Laboratory Investigation

Sepsis reduces isoflurane MAC in a normotensive animal model of sepsis

Ravi Gill FRCA, Claudio Martin FRCPC,
Ted McKinnon FRCPC, Calvin Lam FRCPC,
David Cunningham FRCPC, William J. Sibbald FRCPC

Patients with sepsis often require anaesthesia for surgical procedures. Anaesthesia can be unpredictable and the most haemodynamically stable agents are used. No data are available for the minimum alveolar concentration (MAC) requirements in such patients or in animal models of sepsis. We have characterized the effect of sepsis on the MAC of isoflurane in a normotensive rodent model of sepsis. The minimum inhibitory concentration (MIC) of isoflurane to an identical stimulus was determined for rodents subjected to caecal ligation and perforation (CLP $n = 8$), or sham laparotomy ($n = 7$). The calculated MAC of isoflurane was reduced in the septic animals compared with the sham animals (MAC of isoflurane, CLP = 0.8%, sham = 1.4% $P < 0.003$). No statistical differences were found in the haemodynamic variables measured in either group. Isoflurane leads to haemodynamic stability during anaesthesia in this animal model of sepsis. However, the MAC requirement for isoflurane is reduced by sepsis.

En cas de sepsis, il faut souvent recourir à la chirurgie. Les répercussions anesthésiques sont imprévisibles et il faut utiliser des agents qui assurent la meilleure stabilité hémodynamique possible. Nous ne possédons pas de données sur la concentration alvéolaire minimale (MAC) requise chez les patients septiques ou sur un modèle animal. Nous avons déterminé l'in-

fluence du sepsis sur le MAC de l'isoflurane sur un modèle de rongeur normotensif. La concentration inhibitrice minimale (MIC) de l'isoflurane à un stimulus identique a été déterminée chez des rongeurs soumis à une ligature et une perforation du caecum (CLP $n = 8$), ou une laparotomie factice ($n = 7$). Le MAC calculé de l'isoflurane diminuait chez les animaux septiques comparativement au groupe factice (MAC de l'isoflurane, CLP = 0,8%, factice = 1,4% $P < 0,003$). Nous n'avons pas trouvé de différence statistique pour les variables hémodynamiques entre les deux groupes. L'isoflurane procure la stabilité hémodynamique sur ce modèle animal de sepsis. Cependant le MAC de l'isoflurane est abaissé.

Patients with sepsis often undergo surgical procedures requiring general anaesthesia. All inhalational anaesthetic agents affect the cardiovascular system.¹ These effects range from changes in myocardial performance to alterations in vascular responsiveness and oxygen consumption.² The combination of these effects and those of sepsis makes anaesthesia for such patients demanding and unpredictable.

Anaesthetic agents with the least cardiovascular effects are usually chosen for patients with sepsis and titrated to avoid haemodynamic instability. Yet there are no published data for MAC requirements in such patients or in animal models of sepsis. Experiments that used anaesthetized animal models of sepsis have administered the same dose of anaesthetic to both the septic and sham animals. This approach to anaesthesia does not take into account inter-animal variation to a specific dose of anaesthesia or that there might be any interaction between sepsis and anaesthesia. Thus, inappropriately high or low doses of anaesthesia may have been administered to septic animals which may confound the haemodynamic results observed.³

Anecdotal teaching in anaesthesia often emphasises the

Key words

ANAESTHETICS, INHALATIONAL: isoflurane;
MAC;
SEPSIS.

From the AC Burton Vascular Laboratory, Victoria Hospital, London, Ontario, Canada.

Address correspondence to: Dr. C. Martin, Victoria Hospital, 375 South St., London, Ontario, N6A 4G5.

Accepted for publication 22nd February, 1995.

reduction in anaesthetic requirement in critically ill patients. We have hypothesized that there may be an interaction between anaesthetic requirement and sepsis. Therefore, we have sought to characterize an anaesthetic technique in a previously reported awake model of normotensive sepsis induced by caecal ligation and perforation in rats.⁴ Previous literature suggests that isoflurane may be the inhalational anaesthetic agent of choice in this setting.^{3,5} We believe that it is important to document any interaction between sepsis and the dose requirement for an anaesthetic agent, while monitoring any cardiovascular changes that occur. Therefore, this study tests the hypothesis that sepsis may alter the MAC requirement of isoflurane.

Methods

Animal model

Following approval from the University of Western Ontario's Animal Care and Use Committee, 15 male Sprague-Dawley rats were anaesthetized with halothane, nitrous oxide and oxygen. Catheters were inserted under direct vision into the right external jugular vein and the right carotid artery. A #1Fr thermocouple was inserted via the right carotid artery so that its tip was in the ascending aorta. The lines were tunnelled subcutaneously to the back and passed through a harness to a swivel device which allowed continuous fluid infusion while permitting the animals to move about freely.⁶ The animals were then allowed to recover from anaesthesia.

Four days later the animals were re-anaesthetized with isoflurane. The animals were randomly assigned to undergo caecal ligation and perforation (CLP $n = 8$) or sham laparotomy ($n = 7$). Through a midline laparotomy, the caecum was identified and ligated distal to the ileo-caecal valve so that bowel continuity was preserved. The ileo-caecal mesentery was separated while ensuring that vessels to the caecum were ligated. Two holes were then made in the ante-mesenteric surface of the caecum using a #18-gauge needle. The caecum was then returned to the peritoneum and the incision closed in two layers. The sham operation consisted for bowel manipulation, but the caecum was not ligated or perforated. The animals were then allowed to recover from anaesthesia. Adequate fluid resuscitation was ensured by administering $2 \text{ ml} \cdot 100 \text{ g}^{-1}$, 0.9% saline during the operative procedure. Following the surgery, 0.9% saline was continued at $1 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{hr}^{-1}$. This infusion contained fentanyl, $1 \mu\text{g} \cdot \text{ml}^{-1}$, to provide adequate analgesia, this analgesic regimen was decided upon after a review of postoperative rodent care with the Animal Veterinary Care Services, Animal quarters, University of Western Ontario. The arterial line was maintained patent by continuous infu-

sion through a flush valve which produced a flow of approximately $0.5 \text{ ml} \cdot \text{hr}^{-1}$, 0.9% saline with heparin one $\text{U} \cdot \text{ml}^{-1}$.

Experimental protocol

Twenty-four hours after CLP or sham laparotomy, the CLP animals were reviewed for clinical signs of the development of sepsis. All CLP animals were lethargic, showed lack of interest in their environment, food, hygiene, displayed piloerection and had crusty exudates around their eyes. In contrast, the sham-operated animals were healthy, moving freely and eating. Following this, cardiac output was measured by thermodilution using a $200 \mu\text{l}$ 0.9% saline bolus at room temperature injected into venous line, the thermocouple output was monitored by a cardiac output computer (Cardiotherm 500, Columbus Instruments, Columbus, Ohio). Mean arterial blood pressure was recorded and the heart rate determined from the blood pressure trace (78353B; Hewlett-Packard, Mississauga, Ontario). Respiratory rate was counted by observing chest movements. Anaesthesia was then induced by placing the animal in a glass-enclosed cage with 4% isoflurane in oxygen. After induction the animals were placed supine and their tracheas were intubated with a #16 G Angiocath connected to a calibrated Datex vapour gas analyzer (Type AA-102-27-80) via a three-way stopcock. This allows for a more accurate calculation of MAC.⁷ Following the 24 hr recovery period and prior to commencement of the isoflurane study, the fentanyl infusion was discontinued in both groups, it was a requirement for ethical approval that all animals subjected to major surgery (i.e., laparotomy) with recovery were maintained on an analgesic regimen whilst awake. Anaesthesia was then maintained through a Bain circuit using a recently calibrated mark IV isoflurane vaporizer (Ohio Medical Products). The animals were allowed to breathe 100% oxygen spontaneously through the circuit with fresh gas flow of $3 \text{ L} \cdot \text{min}^{-1}$.

The initial isoflurane concentration was set to 1.5%. However, it became apparent after the first two septic animals that the CLP group did not require this initial dose of isoflurane, and the initial isoflurane concentration was reduced to 1%. After allowing 30 min for equilibration a further set of haemodynamic data was collected. In addition, end-tidal CO_2 and inspired and expired isoflurane concentrations were recorded. The minimum inhibitory concentration of isoflurane was determined for each animal by clamping a 15 cm haemostat at the first ratchet position on the mid-point of the tail. If purposeful movement, as described by Quasha *et al.*,⁸ was observed within one minute the clamp was removed and a positive response was recorded. The isoflurane concentration was increased or decreased in 10% graduations depending

upon the response.^{8,9} Following a further 30 min equilibration period the process was repeated. This was continued until the minimum inhibitory concentration for each animal had been determined. Arterial blood gases were measured at the conclusion of the experiment. At the termination of the experiment all animals were euthanised with pentobarbital *iv*, and a post-mortem examination was performed.

Statistical methods

Descriptive statistics and normal probability plots of the data were examined to verify that the data were normally distributed. Non-paired *t* tests were used to detect any differences between the two groups (CLP and Sham) for each recorded variable. A fully factorial MANOVA was used to calculate differences between the haemodynamic and respiratory values at awake (unanaesthetized), minimum inhibitory (MIC) and maximum concentration of isoflurane; if differences existed, a pairwise comparison using Bonferroni's test was performed to determine between which groups differences existed (i.e., awake, MIC or maximum concentration of isoflurane). Dose response curves (a Kaplan-Meier plot) were constructed in which the percent of animals that have not demonstrated a positive response is plotted against the isoflurane concentration. Note that the values on the ordinate are in descending order. Thus, as the isoflurane concentration is decreased, the number of animals exhibiting a positive response increases. These curves were compared using the Mantel-Haenszel statistic. Statistical significance was taken at the $P \leq 0.05$. All data are reported as means ($\pm$ SEM).

Results

The minimum inhibitory concentration (MIC) and maximum concentration of isoflurane received were different between the CLP ($n = 8$) and Sham ($n = 7$) animals, ($\text{CLP}_{\text{mic}} = 0.68\% \pm 0.06$, $\text{SHAM}_{\text{mic}} = 1.27\% \pm 0.07$, $P < 0.000$), ($\text{CLP}_{\text{max}} = 1.18\% \pm 0.08$, $\text{SHAM}_{\text{max}} = 1.65\% \pm 0.09$, $P < 0.002$). The Kaplan-Meier plot (Figure) demonstrates an increased sensitivity of CLP animals to isoflurane ($P < 0.005$). The median concentration, i.e., MAC of isoflurane that inhibited positive responses was $1.4 \pm 0.12\%$ in the sham group versus $0.81 \pm 0.3\%$ in the CLP rats ($P < 0.003$).

There were no differences between the two groups in heart rate, mean arterial pressure, cardiac output or central venous pressure. No differences existed within groups for these variables in the awake state or at either concentration of isoflurane (Table I).

There was no difference in respiratory rate between groups. However, within both groups there was a decrease in respiratory rate at both concentrations of isoflurane

FIGURE Kaplan-Meier plot for MAC of isoflurane.

compared with the awake state ($P < 0.001$). End tidal carbon dioxide tension was higher in the sham animals at the MIC of isoflurane ($P < 0.05$). In the awake state, temperature was lower in the CLP group than in the sham group ($P < 0.05$). There was no difference in weight of the animals in either group. Arterial PaO_2 and pH measured at the termination of the experiment were reduced in the CLP group ($P < 0.001$) (Table II).

Post mortem examination confirmed the clinical diagnosis of sepsis, as all the CLP animals revealed a necrotic caecum with purulent ascites. The position of the thermistor probe in the ascending aorta was also confirmed. The duration of anaesthesia ranged from one to three hours and appeared to be well tolerated. There were no deaths while the animals were anaesthetized.

Discussion

We have shown that animals subjected to a septic insult (CLP) have a decreased inhalational anaesthetic requirement, expressed by decreased MAC. In addition, we have characterized the haemodynamic effect of anaesthesia in an animal model of normotensive resuscitated sepsis. The determination of MAC has three basic components, an applied noxious stimulus, a defined response and the measurement of end tidal anaesthetic concentration.¹⁰ In this experiment the applied stimulus and response remained constant, with only end tidal anaesthetic concentration being varied.

Many physiological variables have been shown to affect MAC values.⁸ In our study, the CLP animals were more acidotic and showed an increased A-a gradient (decreased PaO_2 for the same FiO_2) compared with the sham animals. However, no animals were hypoxaemic as they were all breathing 100% oxygen, which does not affect anaesthetic requirements.¹¹ Exogenously produced met-

TABLE I Haemodynamic and respiratory data in the awake state, at the minimum inhibitory and maximum concentration of isoflurane

	CLP (n = 8)			SHAM (n = 7)			
		Isoflurane			Isoflurane		
	Awake	0.68%	1.27%	Awake	1.18%	1.65%	P value
HR (min ⁻¹)	407 ± 34	397 ± 34	384 ± 34	395 ± 15	374 ± 15	372 ± 15	NS
MAP (mmHg)	108 ± 7.6	100 ± 7.6	106 ± 7.6	110 ± 5.5	110 ± 5.5	103 ± 5.5	NS
CVP (mmHg)	7.3 ± 1.4	7.2 ± 1.4	6.5 ± 1.3	4.5 ± 2.3	5 ± 0.7	5.3 ± 0.7	NS
CO (ml · min ⁻¹)	189 ± 25	191 ± 28	185 ± 33	164 ± 24	186 ± 21	231 ± 22	NS
RR (min ⁻¹)	106 ± 6.4	74 ± 6	63 ± 6	102 ± 8.6	58 ± 8	58 ± 8	P < 0.001
PETCO ₂ (mmHg)	-	38 ± 2.8	40 ± 2.6	-	48 ± 2.8	46 ± 2.9	P < 0.05
Temp (°C)	36.5 ± 0.4	38 ± 0.4	36.6 ± 0.4	38.2 ± 0.5	36.6 ± 0.5	36.9 ± 0.5	P < 0.05

HR = heart rate; MAP = mean arterial pressure; CVP = central venous pressure; CO = cardiac output; RR = respiratory rate; PETCO₂ = end-tidal carbon dioxide.

TABLE II Descriptive data and arterial blood gases for septic (CLP) and nonseptic (SHAM) rats (± SEM)

	CLP (n = 8)	SHAM (n = 7)	P value
Weight (g)	420 ± 18.5	407 ± 19.11	NS
PaO ₂ (mmHg)	201.1 ± 28.1	361 ± 27.3	P < 0.000
PaCO ₂ (mmHg)	39.3 ± 3.2	44.9 ± 2.2	NS
pH	7.28 ± 0.02	7.35 ± 0.02	P < 0.05

abolic acidosis reduces the MAC of halothane in dogs,¹⁰ but the cause of this effect is still debated. Hypoxia, by an unknown mechanism, produces narcosis. Profound hypoxaemia decreases MAC in animals.¹² This may be due to the associated metabolic acidosis indicating anaerobic metabolism. Occult tissue hypoxia associated with sepsis producing a metabolic acidosis in our model of sepsis may contribute to the decreased anaesthetic requirement that we observed.

Other differences between the two groups of animals in this experiment included PETCO₂. Hypocarbica and hypercarbia within certain limits (21–95 mmHg), do not affect MAC.^{10,13} There are two possible explanations why PETCO₂ was higher in the sham animals at the MIC of isoflurane. Either metabolic acidosis in the CLP animals induced a respiratory compensation that offset the respiratory depression associated with inhalational anaesthesia: in both groups at both levels of anaesthesia the respiratory rate was reduced. Alternatively, the lower PETCO₂ in the CLP animals resulted from hyperventilation due to inadequate anaesthetic depth. However, the MIC is the minimum concentration of isoflurane at which animals did not show any response to stimulation in either group. Therefore, it is unlikely that animals in different groups were at different levels of anaesthesia. In the awake state the CLP animals' temperature was lower than that of the sham animals. This is a repro-

ducible finding in this model of sepsis,⁴ but, during the experimental phase, there were no temperature differences between groups.

Hypotension is associated with a reduced anaesthetic requirement in dogs.¹⁴ However, in this study, mean arterial pressure remained normal, as the model is volume-resuscitated and normotensive. There were no differences in cardiac output between the groups at any time during the study, a finding which we have seen in our model before. However, it should be noted that, due to the small number of animals in each group, this study may not have had the statistical power to determine differences in cardiac profiles.

The haemodynamic response of septic animals to anaesthesia has been examined in two previous studies. Unfortunately, both used endotoxic, hypotensive, shock models of sepsis.^{2,3} Our CLP model satisfies the criteria for the diagnosis of the sepsis syndrome¹⁵ and it is inappropriate to compare our results with these previous studies. It should be noted that both studies^{2,3} showed a protective effect of anaesthesia in a shock model of sepsis.

It is now rare to see patients in septic shock, because of improvements in the recognition of the disease and early fluid resuscitation. From the results of this study on the MAC requirement in sepsis, isoflurane appears to have a broad safety range, as haemodynamic variables were unchanged between either group of animals over a wide concentration range. In conclusion, we have demonstrated the MAC requirement of isoflurane is reduced in a rodent model of normotensive sepsis.

References

- 1 Hoffman W, Banks SM, Alling DW, et al. Factors that determine the hemodynamic response to inhalational anesthetics. *J Appl Physiol* 1991; 70: 2155–63.
- 2 Van der Linden P, Gilbert E, Engelman E, Schmartz D,

- Rood M, Vincent J-L. Comparison of halothane, isoflurane, alfentanil, and ketamine in experimental septic shock. *Anesth Analg* 1990; 70: 608-17.
- 3 Schaefer CF, Brackett DJ, Tompkins P, Wilson MF. Anesthetic-induced changes in cardiovascular and small intestinal responses to endotoxin in the rat. *Circ Shock* 1984; 12: 125-33.
 - 4 Martin CM, Yaghi A, Sibbald WJ, McCormack D, Paterson NA. Differential impairment of vascular reactivity of small pulmonary and systemic arteries in hyperdynamic sepsis. *Am Rev Respir Dis* 1993; 148: 164-72.
 - 5 Stimpfel TM, Gershey EL. Selecting anesthetic agents for human safety and animal recovery surgery. *FASEB J* 1991; 5: 2099-104.
 - 6 Brackett DJ, Schaefer CF, Tompkins P, Fagraeus L, Peters LJ, Wilson MF. Evaluation of cardiac output, total peripheral vascular resistance, and plasma concentrations of vasopressin in the conscious, unrestrained rat during endotoxemia. *Circ Shock* 1985; 17: 273-84.
 - 7 White PF, Johnston RR, Eger EI II. Determinations of anesthetic requirements in rats. *Anesthesiology* 1974; 40: 52-7.
 - 8 Quasha AL, Eger EI II, Tinker JH. Determination and applications of MAC. *Anesthesiology* 1980; 53: 315-34.
 - 9 Mazze RI, Rice SA, Baden JM. Halothane, isoflurane, and enflurane MAC in pregnant and nonpregnant female and male mice and rats. *Anesthesiology* 1985; 62: 339-41.
 - 10 Eger EI II, Saidman LJ, Brandstater B. Minimum alveolar anesthetic concentration: a standard of anesthetic potency. *Anesthesiology* 1965; 26: 756-63.
 - 11 Shim CY, Andersen NB. The effect of oxygen on minimal anaesthetic requirements in the toad. *Anesthesiology* 1971; 34: 333-7.
 - 12 Cullen DJ, Eger EI II. The effects of hypoxia and isovolemic anemia on the halothane requirement (MAC) of dogs. The effect of hypoxia. *Anesthesiology* 1970; 32: 28-34.
 - 13 Bridges BE Jr, Eger EI II. The effect of hypocapnia on the level of halothane anesthesia in man. *Anesthesiology* 1966; 27: 634-7.
 - 14 Tanifuji Y, Eger EI II. Effect of arterial hypotension on anaesthetic requirement in dogs. *Br J Anaesth* 1976; 48: 947-51.
 - 15 ACCP/SCCM Consensus Conference. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Chest* 1992; 101: 1644-55.