Comparison of Isoflurane and Halothane
Safety Margins in Rats

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In rat experiments, the dose-effect curves for three different end points of anesthesia [loss of righting reflex (RR), prevention of movement (PM), and heart rate response (HR) to noxious stimuli] and for the lethal effect (LE) due to cardiovascular depression were determined with isoflurane and halothane. The obtained data were used to calculate LD50/ED50 ratios and standard safety margins (SSM) for assessment of each agent's safety.

It was found that isoflurane provides an equal degree of separation between dose-effect curves for different end points of anesthesia as halothane does. However, isoflurane provides greater margins of safety. The margin between the highest of anesthetic doses—the loss of HR response—and the lethal dose for isoflurane was twice that for halothane (LD50/HR ED50 4.3 vs. 2.2, P < 0.01). The standard safety margin for the loss of HR response was also greater with isoflurane (142 vs. 43, P < 0.05). These results suggest that isoflurane may provide greater cardiovascular safety for anesthesia than halothane does. (Key words: Anesthetics, volatile: isoflurane; halothane. Potency, anesthetic: ED50; LD50.)

AS ANESTHESIOLOGISTS take a more active role in controlling the circulation through pharmacologic means, they become progressively less fearful of the cardiovascular depression inherent in anesthesia.1 Nevertheless, the relative margins of safety, the whole range of which can be measured only in animal experiments, remain an important factor in comparing anesthetic agents.

The aim of the present study was to compare the cardiovascular safety margins for isoflurane and halothane. This comparison was based on two criteria of agent safety: 1) separation between median anesthetic and median lethal doses; and 2) separation between maximally effective anesthetic dose and the dose with a minimal danger of fatal outcome.

Methods

In 255 Sprague-Dawley rats (300–350 g), dose-response curves for three different end points of anesthesia and lethal effect (due to cardiovascular failure) were determined with isoflurane and halothane. Experiments were performed from midmorning to mid-afternoon to minimize the possibility of circadian variation. The rats were anesthetized and kept in a clear chamber with the tail protruding from a special opening. An anesthetic-oxygen non-humidified mixture was directed into the chamber at a rate of 4 l/min. Halothane and isoflurane were vaporized in a Drager® vaporizer and the level in the chamber was monitored with an Engstrom-Emma gas analyzer. Both the vaporizer and analyzer were calibrated with a mass spectrometer (Perkin-Elmer MGA 1100®) in our experimental setting over the anesthetic concentration ranges used in the study. For high concentrations of isoflurane, a copper kettle was used. Rat colonic temperature was monitored (Yellow Springs Inst. Co. UL 43) and maintained at 37.0°C with a heating pad. Each rat was exposed to only one predetermined concentration of anesthetic for 30 min, at which time the presence or absence of the end point of anesthesia was determined. For the lethal end point, rats were tracheotomized and ventilated at 60/min (Harvard S680 Rodent Respirator®) through an endotracheal catheter inserted into a tracheostomy. Tidal volume was adjusted to maintain PaCO₂ at 40 ± 5 mmHg (1.8–2.2 ml).

The following end points of anesthesia were used: 1) loss of righting reflex (RR).2 The test was regarded as positive if a rat failed to right itself (with all four feet on the floor) within 15 s after being placed in a side position. 2) Prevention of purposeful movement response to a noxious stimulus (PM).3 The animals were stimulated for 60 s by placement of a 1-kg weight on the middle of the tail (pressure surface of 0.25 cm²). Only the purposeful movement of the head or legs was considered to be a response. Stiffening, coughing, hyperventilating, or vocalizing were not considered. 3)
Prevention of the heart rate increase in response to a noxious stimuli (HR). Stimulation was the same as for the movement response. A cardiograph triggered by the ECG signals provided records of heart rate on a Grass 7-D^® polygraph. An increase in heart rate of greater than one per cent was regarded as a positive response. In the HR series of experiments, animals breathed spontaneously and respiratory depression was possible. Such depression might influence the heart rate responses to stimulation and affect determination of doses blocking heart rate response. To investigate this possibility, we compared HR ED50 for halothane obtained in animals with spontaneous (n = 30) and controlled respiration (n = 50). The obtained results showed that there was no statistically significant difference between these doses (2.1% vs. 2.2%). 4) The end point for the lethal effect (LE) was 7 mmHg (static pressure) in the femoral artery, with artificial ventilation. We have chosen 7 mmHg as an end point because in our pilot experiments, when the heart had stopped, the pressure in the femoral artery was within the range of 7 to 4 mmHg. It was very close to the level of systemic filling pressure reported by Guyton et al. 5,6

With each of the anesthetics, four series of experiments were performed: to determine the righting reflex, purposeful movement response, heart rate response, and lethal effect. In each set of experiments, five to six groups of rats were used consisting of five to seven rats each. In one group of animals, the inspired concentration of the agent was low enough so that all animals remained unaffected, while in another group, it was high enough so that all were affected. In the three to four remaining groups, the concentrations of the agent were spaced equally between the above-mentioned doses.

For calculation of the dose-effect curves, we used the probit method of statistical analysis. The percentage of positive effects was converted into probit values (multiples of the standard deviation) and plotted against a log of the inspired concentrations. This converts the sigmoid dose-effect curves into a straight line and facilitates comparisons. Calculations were performed with the use of the probit procedure in SAS^® on an IBM® 370 computer. The procedure was a nonlinear least-squares technique based on the method of Finney.

To obtain evidence that the high concentrations of isoflurane and halothane to which the animals were exposed were in fact the brain tissue concentrations, we performed determinations of HR and LE dose-effect curves based on brain anesthetic concentrations. After determination of presence or absence of the end point of anesthesia, the rats were killed while in the chamber by placing a clamp around the neck. The whole brain was removed, homogenized, and transferred to preweighed, capped tubes containing carbon tetrachloride and chloroform. 10,11 After extraction, tissue anesthetic concentration was determined by gas chromatography. 12 Individual values of anesthetic brain concentration and signs of the response were used to generate dose-effect curves. To achieve this, we applied logit quantal analysis which constructs the curve directly from individual observations. 13 All calculations were performed using a logit procedure from SAS.^®

For the assessment of anesthetic safety, we used not only therapeutic ratio (LD50/ED50) but also standard safety margin, 4,14

$$SSM = \frac{LD5 - ED95}{ED95} \times 100$$

that represents the percentage by which the ED95 has
to be increased before LD5 is reached. Since the aim of anesthesia is to achieve the desired anesthetic effect in all individuals without the risk of producing a hazardous effect in any, the standard safety margin has a definite advantage over therapeutic ratio. In contrast to LD50/ED50 index, SSM is influenced not only by the distance between central points of the anesthetic and lethal dose-effect curves, but also by the slopes of these curves. This can be illustrated in figure 1 based on our dose-effect data obtained with the use of thiopental and diazepam. If LD50/HR ED50 was the criterion of agent safety for the effect of prevention of heart rate increase in response to painful stimulation in rats, thiopental and diazepam would be judged equally safe since the distance between HR ED50 and LD50 is approximately the same for both agents. An entirely different evaluation of relative safety is reached, however, if we compare these agents on the bases of their respective SSM. The slope of HR dose-effect curve for thiopental is sufficiently steep so that there is a significant separation between the maximally effective dose and minimally lethal dose. The slope of the HR dose-effect curve for diazepam is much flatter than that for thiopental and the lethal dose-effect curve for diazepam is also flatter. As a consequence, the upper part of the HR dose-effect curve for diazepam overlaps the lower part of its lethal curve (fig. 1). In the case of diazepam, HR dose-effect curve does not parallel the lethal dose-effect curve for thiopental.5

**Fig. 2.** Isoflurane dose-effect curves for different end points of anesthesia and lethal effect. The ordinate represents the percent of animals (on a probit scale) that reached the required end point. The abscissa shows the dose (inspired per cent) on a logarithmic scale. RR = loss of righting reflex; PM = prevention of purposeful movement in response to a noxious stimuli; HR = prevention of heart rate increase in response to a noxious stimuli; and LE = lethal effect due to cardiovascular depression.

**Fig. 3.** Halothane dose-effect curves for different end points of anesthesia and lethal effect.
ISOFLURANE AND HALOTHANE SAFETY MARGINS

TABLE 1. ED50 of Isoflurane and Halothane for Different End Points of Anesthesia and Lethal Effect in Rats

<table>
<thead>
<tr>
<th>End Point</th>
<th>Isoflurane (ED50)</th>
<th>Halothane (ED50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR</td>
<td>0.8 (0.7–0.9)*</td>
<td>0.6 (0.6–0.7)</td>
</tr>
<tr>
<td>PM</td>
<td>1.7 (1.6–1.8)</td>
<td>1.1 (1.0–1.3)</td>
</tr>
<tr>
<td>HR</td>
<td>2.9 (2.6–3.2)</td>
<td>2.1 (2.0–2.5)</td>
</tr>
<tr>
<td>LE</td>
<td>12.5 (11.1–14.6)</td>
<td>4.7 (4.6–4.9)</td>
</tr>
</tbody>
</table>

*Ninety-five per cent fiducial limits.

RR = loss of righting reflex; PM = prevention of purposeful movement in response to a noxious stimulus; HR = prevention of increase in heart rate in response to a noxious stimulus; and LE = lethal effect (due to cardiovascular depression).

effect curve, and the ratio between lethal and HR effect doses is different at different levels of response. This is an illustration that LD50/ED50 index may sometimes give a false assessment of the relative safety of agents. At the same time LD5/ED95 ratio (SSM) reflects the slopes of the curves and, therefore, can give additional valuable information regarding agent safety.

Results

Dose-effect curves reflecting different end points of anesthesia and lethal effect (due to cardiovascular depression) for isoflurane and halothane are presented in figures 2 and 3. Central points of the dose effect curves represent median effective doses (ED50 or LD50). These points are summarized numerically in table 1. According to this table, the degree of separation between doses producing the loss of righting reflex, prevention of purposeful movement response, and prevention of heart rate response was similar for isoflurane and halothane. As a result, we could not find statistically significant differences between the ratio HR ED50/RR ED50 (or HR ED50/PM ED50) for isoflurane and halothane (see table 2). However, the margin between any of the median anesthetic doses (RR ED50, PM ED50, and HR ED50) and the median lethal dose (LD50) was greater for isoflurane than for halothane.

Standard safety margins at different end points of anesthesia for isoflurane and halothane are presented in figure 4, which shows that isoflurane standard safety margins are wider than those of halothane. The SSM with the highest of anesthetic doses—the loss of HR response—was approximately three times greater for isoflurane than for halothane—142 vs. 43, P < 0.05 (fig. 5). HR and LE dose-response curves based on brain anesthetic concentrations also demonstrated a greater margin of safety with isoflurane (fig. 6).

Discussion

Our results show that isoflurane provides a degree of separation between dose-effect curves for different end points of anesthesia which are comparable to halothane. If we define the distance between the dose that induces the loss of righting reflex in the most sensitive animals and the dose that induces the abolition of the heart rate response in the least sensitive animals as the range of anesthetic doses, it is possible to say that both agents have similar width of the range. At the same time, the location of the lethal dose-effect curve along the dose axis regarding this range of anesthetic doses was different for both drugs. Since the lethal effect was determined in our experiments with controlled ventilation, death was a result of cardiovascular depression.

**Table 2. Ratios Reflecting Separation between Median Effective and Lethal Doses for Isoflurane and Halothane**

<table>
<thead>
<tr>
<th>Ratios</th>
<th>Isoflurane</th>
<th>Halothane</th>
<th>Statistical Comparison [t vs. HR*]</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR ED50/RR ED50</td>
<td>3.58</td>
<td>3.35</td>
<td>NS</td>
</tr>
<tr>
<td>HR ED50/PM ED50</td>
<td>1.66</td>
<td>1.86</td>
<td>NS</td>
</tr>
<tr>
<td>LD50/RR ED50</td>
<td>15.35</td>
<td>7.40</td>
<td>0.01</td>
</tr>
<tr>
<td>LD50/PM ED50</td>
<td>7.10</td>
<td>4.15</td>
<td>0.01</td>
</tr>
<tr>
<td>LD50/HR ED50</td>
<td>4.32</td>
<td>2.21</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* Statistical values denote P less than stated; NS indicates P > 0.05.

**Figure 4. Standard safety margins (SSM) for isoflurane and halothane with different end points of anesthesia. SSM shows the percentage by which ED95 has to be increased before LD5 is reached. RR = righting reflex; PM = purposeful movement response; and HR = heart rate response to a noxious stimuli.**
Fig. 5. Isoflurane and halothane standard safety margins for the heart rate effect calculated on the basis of inspired anesthetic concentrations. The ordinate represents (on a probit scale) the per cent of animals that reached the end point. The abscissa shows (on a logarithmic scale) the inspired anesthetic concentration. HR = prevention of heart rate increase in response to a noxious stimulus; and LE = lethal effect.

Fig. 6. Isoflurane and halothane standard safety margins for the heart rate effect calculated on the basis of brain anesthetic concentrations. The ordinate represents (on a probit scale) the per cent of animals that reached the end point. The abscissa shows (on a logarithmic scale) the brain anesthetic concentrations.

The results show that the lethal dose-effect curve for isoflurane differs significantly from that for halothane. First, the distances between the anesthetic dose-effect curves and the lethal dose-effect curve are greater with isoflurane. Second, the slope of the isoflurane lethal dose-effect curve is flatter than that of halothane.

With all three end points of anesthesia—loss of righting reflex, abolition of movement response, and abolition of heart rate response—SSM for isoflurane was always greater than for halothane (see fig. 4). SSM for the heart rate response was calculated not only on the basis of inspired anesthetic concentrations, but also on the basis of brain anesthetic concentrations (compare figs. 5 and 6). In both cases, SSM was greater with isoflurane than with halothane.

Our data on the greater distance between anesthetic and lethal doses with isoflurane are in agreement with the results reported by Wolfson and associates.11 In rat experiments, they measured the brain concentration of anesthetics at the end point of abolition of movement response to tail clamping and at the end point of significant electrocardiographic changes (bradycardia, heart block, or deepened S wave). According to their results, the ratio of toxic concentration to the anesthetic concentration was greater with isoflurane as compared with halothane.
Thus, halothane and isoflurane have a similar degree of separation between dose-response curves for anesthetic end points. At the same time, isoflurane has a greater separation between dose-response curves for anesthetic effects on one hand, and cardiovascular failure on the other. One can speculate that this is because the difference between the CNS and the heart in sensitivity to the anesthetic is greater with isoflurane than with halothane. Such an explanation agrees with the fact that with equianesthetic doses, isoflurane decreases cardiac output to a lesser degree than halothane.\(^1\)\(^5\) Although the variability in anesthetic requirements from species to species is remarkably small, the extent to which our data relate to humans is uncertain. The presented results suggest that isoflurane provides greater cardiovascular safety for anesthesia than halothane.

References