiology and Chronic Health Evaluation, APACHE; Age and APACHE II are continuous variables; *At 24 hours

cardiothoracic surgery. The study did not contain trauma patients, and their overall ICU mortality of 11% is very low, likely drive by a preponderance of elective cases. Our results did find a similar incidence of IAH but a significantly higher overall mortality.

In our study, IAH was equally distributed between medical, surgical and trauma admissions, confirming the hypothesis that intra-abdominal pathology is not required for the development of IAH and that a broad patient population is affected (6,19,20). Patients diagnosed with IAH differed from non-IAH patients in a number of ways, including: BMI, co-morbidities, APACHE II score, MODS, and being mechanically ventilated. While the difference in age was found to be statistically significant, it is not clinically meaningful. Variables which explained the presence of IAH that were consistent with previous literature included: sepsis, obesity, fluid balance greater than three liters at 24 hours and mechanical ventilation (9–11). All but obesity are related to the underlying disease process, and serve, at least in part, as surrogate measures of illness severity. In particular, fluid balance was an important predictor, as patients with IAH received nearly one litre more on admission than those without IAH. As one of the management strategies of IAH is to avoid over-resuscitation, this finding supports the early implementation of vasopressors, particularly in sepsis, to avoid IAH, and potentially death (21).

IAH has been suggested to be an independent risk factor for mortality. While no prospective, goal directed, randomized control trials have been performed, centres that regularly monitor IAH and adhere to clinical guidelines appear to

experience a reduced rate of ACS (13,22). One large prospective, observational study demonstrated a reduced incidence of ACS, reduction in ventilator days and survival to discharge (both raw and severity-adjusted) with the use of protocoled bladder pressure monitoring and treatment according to consensus guidelines (22). There continues to be conflicting reports on the use of IAH as a predictor of mortality (1,4–9). We have demonstrated that even when controlling for other known predictors of mortality, IAH is an independent predictor of mortality. This is contradictory to Iyer *et al* (9), likely because of their patient population (largely elective cardiac surgery patients), and the low incidence of higher grades of IAH. When considering Grades II and above in their study, IAH was predictive of mortality.

ACS was seen in eight patients (3%), of whom half underwent decompressive laparotomy, yet only one patient survived. Predictors for the development of ACS were not considered in the current study, due to the relatively low number of outcomes: a challenge that plagues much of the literature on this topic (10,11). Clearly not all patients with IAH progress to ACS and it is difficult to predict which patients with IAH will progress to ACS. Clinically, the assumption is that if the progression from IAH to ACS can be prevented with treatment, mortality will be reduced (23).

The major strengths of our study are its prospective natures and its mixed medical-surgical, high acuity participants. Furthermore, the study design was pragmatic, where individual nurses were measuring IAP, rather than study

personnel, thereby increasing real-world applicability. Finally, the study was appropriately powered to determine IAH accurately.

The study was not without limitations. One of these was that only 75% of all IAP were completed. While this may reflect real world practice, it may limit interpretation of the incidence. The reasons for such compliance rates are variable, and related both to systems and personnel issues. Fortunately, less than 5% of patients were excluded due to lack of an IAP measurement on admission. In addition, the study was also single-centered, with IAP measurements only performed every 12 hours.

Our study continues to expand the body of literature that identifies IAH as a common entity in *all* critically ill patients. Our reporting adheres to the WSACS guidelines and definitions, and provides a well-documented approach to the epidemiology of IAH in a large, diverse patient population using pragmatic methods. There is a clear association between IAH and ICU mortality in our patient population. Future work should evaluate the effect of intervention on clinical outcomes.

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CHAPTER 3

CARBON MONOXIDE AND HYDROGEN SULPHIDE AS POSSIBLE THERAPEUTICS FOR ABDOMINAL COMPARTMENT SYNDROME

CHAPTER 3: CARBON MONOXIDE AND HYDROGEN SULPHIDE AS POSSIBLE THERAPEUTICS FOR ABDOMINAL COMPARTMENT SYNDROME

3.1 INTRODUCTION

Critically ill patients are at risk of intra-abdominal hypertension (IAH), resulting in metabolic, vascular and organ derangements, both at local and systemic levels (1). In a subset of patients, abdominal compartment syndrome (ACS) may result. ACS is defined by abdominal pressures greater than 20mmHg and new onset end-organ failure. While ACS was originally described in post-operative abdominal aortic aneurysm patients (2), the syndrome is not limited to surgical patients. Recent data would suggest that the incidence of ACS in critically ill patients with and without intra-abdominal pathology is in the range of 1-5%. Although various medical efforts may be employed, the mainstay of treatment for ACS remains decompressive laparotomy (3–5), with on-going organ failure.

ACS results in decreased cardiac output, hypoperfusion of hepatic microvasculature, cessation of the renal filtration gradient, and increased mucosal permeability with bacterial translocation from the bowel (5). The resulting injury is related to both, the ischemia caused by high abdominal pressures (to which the gut is very sensitive), and the reperfusion injury following pressure relief (6–8). Several animal models have attempted to simulate the ischemia-reperfusion (I/R) of ACS by various methods, although pneumoperitoneum appears to be the most

common (9–16). Previous work in our laboratory had demonstrated significant hepatic microvascular changes, in addition to evidence of multi-organ dysfunction and metabolic derangement, in a rat model of ACS (17).

Non-surgical treatments of ACS currently do not exist in a clinical setting. Ideally, the incidence of IAH should be minimized by adhering to consensus guidelines (1), but, as established in Chapter 2, it remains high. Decompressive laparotomy is the only available method to effectively relieve the pressure and restore end-organ perfusion; however, mortality remains high at ~80% (1,18). While the mortality associated with ACS is multifactorial, it likely includes an element of I/R injury. Previously, a number of compounds have been tested, including minocycline, octreotide, dopamine, and glutamine (19–22). Recently, carbon monoxide (CO) and hydrogen sulphide (H_2S) have demonstrated promise in reducing cellular injury and microvascular perfusion derangements in rat models of acute limb compartment syndrome (23,24). CO and H_2S are both produced endogenously, protecting against I/R injury via anti-oxidant and anti-inflammatory mechanisms that continue to be elucidated (25,26). CO- and H_2S-releasing molecules (CORM-3 (27–29) and GYY4137 (30,31), respectively) can be used to deliver both compounds systemically and have been shown effective in rat models of acute limb compartment syndrome (23,24).

The purpose of this study was to determine the effect, of CO and H_2S on hepatic microvasculature, hepatic cell death, and inflammatory, metabolic and renal dysfunction in a rat model of ACS. The ultimate goal is the development of

non-surgical (pharmacological) therapy for ACS. We hypothesized CO and H2S would reduce the systemic and organ injury as a result of ACS.

3.2 MATERIALS AND METHODS

The experimental protocol was approved by the Animal Use Subcommittee of Canadian Council on Animal Care at the University of Western Ontario (Appendix III). Animals were housed in clear plastic cages in pairs, at 12:12 light:dark cycle, and had free access to food and water. The sample size was calculated with leukocyte rolling as the primary outcome based on the expected results from Chadi *et al*. (17).

3.2.1 Animal Preparation

Male Wistar rats were anaesthetised by inhalational isoflurane (5% induction 2% maintenance) in a 1:1 oxygen/nitrogen mixture. The left carotid artery was cannulated to allow for systemic monitoring, fluid administration and continuous blood pressure monitoring. The body temperature of the animal was monitored via a rectal probe, and maintained constant at 37°C by the means of a heat lamp. A 16-gauge and 14-gauge angiocatheters were inserted into the peritoneum; these were used to generate pneumoperitoneum and to continuously monitor the intra-abdominal pressure, respectively (Figure 3.1). At the conclusion of the experiment, all animals were euthanized by an overdose of anaesthetic agent.

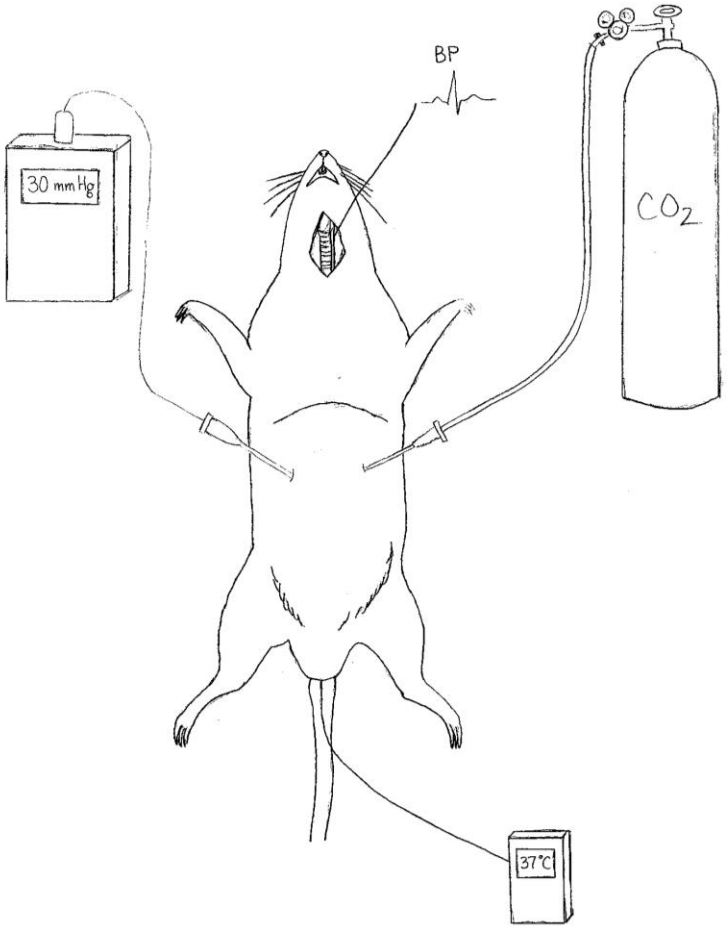

Figure 3.1. Schematics of the experimental setup for rat abdominal compartment syndrome.

3.2.2 CORM-3 and GYY4137

A water soluble CO donor, CORM-3 (tricarbonylchloro-glycinate-ruthenium(II), [$Ru(CO)_3Cl$-glycinate]; molecular weight 295 $gmol^{-1}$) was synthesized by us, according to an established protocol (26). Fresh CORM-3 was prepared by dissolving it in isotonic saline just prior to injection. The inactive form (iCORM-3) was prepared by dissolving CORM-3 in saline 72 hours prior to injection, to allow the release of all CO from solution.

A water soluble H_2S donor, GYY4137 (Cayman Chemicals, Ann Arbor, Michigan, USA), was dissolved in a normal saline solution, just prior to the administration (30,31).

3.2.3 Elevation of IAP as a Model of ACS

A fitted Plaster of Paris cast was applied around the abdomen of each rat to limit distension of abdominal cavity. Care was taken to ensure the diaphragm and rib cage of each rat was not obstructed. The plaster cast was activated with water and moulded to the animal's contours. The intra-abdominal pressure (IAP) was raised to 20-30mmHg by insufflation with CO_2, while an electronic compartmental pressure monitoring system was used to continuously monitor the intra-abdominal pressure (Synthes, USA). The elevated IAP was maintained for 2 hours. Sham animals were subjected to the same preparation, but the IAP was kept at baseline of 0mmHg. Following the 2 hour period of ACS a midline laparotomy was performed to decompress the abdomen and begin data collection via IVVM and blood draws.

3.2.4 Experimental Groups

Rats were randomly assigned to one of four groups: 1. sham (n=4), 2. ACS+iCORM-3 (n=5), 3. ACS+CORM-3 (n=5), and 4. ACS+GYY4137 (n=5). Randomization was based on day of the week. CORM-3 and GYY4137 were administered at the dose of 10mg/kg and 50mg/kg, respectively, via the carotid artery. Inactive CORM-3 served as the experimental control, to demonstrate the physiological effects of ACS. Doses were chosen based on previous work on limb compartment syndrome (23,24).

3.2.5 Intravital Video Microscopy (IVVM)

Abdominal organs were exposed through midline laparotomy. The animal was then transferred to the stage of an inverted microscope (Nikon) and the liver was exteriorized into a saline bath containing 5μg/mL fluorescent vital dye, propidium iodide (PI, excitation 482nm; emission 616nm). PI stains all nuclei of cells with severely compromised cell membranes. A plastic film was used to cover the preparation and prevent the organ from drying. The temperature of the organ bath was maintained by the means of a heat lamp at 32°C. Care was taken to ensure that the time from laparotomy to first microscopy reading was minimized (<5 minutes).

Hepatic sinusoidal perfusion and leukocytes within the post-sinusoidal venules were recorded by translumination with 20x and 40x objectives, respectively, in eight to twelve fields of view containing a complete hepatic vascular

unit. Fluorescence microscopy was used to visualise the PI staining from the same fields of view as those used to assess the sinusoidal perfusion. All videos were captured into the computer for offline analysis. The entire IVVM recording lasted 20-30 minutes per animal.

3.2.6 Offline Video Analysis

Sinusoidal perfusion was assessed by counting the number of continuously perfused (CPS), intermittently-perfused (IPS) and non-perfused (NPS) sinusoids within a 36-point stereological grid, using standard stereological techniques and was expressed at the % of total sinusoids. Sinusoidal diameters (D) were measured using ImageJ (NIH, Bethesda, MD) software, by averaging 3 different points along each sinusoid, and expressed in µm. Centreline velocity of red blood cells (RBC) (V) was assessed within each sinusoid by using frame-by-frame analysis and expressed as µm/s. Volumetric flow (VQ) in pL/s, and shear (γ) (s^{-1}) were calculated using the formulas $VQ = \pi r^2 \times V$ and $\gamma = 8V/D$, respectively. Hepatic injury was assessed by counting the number of PI-labelled cells within a field of view and expressed per volume of tissue. Leukocyte activation was assessed by counting the number of adherent and rolling leukocytes within post-sinusoidal venules, and expressed per 10,000 μm^2. Venular area was measured using ImageJ (NIH, Bethesda, MD). A leukocyte was considered adherent if it remained stationary for at least 30 seconds, and rolling if the cell remained in contact with the wall of the vessel during movement. The data analyst was blinded to the intervention group.

3.2.7 Blood Sample Analysis

At the conclusion of the experiment, arterial blood samples were taken from each rat for the assessment of serum levels of alanine transaminase (ALT), aspartate aminotransaminase (AST), alkaline phosphatase (AlkPhos), blood urea nitrogen (BUN), creatinine, carboxyhemoglobin (COHb) and arterial blood gases (pH, pCO_2 and pO_2), just prior to euthanasia.

3.2.8 Tissue Level of Myeloperoxidase (MPO) Activity

MPO was measured enzymatically, as published previously (17). Briefly, MPO was extracted from lung, liver and small intestinal tissues by homogenization in 0.1M phosphate buffered saline (PBS) at a 1:10 ratio, followed by centrifugation at 6,000xg for 20min at 4ºC. The pellet was then reconstituted and sonicated in acetic acid/CETOH buffer, and centrifuged at 6,000xg at 4ºC. The resulting supernatant was then added to MPO cocktail (acetic acid and tetramethyl benzidine (TMB)) and warmed to 37ºC. The enzymatic reaction was initiated by the addition of hydrogen peroxide and stopped 3min later by catalase. Following the addition of 0.2M glacial acetic acid, the samples were analysed spectrophotometrically, at 655nm (Beckman DU-640). The MPO activity was expressed as Units/ml tissue extract.

3.2.9 Statistical Analysis

All statistical analyses were carried out using Prism 5.0 for Mac software (GraphPad Inc.). All parameters were expressed as means ± standard error of the mean (SEM), and analysed using one-way analysis of variance (ANOVA), with the Tukey post-hoc test where appropriate to perform single-step multiple comparisons. The significance level was set at $p<0.05$.

3.3 RESULTS

3.3.1 Systemic Leukocyte Count and COHb

Elevation of IAP led to a significant rise in systemic leukocyte count; CORM-3 and GYY4137 treatment were able to decrease the severity of this response (Figure 3.2). Application of CORM-3, GYY4137 or iCORM-3 had no effect on COHb levels (Figure 3.2).

3.3.2 Hepatic Microvascular Perfusion

Elevation of IAP resulted in significant changes to hepatic sinusoidal perfusion, as shown in Figure 3.3. The number of continuously-perfused sinusoids decreased from 93±3% in sham to 69±9% in ACS+iCORM-3 ($p<0.01$), while the number of non-perfused sinusoids increased from 2±1% in sham to 13±6% in ACS+iCORM-3 ($p<0.01$). CORM-3 and GYY4137 treatments were able to restore the number of continuously-perfused capillaries to 88±3% and 95±2%, respectively ($p<0.01$), while iCORM-3 had no effect. Sinusoidal diameters, RBC velocity, VQ and shear did not significantly differ in any group (Table 3.1).

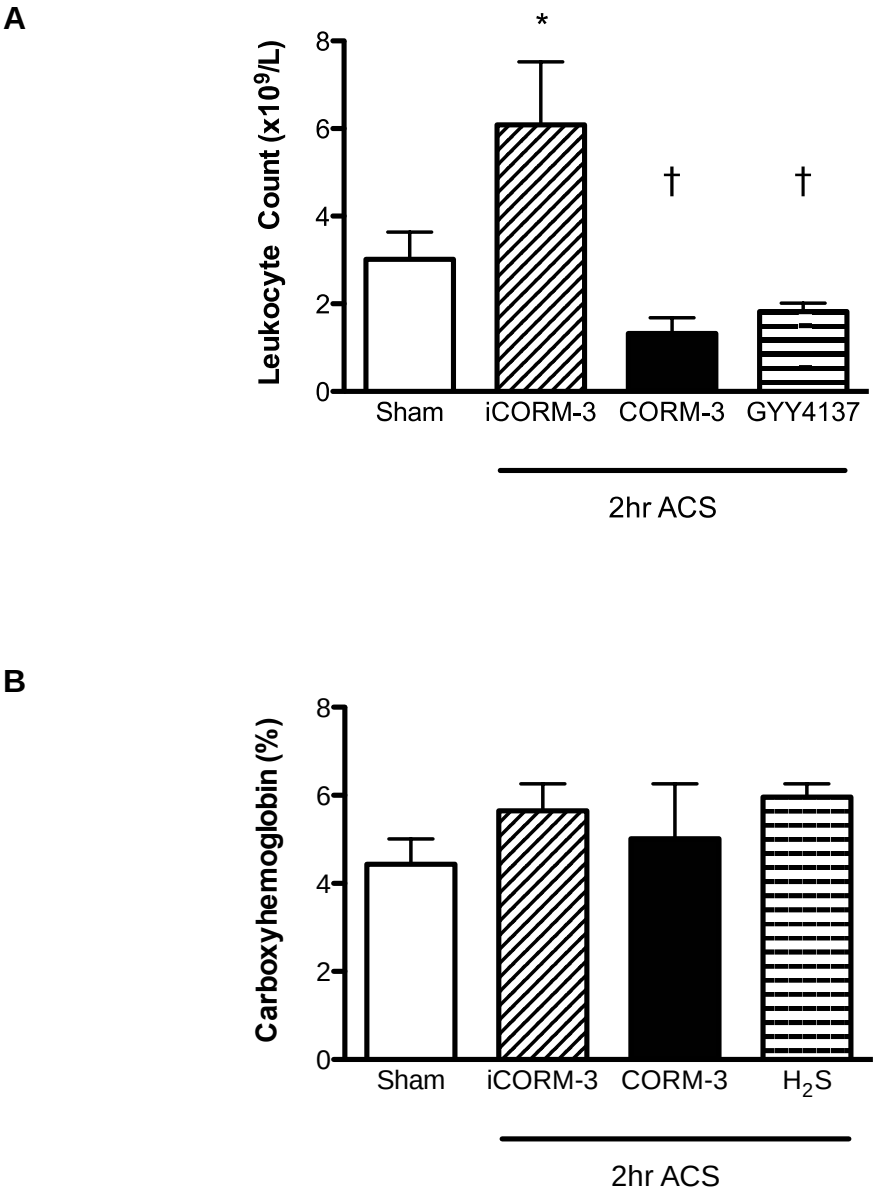

Figure 3.2. The effect of CORM-3 and GYY4137 on (A) total systemic leukocyte count and (B) COHb levels. *ACS-associated inflammation was reversed by CO and H_2S application, while having no effect on COHb levels* (one-way ANOVA $p<0.05$; *$p<0.05$ from sham; †$p<0.05$ from ACS+iCORM-3).

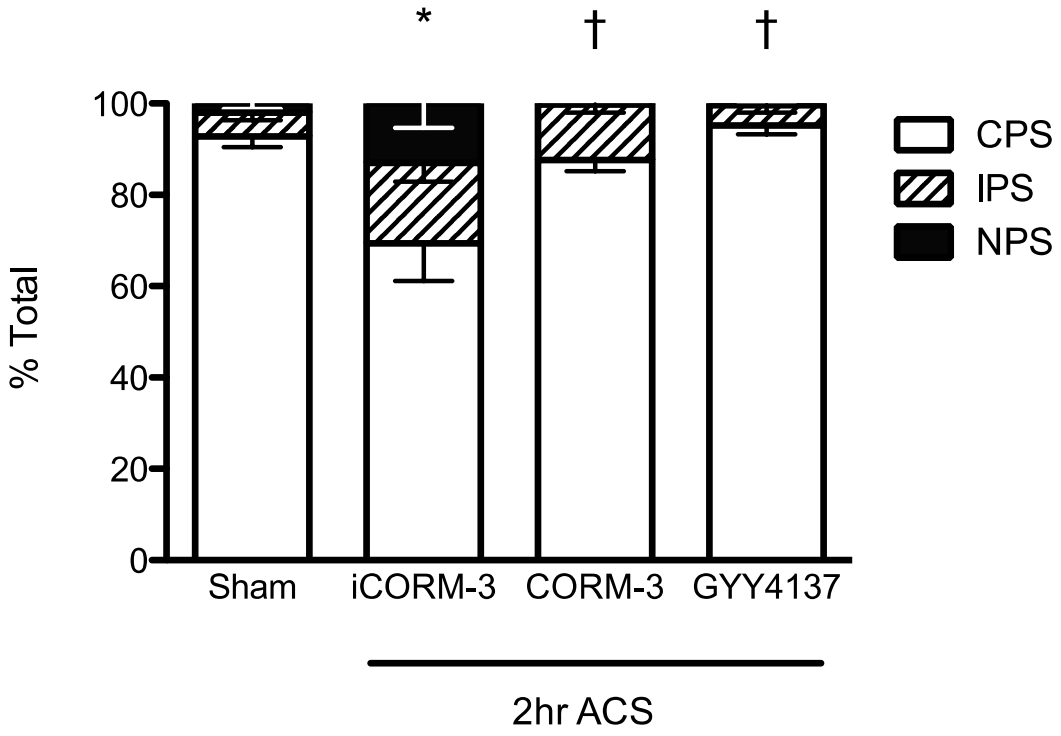

Figure 3.3. The effect of CORM-3 and GYY4137 on liver microvascular perfusion following ACS. A*CS-associated perfusion changes were reversed by CO and H_2S application* (one-way ANOVA $p<0.05$; *$p<0.05$ from sham; †$p<0.05$ from ACS+iCORM-3). *CPS*, continuously perfused sinusoids; *IPS*, intermittently perfused sinusoids; *NPS*, non-perfused sinusoids.

Table 3.1. The effect of CORM-3 and GYY4137 on hepatic sinusoidal diameters, centreline RBC velocity, volumetric flow and shear in rat model of ACS. There were no significant differences among the groups.

		2hr ACS			
Parameter	**Sham**	**iCORM-3**	**CORM-3**	**GYY4137**	**p-value**
Sinusoidal Diameter (D) (μm)	8.6±0.9	8.5±0.8	8.0±0.5	7.2±0.4	0.49
Centreline Velocity (V) (μms^{-1})	203±10	187±20	240±22	279±57	0.27
Volumetric Flow (VQ) (pLs^{-1})	7.8±1.5	7.2±1.3	8.1±1.5	7.1±0.5	0.93
Shear (γ) (s^{-1})	125.6±16.7	118.1±17.4	154.8±16.7	211.2±59.4	0.24

$VQ = \pi r^2 \times V$
$\gamma = 8V/D$

3.3.3 Hepatocellular Death

Hepatocellular death, as measured by PI, significantly increased from 29±9 cells/0.1mm^3 in sham to 489±188 cells/0.1mm^3 ($p<0.05$) in ACS+iCORM-3 group. CORM-3 and GYY4137 treatments were able to diminish tissue death to 88±44 cells/0.1mm^3 and 127±50 cells/0.1mm^3, respectively ($p<0.05$) (Figure 3.4).

3.3.4 Inflammation

Elevation of IAP led to significant leukocyte activation, as demonstrated by the adhesive interactions with liver vascular endothelium. Leukocyte adherence in the post-sinusoidal venules was increased from 0.5±0.2 in sham to 17.4±5.2 leukocytes/30s/10,000μm^2 in ACS+iCORM-3 ($p<0.01$). Leukocyte rolling also increased from 1.5±1.2 to 6.6±1.4 leukocytes/30s/10,000μm^2. CORM-3 treatment led to a significant, 17-fold decrease in leukocyte adherence and 6-fold decrease in leukocyte rolling (0.4±0.2 adherent leukocytes/30s/10,000μm^2 and 1.0±0.5 rolling leukocytes/30s/10,000μm^2, respectively, $p<0.01$). GYY4137 treatment led to a significant, 8-fold decrease in leukocyte adherence, while having no effect on leukocyte rolling (2.4±0.4 adherent leukocytes/30s/10,000μm^2, $p<0.01$, and 6.4±1.7 rolling leukocytes/30s/10,000μm^2, not significant) (Figure 3.5).

3.3.5 Tissue MPO

Lung, liver and small intestine MPO activity increased from 25.7±3.9, 13.9±0.7 and 25.2±4.4U/ml, respectively in sham to 110.3±9.9, 20.7±1.3 and 46.1±10.1U/ml, respectively in ACS+iCORM-3 ($p<0.05$). CORM-3 treatment led

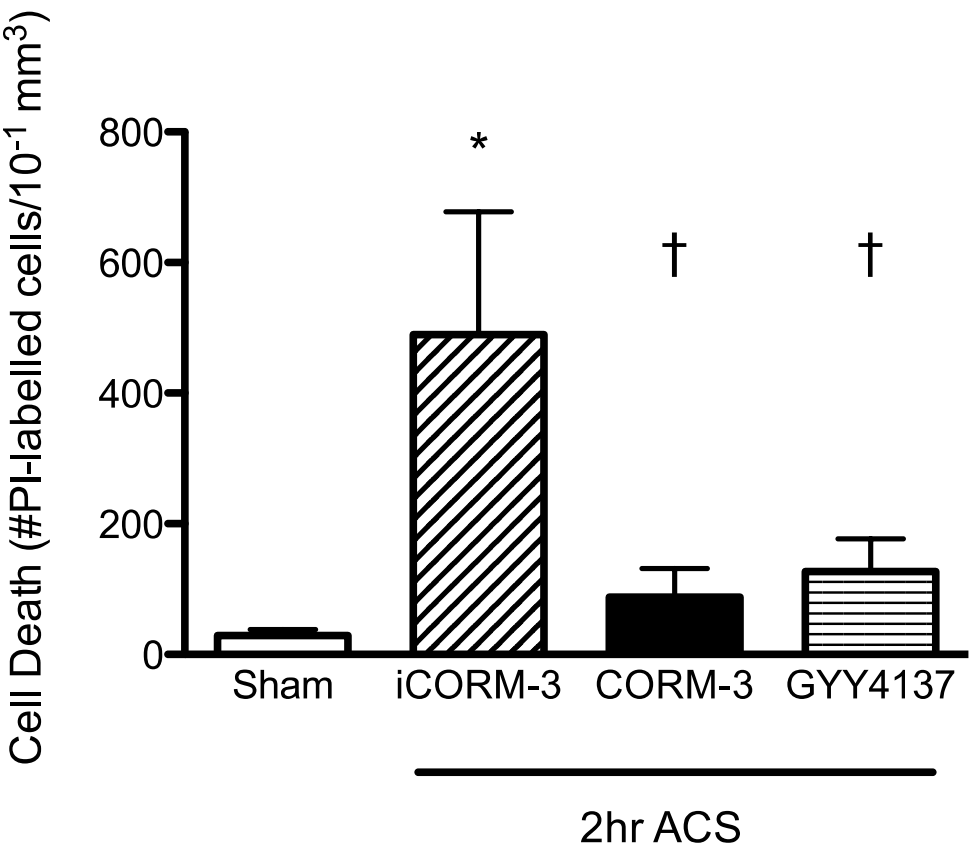

Figure 3.4. The effect of CORM-3 and GYY4137 on hepatocellular death following ACS. *ACS-associated hepatocellular death was reversed by CO and H_2S application* (one-way ANOVA $p<0.05$; *$p<0.01$ from sham; †$p<0.01$ from ACS+iCORM-3).

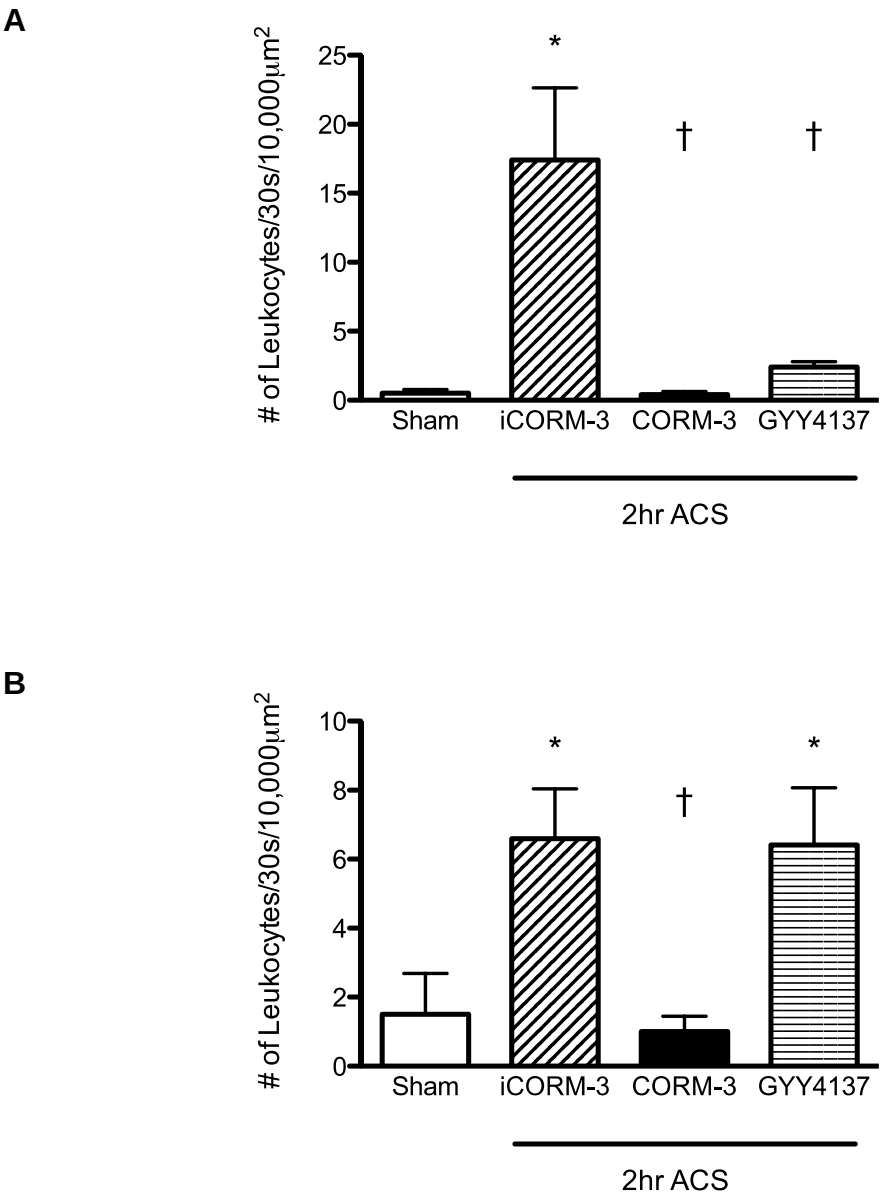

Figure 3.5. The effect of CORM-3 and GYY4137 on leukocyte activation following ACS: (A) adherence, (B) rolling. *ACS-associated inflammation was reversed by CO and H_2S application* (one-way ANOVA $p<0.05$; *$p<0.05$ from sham; †$p<0.05$ from ACS+iCORM-3).

to a significant decrease in MPO (65.3±11.0U/ml in lung, 16.5±0.6U/ml in liver and 23.1±3.3U/ml in small intestine, $p<0.05$), while GYY4137 had no effect (92.0±17.7U/ml, 16.4±2.3U/ml and 40.6±11.1U/ml in lung, liver and small intestine, respectively, not significant) (Figure 3.6).

3.3.6 Organ Function and Blood Gases

Elevation of IAP resulted in a significant increase in ALT and a trend towards an increase in AST, from 37.2±2.6 0U/L and 70.5±11.5U/L in sham to 237.3±150.0U/L ($p<0.05$) and 508.3±333.1 U/L in ACS+iCORM-3 ($p<0.05$), while having no effect on levels of AlkPhos or bilirubin (Figure 3.7). BUN and creatinine also increased, from 5.6±0.5mmol/L and 12.0±1.2μmol/L, respectively, in sham to 16.8±1.0mmol/L ($p<0.05$) and 16.5±2.5μmol/L in ACS+iCORM-3 (Figure 3.8). CORM-3 and GYY4137 treatments significantly attenuated the liver and kidney damage (Figures 3.7 and 3.8).

Elevated IAP led to alterations in arterial blood gases (particularly pCO_2) and blood pH compared to sham; arterial blood gases and blood pH were significantly improved by CORM-3 and GYY4137 treatments (Figure 3.9).

3.4 DISCUSSION

Abdominal compartment syndrome poses a challenge in critically ill patients; decompressive laparotomy remains as the mainstay of treatment. In our

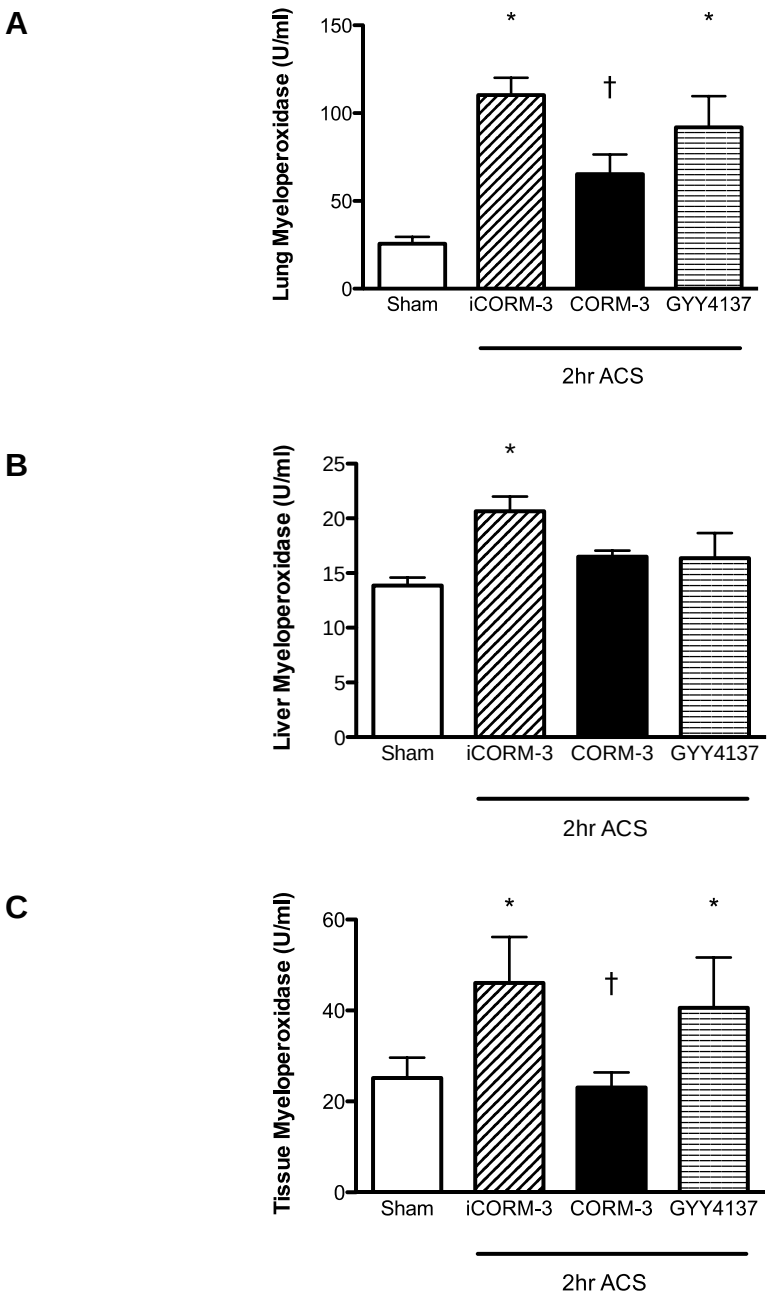

Figure 3.6. The effect of CORM-3 and GYY4137 on tissue MPO in (A) lung, (B) liver and (C) small intestine following ACS. *ACS-associated increase in MPO was attenuated by CO, but not H_2S application* (one-way ANOVA $p<0.05$; *$p<0.05$ from sham; †$p<0.05$ from ACS+iCORM-3).

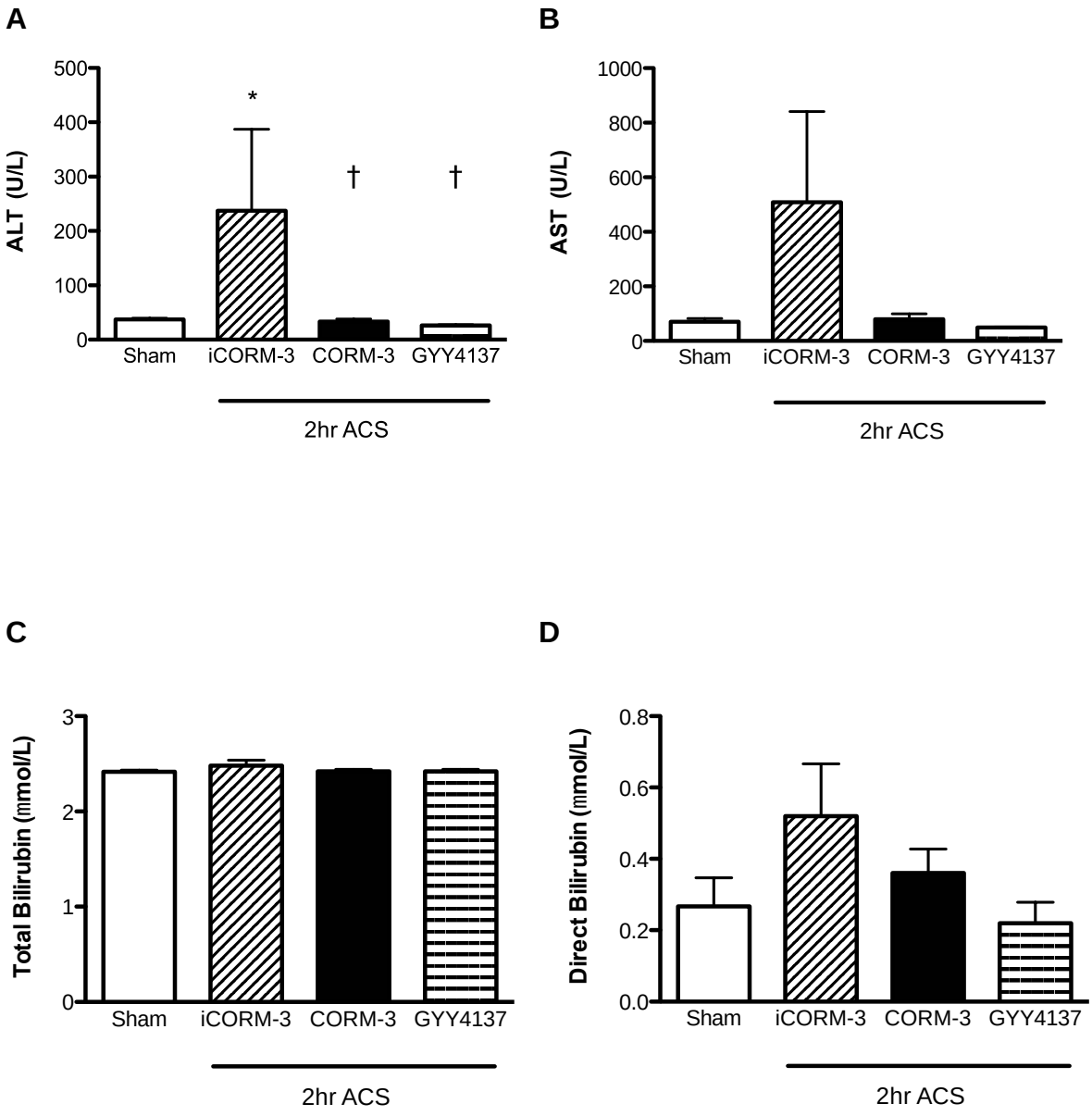

Figure 3.7. The effect of CORM-3 and GYY4137 on liver function tests following ACS: (A) ALT, (B) AST, (C) total bilirubin, (D) direct bilirubin. (one-way ANOVA $p<0.05$; *$p<0.05$ from sham; †$p<0.05$ from ACS+iCORM-3).

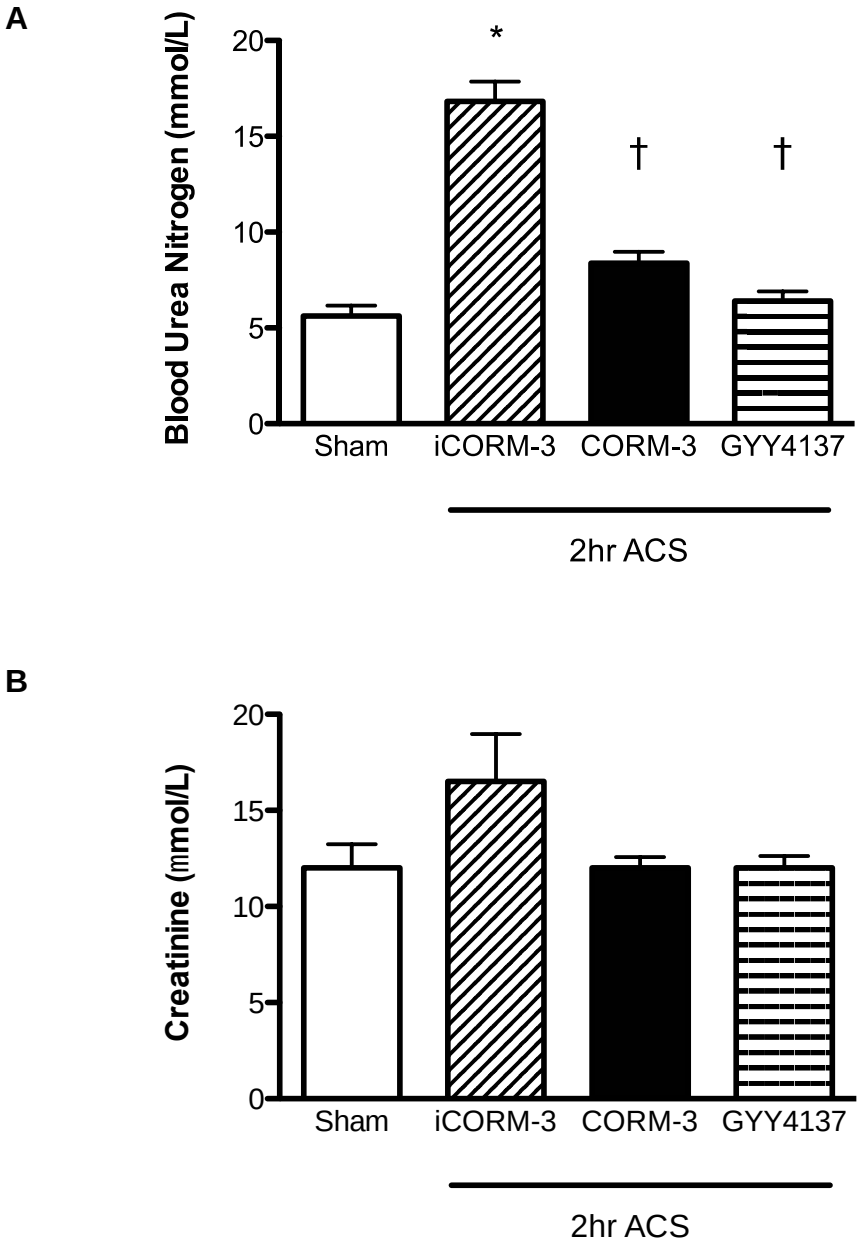

Figure 3.8. The effect of CORM-3 and GYY4137 on kidney function following ACS: (A) blood urea nitrogen, (B) creatinine. (one-way ANOVA $p<0.05$; *$p<0.05$ from sham; †$p<0.05$ from ACS+iCORM-3).

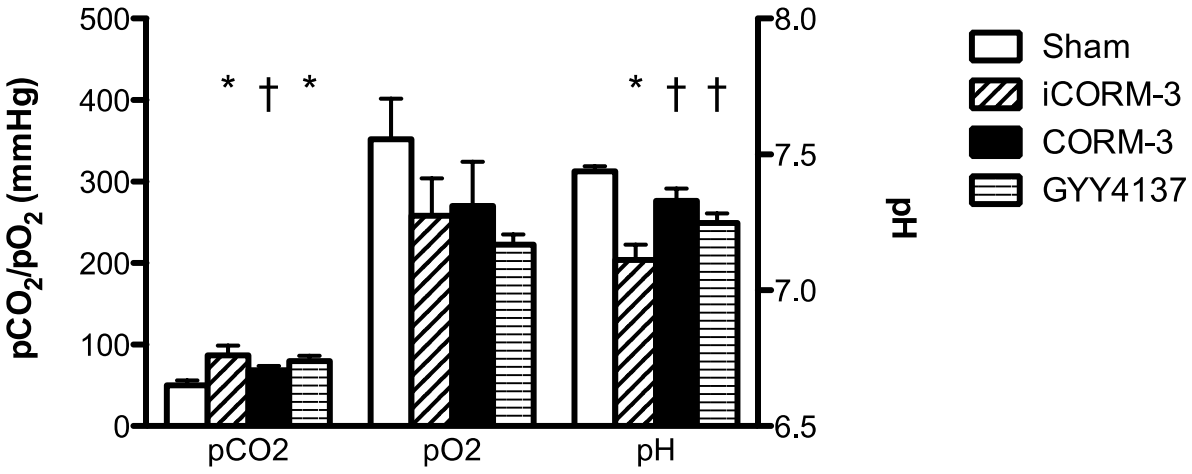

Figure 3.9. The effect of CORM-3 and GYY4137 on arterial blood gases parameters. A*CS-associated acidosis was reversed by CO and H_2S application* (one-way ANOVA p<0.05; *p<0.05 from sham; †p<0.05 from ACS+iCORM-3).

study, we investigated the impact of two possible novel pharmacologic therapeutics in a rat model of ACS.

Sustained elevation of IAP resulted in significant and negative physiologic effects at the microvascular, organ and systemic levels. These were most likely secondary to ischemia-reperfusion (I/R) injury. At the microcirculatory level, we found that the elevated IAP led to a significant increase in the heterogeneity of sinusoidal perfusion after decompression, with a shift from continuous perfusion normal to sham towards intermittent and non-perfused sinusoids in ACS group. This effect was partially lessened by both CO and H_2S donors CORM-3 and GYY4137, respectively (Figure 3.3). While we found a decrease in the continuous perfusion, the volumetric flow and sinusoidal diameters remained unchanged (Table 3.1). The change in sinusoid perfusion has been classically described in models of liver transplantation (32), where the effect appears to be secondary to hypoperfusion and reperfusion (33). Hypoperfusion represents a state of ischemia caused by the diminished blood flow, where liver sinusoidal endothelial cells become damaged due to a lack of energy substrate and hypoxia (33). Ischemia is known to result in an increased vascular permeability, leading to the formation of local edema (34), which then further contributes to non-perfused segments of microcirculation. In our rat model of ACS, the blood flow was not completely occluded by the elevation of IAP, but rather the liver perfusion was subjected to the state of low flow, until normal blood flow was restored on laparotomy. The subsequent reperfusion injury then accentuated the microvascular dysfunction (33) triggered during the period of IAP elevation by an inflammatory response and

neutrophil accumulation (35), in response to hepatocellular death and the subsequent release of pro-inflammatory cytokines/chemokines (36).

The protective mechanism through which both CORM-3 and GYY41317 work is likely multifactorial. While both molecules possess vasodilatory properties (37,38), no changes in sinusoidal diameters were observed in our study (Table 3.1). However, vasodilation of larger, more proximal vessels cannot be ruled out. It has been postulated that there are synergistic pathways through which the gasotransmitters exhibit their effects. The anti-inflammatory and anti-oxidant properties of both CORM-3 and GYY4137 have been well described (27,39,40). One of the possibilities is that the scavenging of reactive oxygen species and the reduction of neutrophil activation had a significant impact on liver microvasculature. Additionally, attenuation of liver sinusoidal perfusion failure may have also been mediated through a nitric oxide pathway, which both CO and H_2S are known to influence (41).

Hepatic cells undergo cell death in response to ischemia, but more so upon reperfusion (42). The mechanism for hepatocellular death remains controversial, but may be due to apoptosis, necrosis or a pathway common to both, termed necroptosis (43). In our study, hepatocellular death in both the CORM-3 and GYY4137 groups was significantly less than that in the iCORM-3 group, but greater than in sham (Figure 3.4). Given the timing of our pharmacologic therapy administration (i.e. just before decompression), the data suggests that the reperfusion injury is responsible for the majority of cell death, reduced by both CORM-3 and GYY4137. Again, the effect is likely multi-factorial, due to attenuation

of inflammation, but also through direct suppression of apoptotic and necrotic cell pathways (31,44–46).

Leukocytosis was demonstrated in post-sinusoidal venules (rolling and adhesion to the endothelium), as well as through measurement of MPO in liver, small intestine and lung after ACS (Figure 3.5 and Figure 3.6, respectively). Our results are consistent with those of previous animal models of ACS demonstrating an inflammatory response, which then led to multi-organ failure (47). While we did not directly measure pro-inflammatory cytokines in this particular study, it has been well established that I/R results in upregulation of TNF-α, interleukins and various chemokines, which, in turn, leads to downstream activation of leukocytes (47). In our study, we found a differential effect of CORM-3 and GYY4137 on leukocyte rolling. CORM-3, but not GYY4137, reduced leukocyte rolling in postsinusoidal venules. Leukocyte rolling and adhesion are mediated by differential expression of various adhesion molecules (selectins, Ig superfamily, respectively), both of which pre-empt transmigration (48). The evidence for CO (and CORM-3) on leukocyte rolling and adhesion is more straightforward than that found for H_2S: there are consistent findings of anti-inflammatory effects, including down-regulation of selectins and integrins (49–51). The actions of H_2S are less straightforward. Experimental evidence has identified H_2S as a pro-inflammatory or anti-inflammatory, depending on the administered dose, rate of release and donor molecule (38,52–54). Our data certainly suggests that GYY4137 has anti-inflammatory effects, and is consistent with other reports on this donor molecule (31,38,54). Given that we did not investigate the molecular mechanics of

inflammation in our *in vivo* studies, it is challenging to draw firm conclusions on the differential effect of CO and H_2S on leukocyte rolling; however the effect of both substances on leukocyte adhesion is clear.

Further evidence that H_2S and CO in fact acted as anti-inflammatory agents in our experiment was seen with regards to their effect on tissue MPO levels. MPO reflects accumulation of tissue polymorphonuclear cells (PMN), as MPO in neutrophils is constant (55). In our study, we found that ACS resulted in significant increases of MPO, particularly in lung, liver and small intestine. MPO has been shown to be elevated in response to ischemia, but even more so in reperfusion (56). In addition, elevation of MPO has been suggested as a marker of organ damage rather than that of PMN aggregation (57). Previously, CORM-3 has been shown to not only reduce MPO release, but also ameliorate the catalytic activity of MPO on endothelial cells (58). While the degree of MPO reduction is less for GYY4137 compared to CORM-3, this may be due to differences in local cellular levels. In animal models using H_2S donor NaHS, MPO levels were found to actually increase (59,60), providing further evidence for the biphasic action of H_2S (54). Without measuring tissue levels of H_2S or demonstrating a dose response, it is challenging to draw conclusions regarding the reduced anti-inflammatory effect of H_2S compared to CO, other than to conclude that at the doses given, CORM-3 had a greater effect overall.

The ACS-associated rise in serum markers of tissue injury and organ function was significantly reduced in GYY4137 and CORM-3 groups, as evident by levels of liver and renal enzymes (Figure 3.7 and Figure 3.8, respectively). Their

effect of hepatic hypoperfusion may be significantly less, compared to the reperfusion injury, similar to what we had observed with MPO levels (19). The acute renal failure associated with ACS is usually related to renal vein constriction (61); in our study, the effect was attenuated by the administration of CORM-3 and GYY4137 (28,31,62,63). This would suggest that the mechanism of protection may be related to the anti-oxidant properties of these molecules, contributing to the reduction in reactive oxygen species. Multiple authors have demonstrated previously, in cardiac ischemia-reperfusion injury, that CORM-3 was able to reduce tissue oxidative stress (29,64). Similar results have been found for GYY4137 in cardiac I/R models (65) and endotoxic shock (31), where similar protective effects on serum biomarkers, as well as liver and renal function were observed.

Our study was not without limitations. First, we used only one dose of GYY4137 and CORM-3, and therefore did not generate a dose-response relationship, although we surmised, based on earlier experiments, that one may exist (28,31). Another limitation is that our model of ACS represented a single insult, although in the clinical settings the IAP can fluctuate. Finally, GYY4137 and CORM-3 were given after a period of elevated IAP, and while significant protective effects were demonstrated, the role of administration prior to the development of ACS is unclear. It is possible that identification of patients at risk of IAH/ACS, and pre-emptively medicating them may be a better alternative than treating them after the syndrome had already developed.

Development and testing of therapeutics to reduce tissue injury in the setting of abdominal compartment syndrome is important, particularly with

increased use of early decompressive laparotomy for ACS (66,67). The high mortality associated with ACS is likely multifactorial, and related not only to the underlying pathology causing elevated abdominal pressures, systemic organ failure and tissue ischemia, but also the reperfusion injury following decompression (68). Our results suggest both CORM-3 and GYY4137 are promising pharmacologic avenues for further investigation, capable of reduction of the ischemia-reperfusion injury in ACS. Future work should evaluate the use of these substances in the setting of on-going IAH, which is more common clinically.

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CHAPTER 4

GENERAL DISCUSSION

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4.1 OVERVIEW OF RESULTS

Intra-abdominal hypertension is common; 45% of 285 patients had at least one episode of IAH during admission to the intensive care unit. The type of patient, medical, surgical or trauma did not impact rates of IAH. Further, in our mixed medical-surgical patient population IAH was an independent predictor or mortality; patients with IAH were nearly three times more likely to die in the ICU compared to patients without IAH. While our patient population was different compared to recent reports on IAH, our reported incidence was very similar (Table 2.2). While our study found a relationship between IAH and mortality this has been inconsistent through the literature. The utility of treating IAH aggressively, to potentially reduce mortality, has not been examined. Our final conclusions cannot infer whether IAH is a marker of illness severity or an entity which, when treated, will improve patient outcomes. Indeed, while many non-operative management and preventative strategies have been described, the evidence for effectiveness at reducing IAP and the impact on patient outcomes is limited.

Our study was not designed to examine factors that would predict progression to ACS and had only eight patients (3%) that developed ACS, seven of whom died in the intensive care unit. Drawing conclusions regarding risk factors for ACS, or whether progression to ACS during admission might be preventable is challenging given the small event rate. Further, the nature of a patient's underlying disease process can cloud interpretation of determining management by

laparotomy, as this is against some goals of patient care. Several researchers do suggest that given the improved techniques to manage the open abdomen, decompressive laparotomy should be considered earlier than current recommendations (1). Non-operative strategies for the management of ACS do not currently exist, nor are there options for preventing the reperfusion injury associated with ACS.

The second objective of this thesis was to investigate two potential therapeutics for ACS, CORM-3 and GYY4137. In our rat model of ACS both CORM-3 and GYY4137 reduced the physiologic consequences of ACS. These results corroborate similar animal studies of acute limb compartment syndrome, and add to the evidence surrounding the potential for pharmacologic strategies to be used in areas where surgery has traditionally been mandated. In addition, our results postulate that there may be a role for CORM-3 and/or GYY4137 use in conjunction with surgical treatment, in order to reduce the ischemia-reperfusion injury associated with decompression.

4.2 FUTURE DIRECTIONS

A number of hypotheses were generated from the present work. It remains unknown whether the IR injury of ACS and compartment syndrome in general is clinically relevant at lower IAPs. The fluctuation of IAP over time may be introducing cyclical IR injury. It remains unclear which patients have clinically relevant IAH. Identifying patients in whom IAH is clearly affecting physiology or

who will progress to ACS will be paramount in further study of potential non-operative therapeutics for IAH. More rigorous trials are needed to determine if the measurement and treatment of IAH lead to improved outcomes, such as survival. This should be done in a randomized fashion where new patients to the ICU are randomized to receive IAP monitoring and treatment, or not.

Pharmaceuticals for the treatment of elevated IAP and compartment syndrome are still in early phases of development. The next steps include extension to larger animals, such as pigs, and testing in IAH rather than ACS. The potential population who would benefit is much larger for IAH than ACS. Finally, a combination treatment with both CORM-3 and GYY4137 has shown promise in acute limb compartment syndrome (2).

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APPENDICES

APPENDIX I. RESEARCH ETHICS BOARD APPROVAL

Western Research

Research Ethics

Western University Health Science Research Ethics Board
HSREB Delegated Initial Approval Notice

Principal Investigator: Dr. Kelly Vogt
Department & Institution: Schulich School of Medicine and Dentistry\Surgery,London Health Sciences Centre

HSREB File Number: 106031
Study Title: Incidence of intra-abdominal hypertension in the critically ill
Sponsor:

HSREB Initial Approval Date: December 15, 2014
HSREB Expiry Date: December 15, 2015

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Other		2014/10/14
Data Collection Form/Case Report Form		2014/11/08
Letter of Information & Consent		2014/12/02
Sponsor Protocol	Received for Information	2014/10/14
Western University Protocol		

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Datenoted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB ContinuingEthics Review. If an Updated Approval Notice is required prior to the HSREB Expiry Date, the Principal Investigator is responsible for completing andsubmitting an HSREB Updated Approval Form in a timely fashion.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), theInternational Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical PracticePractices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health CanadaMedical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when theyare presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

icer, on behalf of Dr. Marcelo Kremenchutzky, HSREB Vice Chair

Ethics Officer to Contact for Further Information

Erika Basile	Grace Kelly	Mina Mckhail	Vikki Tran
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APPENDIX II. PATHOPHYSIOLOGY OF IAH

II.1 THE EFFECTS OF ELEVATED IAP ON ORGANS AND SYSTEMS

II.1.1 Gastrointestinal Tract

The arterial and venous blood flow of the gastrointestinal tract is sensitive to elevation of IAP, even with normal cardiac output. Mesenteric artery blood flow and intestinal mucosal blood flow are reduced significantly at pressures of 20mmHg (1,2). Microperfusion of the gut results in significant bacterial translocation after periods of high IAP (1). Evidence of intestinal damage in the mucosa has been determined by the acidosis associated with elevations in IAP; this was further correlated with sepsis, hypotension, renal derangements and death (3). The effect of severe acidosis has been demonstrated in both humans and animals.

The splanchnic ischemia and resulting necrosis of bowel may play a key role in multi-organ dysfunction (4). An uncontrolled inflammatory response in multi-organ dysfunction syndrome (MODS) has been identified as an important pathogenic component (5).

II.1.2 Liver

Hepatic microcirculation is diminished during laparoscopy and is rapidly restored following abdominal deflation (6). In a porcine model of IAH/ACS, hepatic artery and portal venous blood flow were significantly diminished even at pressures as low as 10mmHg (7). Liver function tests following laparoscopy have

been found to be significantly elevated, compared to open operations, and elevated IAP seems to be the cause. The elevations are likely not clinically significant and return to baseline within 48 hours, but this suggests that even transient increases in IAP can influence liver function (8,9). Animal models of ACS have demonstrated significant elevation in liver enzymes, coupled with changes in hepatic microvasculature following periods of high IAP (10).

II.1.3 Renal System

Kron *et al* (11) originally diagnosed ACS following open abdominal aortic aneurysmal repair by oliguria in the setting of adequate resuscitation. The progress to anuria, despite volume expansion, characterizes ACS clinically. While anuria typically does not occur until IAP of 25-30mmHg, oliguria can occur between 15-20mmHg (12,13). The renal system relies on adequate cardiac output, coupled with proportional arterial flow and resistance; these are negatively affected with increasing IAP. Hence, direct compression of the renal veins and cortical arterioles will limit the normal perfusion. even with an adequate cardiac output (14). It has been demonstrated, in a porcine model of ACS, that elevation of renal vein pressure to 30mmHg resulted in a significant decrease in renal arterial blood flow and glomerular filtration, and could only partially be reversed with decompression. In addition, the decreased renal flow naturally increased the circulating levels of anti-diuretic hormone and aldosterone, further contributing to an increase in vascular resistance (15,16).

Clinically, the effect on the renal system (as observed by urinary output) is one of the most profound, both with respect to the oliguria/anuria during ACS, and the almost immediate diuresis following abdominal decompression.

II.1.4 Respiratory System

The relationship between abdominal and thoracic pressures was an early discovery (17). Clinically, IAH and ACS are typically characterized by difficulty to ventilate, with high settings, hypoxia and hypercapnia (18–20). The mechanism appears to be due to diaphragmatic elevation leading to a reduction in intra-thoracic space and dynamic lung compliance (21,22).The resulting ventilation-perfusion anomalies result in hypoxia. Increased thoracic pressure leads to an increased pulmonary vascular resistance (19,20), resulting in pulmonary hypertension (19,20). The effects of IAH/ACS on the respiratory system are perhaps best demonstrated by the almost immediate recovery of respiratory function and ease of ventilation following decompressive laparotomy (11,19).

II.1.5 Cardiac

As abdominal pressure rises, equal force is exerted on all abdominal structures, as established by Pascal's laws. Preload and eventual cardiac output are determined by venous return, which can be impaired at IAP as low at 15mmHg (23). Venous return from both the inferior vena cava and portal system is reduced. Increased thorax pressure and diaphragmatic elevation also impede the superior vena cava and reduce ventricular compliance, shifting the Starling

curve, particularly at high pressures (i.e. >30mmHg) (24). The decrease in cardiac output is compounded by an increase in afterload secondary to IAH (18,23,25–27). Thus, the overall impact on cardiac output is dependent on a number of factors, including volume status, where hypovolemia and PEEP exacerbate the reduction in cardiac output.

II.1.6 Neurological

Reduced cerebral perfusion secondary to elevated abdominal pressure can lead to intra-cranial derangement independent of pulmonary and cardiovascular function (28–30). Intracranial pressure (ICP), as related to IAP, appears to be mediated via raised intra-thoracic pressure, as animals with a median sternotomy do not demonstrate any elevation in ICP with increasing IAP. In the non-critically ill setting, chronic IAH has been suggested to be a cause of benign intra-cranial hypertension in the morbidly obese patients (31).

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APPENDIX III: ANIMAL PROTOCOL APPROVAL LETTER

11.01.14
This is the original approval for this protocol
A full protocol submission will be required in 2018

Dear Dr. Lawendy:

Your animal use protocol form entitled:

Direct and Remote Organ Injury Following Compartment Syndrome

Funding agency Orthopaedic Trauma Association – Direct and Remote Organ Injury Following Compartment Syndrome – Grant #R4889A04 has been approved by the University Council on Animal Care.

This approval is valid from **11.01.13 to 03.31.18** with yearly renewal required.

The protocol number for this project is **2009-083**.

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

ANIMALS APPROVED FOR 4 YEARS

Species	Strain	Other Detail	Pain Level	Animal # Total for 4 years
Rat	Wistar	150-350 g	C	680
Pig	Yorkshire-Landrace	50-60 kg	B	30

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

c.c. R Bihari, T Carter, K Bothwell, P Coakwell

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