Halothane Reversibly Inhibits Human Neutrophil Bacterial Killing

William D. Welch, Ph.D.*

The effect of halothane, at clinically relevant concentrations, on the ability of human polymorphonuclear leukocytes (PMNL) to kill the most frequently isolated gram-negative organisms responsible for human bacteremias, *Escherichia coli* and *Klebsiella pneumoniae*, was studied. Exposure of PMNL to 0.75 per cent halothane in air significantly inhibited the killing of *E. coli* (from 81 per cent to 65 per cent) but not *K. pneumoniae*. At 1.0 per cent halothane there was no killing of *E. coli* and the killing of *K. pneumoniae* was reduced from 98 per cent to 82 per cent. With 1.5 per cent halothane, the killing of *K. pneumoniae* by PMNL was further reduced to 65 per cent. This inhibition of bacterial killing could be reversed after exposure of halothane-treated PMNL to air. The mechanism of inhibition may be due in part to a deleterious effect of halothane on the oxidative microbicidal activity of human PMNL. Although halothane reversibly inhibits the ability of PMNL to kill bacteremic culture isolates, the degree of susceptibility of bacteria to halothane-treated PMNL may vary. (Key words: Anesthetics, volatile: halothane. Bacteria: killing. Blood: neutrophils.)

POLYMORPHONUCLEAR LEUKOCYTES (PMNL) provide a crucial defense against invading microorganisms. Deter- rimental effects of commonly used general anesthetic agents on PMNL function are thus of obvious importance. The volatile anesthetic, halothane, has been shown to inhibit PMNL chemotaxis and either reduce or have no effect on PMNL phagocytosis. In the latter phagocytosis studies, bacterial killing by PMNL was not determined; only the ingestion of bacteria or latex beads by PMNL following halothane exposure was measured. The present report investigates the effect of clinical concentrations of halothane on the ability of human PMNL to destroy bacteria. The two most commonly isolated etiologic agents of gram-negative bacteremias, *Escherichia coli* and *Klebsiella pneumoniae*, were chosen as test organisms. Using a standard bactericidal assay consisting of human serum, bacteria, and PMNL, a dose-dependent significant inhibition of PMNL bacterial killing was seen with halothane. This inhibition was reversed by exposure to air.

Materials and Methods

PREPARATION OF HUMAN POLYMORPHONUCLEAR LEUKOCYTES (PMNL) AND POOLED HUMAN SERUM

Venous blood was obtained from normal healthy volunteers. PMNL were separated from heparinized blood by sedimentation with plasmagel† at a ratio of 1:4 plasmagel to blood for 45 min at room temperature. The PMNL-rich supernatant was then further processed in Hanks buffer pH 7.40, to yield approximately 85–90 per cent PMNL as previously described.  

For preparation of pooled human serum, venous blood from six donors was allowed to clot for 30 min at room temperature and then centrifuged at 270 × g for 10 min. The individual donor serum was removed, combined in equal volumes and kept at −80°C in 0.5-ml aliquots until needed.

BACTERIAL ISOLATES

*Escherichia coli* 0101 K nontypable is a blood culture isolate from the University of California, Los Angeles, Medical Center. *Klebsiella pneumoniae* is a blood culture isolate obtained from the University of California, Irvine Medical Center. Both isolates were cultured on blood agar plates and stored in whole human blood at −80°C. For bactericidal assays, the isolates were transferred to brain-heart infusion broth, incubated for 2 to 3 h at 37°C, washed twice with sterile saline, and adjusted to a concentration of 1 × 10⁸ bacteria/ml by comparing to an appropriate McFarland Standard.

BACTERICIDAL ASSAY

The bactericidal assay used was a modification of the method described by Cross and Lowell. Briefly, the reaction mixture consisted of 0.05 ml of 1 × 10⁷ PMNL/ml, 0.01 ml of 1 × 10⁸ bacteria/ml 0.01 ml of pooled human serum, and Hanks buffer to a final volume of 0.1 ml. The assay was performed in sealed microtiter plates which were gently mixed at 37°C on a Cooke microtiter shaker at the #3 setting. After the incubation period, dilutions of the reaction mixtures were made in cold, sterile, distilled water and the number of viable bacteria (colony forming units, [CFU]) determined by growth in trypticase soy agar pour plates. The net change (Δ) in CFU was determined as follows: ΔCFU = CFU at end of assay − CFU at beginning of assay. The percentage of bacteria killed was calculated by the following equation:

\[
\text{per cent bacteria killed} = 100 \times \frac{(\text{CFU at beginning of assay} - \text{CFU at end of assay})}{\text{CFU at beginning of assay}}
\]

† Roger Bellon Laboratories, Neuilly, France.
§ Serotyped by Dr. Bertil Kaijer, Institute of Microbiology, University of Goteborg, Sweden.

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Address reprint requests to Dr. Welch.

† This study was approved by the Institutional Human Subjects Review Committee.

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650
INHIBITION OF NEUTROPHIL BACTERIAL KILLING

TABLE 1. Effect of Halothane on PMNL Killing of Escherichia coli

<table>
<thead>
<tr>
<th>Components in Individual Experiments</th>
<th>Per Cent Halothane (H)/Time Exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 Per Cent H/60 min</td>
</tr>
<tr>
<td>PMNL, E. coli + pooled human serum*</td>
<td>76 ± 7; (−548 ± 90)††</td>
</tr>
<tr>
<td>E. coli + pooled human serum</td>
<td>0 ; (+208 ± 34)</td>
</tr>
<tr>
<td>E. coli only</td>
<td>0 ; (−91 ± 9)</td>
</tr>
<tr>
<td></td>
<td>0.75 Per Cent H/60 min</td>
</tr>
<tr>
<td>PMNL, E. coli + pooled human serum</td>
<td>67 ± 12; (−322 ± 101)‡</td>
</tr>
<tr>
<td>E. coli + pooled human serum</td>
<td>0 ; (+121 ± 12)</td>
</tr>
<tr>
<td>E. coli only</td>
<td>0 ; (+102 ± 11)</td>
</tr>
<tr>
<td></td>
<td>1.0 Per Cent H/60 min</td>
</tr>
<tr>
<td>PMNL, E. coli + pooled human serum</td>
<td>0 ; (+94 ± 19)§</td>
</tr>
<tr>
<td>E. coli + pooled human serum</td>
<td>0 ; (+110 ± 22)</td>
</tr>
<tr>
<td>E. coli only</td>
<td>0 ; (+98 ± 11)</td>
</tr>
<tr>
<td></td>
<td>1.5 Per Cent H/60 min</td>
</tr>
<tr>
<td>PMNL, E. coli + pooled human serum</td>
<td>0 ; (+152 ± 19)§</td>
</tr>
<tr>
<td>E. coli + pooled human serum</td>
<td>0 ; (+99 ± 10)</td>
</tr>
<tr>
<td>E. coli only</td>
<td>0 ; (+56 ± 11)</td>
</tr>
</tbody>
</table>

* E. coli and pooled human serum were added after PMNL had been exposed to halothane.
† Number before semi-colon are per cent values of bacteria killed. Numbers after semi-colon are the net increases (+) or decreases (−)
‡ P < 0.05, one-way analysis of variance.
§ P < 0.001, one-way analysis of variance.

EXPOSURE OF BACTERICIDAL ASSAY TO HALOTHANE

Bacterial assay reaction components were placed in a gas tight chamber and exposed to the desired concentrations of vaporized halothane in air (Ohio® DM 5000 vaporizer) at a flow rate of 400 ml/min via inflow and exit valves. Halothane concentrations were monitored at the chamber exit valve using a Hewlett Packard® 5730 A gas chromatograph (50/80 Poropak Q® glass column) with a 3380S integrator. For control experiments only air was administered to the chamber with reaction components. To determine if the air halothane mixture altered pH, $P_{O_2}$ or $P_{CO_2}$ of the buffer, these variables were measured after exposure of the buffer to air or halothane for one hour, using an IL 213 pH blood-gas analyzer. No significant difference in pH, $P_{O_2}$ or $P_{CO_2}$ was seen between halothane and air-exposed buffer.

DETERMINATION OF THE PARTITION COEFFICIENT BETWEEN HALOTHANE AND HANKS BUFFER

In order to quantitate the actual amount of halothane in Hanks buffer, which was used in all PMNL studies, the partition coefficient between halothane and Hanks buffer was determined. Using the method of Fink and Morikawa, the partition coefficient between Hanks buffer and halothane at 24°C was found to be 1.74 ± 0.20 (n = 10). The partition coefficient was calculated at 24°C as this was the temperature during anesthetic exposure of the bactericidal assay components and could be performed without the elaborate equipment or experimental design that would be required for a similar determination at 37°C. Although bactericidal experiments were carried out at 37°C, the per cent halothane exposure of the bactericidal assays were in accordance with in vivo clinical blood levels based upon the partition coefficient at 24°C. The bactericidal assay at 24°C demonstrated identical trends to those seen at 37°C (tables 1 and 2), but took considerably longer to achieve, approximately 2.5 to 4 h (data not given).

STATISTICAL ANALYSIS

Mean, standard deviation, and one-way analysis of variance were determined using a Monroe® 1860 calculator.

Results

EFFECT OF HALOTHANE ON HUMAN PMNL BACTERICIDAL ACTIVITY

In the absence of PMNL, blood culture isolates of E. coli and K. Pneumoniae were not killed with or without
### Table 2. Effect of Halothane on PMNL Killing of Klebsiella pneumoniae

<table>
<thead>
<tr>
<th>Components in Individual Experiments</th>
<th>0.5 Per Cent H/60 min</th>
<th>0.75 Per Cent H/60 min; Air/30 min</th>
<th>1.5 Per Cent H/60 min; Air/30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMNL, <em>K. pneumoniae</em> + pooled human serum*</td>
<td>92 ± 7; (−221 ± 31)†</td>
<td>96 ± 5; (−259 ± 42)</td>
<td>96 ± 4; (−240 ± 30)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> + pooled human serum</td>
<td>0; (−123 ± 21)</td>
<td>0; (−189 ± 16)</td>
<td>0; (−108 ± 21)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> only</td>
<td>0; (−203 ± 20)</td>
<td>0; (−269 ± 21)</td>
<td>0; (−141 ± 29)</td>
</tr>
<tr>
<td>PMNL, <em>K. pneumoniae</em> + pooled human serum</td>
<td>89 ± 1; (−200 ± 20)</td>
<td>97 ± 3; (−216 ± 21)</td>
<td>95 ± 2; (−210 ± 19)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> + pooled human serum</td>
<td>0; (−162 ± 54)</td>
<td>0; (−185 ± 18)</td>
<td>0; (−102 ± 16)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> only</td>
<td>0; (−140 ± 28)</td>
<td>0; (−234 ± 22)</td>
<td>0; (−121 ± 26)</td>
</tr>
<tr>
<td>PMNL, <em>K. pneumoniae</em> + pooled human serum</td>
<td>82 ± 6; (−490 ± 141)‡</td>
<td>99 ± 2; (−536 ± 47)</td>
<td>98 ± 1; (−640 ± 103)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> + pooled human serum</td>
<td>0; (−199 ± 30)</td>
<td>0; (−252 ± 99)</td>
<td>0; (−139 ± 19)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> only</td>
<td>0; (−201 ± 80)</td>
<td>0; (−290 ± 72)</td>
<td>0; (−90 ± 15)</td>
</tr>
<tr>
<td>PMNL, <em>K. pneumoniae</em> + pooled human serum</td>
<td>65 ± 8; (−326 ± 49)§</td>
<td>97 ± 2; (−605 ± 104)</td>
<td>94 ± 2; (−580 ± 96)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> + pooled human serum</td>
<td>0; (−138 ± 61)</td>
<td>0; (−298 ± 22)</td>
<td>0; (−150 ± 25)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> only</td>
<td>0; (−140 ± 29)</td>
<td>0; (−229 ± 36)</td>
<td>0; (−170 ± 30)</td>
</tr>
</tbody>
</table>

* K. pneumoniae and pooled human serum were added after PMNL had been exposed to halothane.
† Numbers before semi-colon are per cent values of bacteria killed. Numbers after semi-colon are net increases (+) or decreases (−) in colony-forming units × 10^2 at the conclusion of the experiment. Results shown represent the mean ± SD of four separate experiments.
‡ P < 0.05, one-way analysis of variance.
§ P < 0.001, one-way analysis of variance.

10 per cent (v/v) pooled human serum in the reaction mixture, or after exposure to 0.5, 0.75, 1.0, or 1.5 per cent air-vaporized halothane, or air only (tables 1, 2). However, a slight decrease in the number of CFU of *E. coli* was observed with increasing halothane concentrations (table 1). Exposure of PMNL to 0.50 per cent halothane for 60 min did not significantly reduce their ability to kill *E. coli* or *K. pneumoniae* isolates. PMNL exposed to 0.75 per cent halothane demonstrated significantly less killing of *E. coli* but not *K. pneumoniae* as compared to air exposed controls. If a 30-min air exposure was given to PMNL after the halothane exposure, the inhibition of killing of *E. coli* was completely removed. Increasing the halothane concentration to 1.0 per cent abolished all PMNL killing of *E. coli* (zero per cent bacteria killed; an increase of 94 × 10^5 CFU over the initial inoculum), and also PMNL killing of *K. pneumoniae* (P < 0.05). The complete inhibition of killing of *E. coli* was significantly reduced by exposing the reaction mixture to air for 30 min following halothane exposure (from 0 to 80 per cent bacteria killed). Increasing the halothane exposure concentration to 1.5 per cent in the greatest inhibition of killing for both *E. coli* (zero per cent killed with an increase of 152 × 10^3 CFU over the initial inoculum) and *K. pneumoniae* (65 per cent bacteria killed with a decrease of only 326 × 10^3 CFU as compared to 82 per cent bacteria killed and a decrease in CFU of 489 × 10^3 at a halothane concentration of 1 per cent). Again, this inhibition was completely reversed after exposure to air.

**Discussion**

The question of whether or not general anesthetic agents can predispose the host to infection via depression of the reticuloendothelial system has been the subject of many investigative efforts since the 1900s. Graham, for example, found a significant inhibition of phagocytosis of streptococci by human leukocytes in the presence of ether, and Hamburger showed that chloroform inhibited the ability of equine leukocytes to phagocytose carbon particles. A correlation between anesthesia, surgery, and reduced phagocytic function was suggested by Kosciolk in studies where PMNL from patients anesthetized with halothane demonstrated a decrease in phagocytosis of *S. aureus* for up to 24 h after surgery. The possibility that anesthesia and surgery may increase the chances of bacterial infection was implied by Cruse who observed a greater incidence of wound infection in patients undergoing long periods of anesthesia and surgery. Although some aspects of the effect of halothane on phagocyte microbicidal function have been reported,
the effect of halothane or other general anesthetics on in vitro PMNL bactericidal activity has not. Most in vitro studies have examined the effect of halothane on the ability of the PMNL to phagocytose either the bread yeast Saccharomyces cerevisiae or latex beads.

In the present study, exposure of human PMNL to 1.0 and 1.5 per cent halothane caused a significant decrease in the ability of PMNL to kill blood culture isolates of E. coli and K. pneumoniae. These organisms were chosen based on their high frequency of isolation from bacteremic patients and thus represent bacterial pathogens likely to be encountered by PMNL. A significant inhibition of PMNL killing of E. coli but not K. pneumoniae was also seen with 0.50 and 0.75 per cent halothane. However, even at the most inhibitory halothane concentration tested (1.5 per cent), exposure of halothane-treated PMNL to air resulted in an improvement of bacterial killing of E. coli from 0 to 65 per cent bacteria killed (table 1) and for K. pneumoniae from 65 to 97 per cent bacteria killed (table 2).

The minimal alveolar concentration (MAC) of halothane is 0.77 per cent. Since the partition coefficient between halothane and blood is 2.3,15 1 and 1.5 MAC of air-vaporized halothane would result in 1.77 and 2.65 per cent halothane in blood, V/V. In our bactericidal assay, Hanks buffer was used which was found to have a partition coefficient of 1.74 ± 0.20 with halothane. Thus, the per cent dissolved halothane that inhibits PMNL killing of E. coli was 1.3 per cent (0.75 per cent air/halothane) and for K. pneumoniae 1.75 per cent (1 per cent air/halothane), both of which could be achieved in vivo with 1 MAC halothane.

The microbicidal activity of phagocytic cells, especially PMNL, is primarily dependent upon oxidative pathways which are capable of generating highly reactive microbicidal species of oxygen such as superoxide anions, hydrogen peroxide, hypohalite ions, and hydroxyl radicals.16,17 PMNL enzyme systems responsible for producing these metabolites include NADPH-oxidoreductase and H2O2-myeloperoxidase. Non-oxidative microbicidal pathways also present in PMNL include lysozyme and cationic proteins which probably serve as adjunct systems.18,19 What microbicidal mechanisms are being effected by halothane in the present report may only be postulated. However, preliminary results from this laboratory have shown an inhibition in the chemiluminescence response of human PMNL after exposure to halothane.18 Chemiluminescence or the emission of light by phagocytosing PMNL is intimately linked to oxidative microbial pathways in PMNL.19,20 The lesser susceptibility of E. coli to halothane-treated PMNL than K. pneumoniae reported here may be due to a higher sensitivity of K. pneumoniae to non-oxidative microbicidal mechanisms.

PMNL tonometered in halothane were shown by Rosenbaum and Orkin10 to ingest the same number of yeast particles as nonhalothane-treated PMNL. PMNL exposed to halothane in vitro and in vivo were reported by Cullen4,21 to have slightly fewer latex beads ingested than control PMNL. However, PMNL which are defective or deficient in the H2O2-myeloperoxidase or NADPH-oxidoreductase microbicidal systems are effectively able to phagocytose, but not kill, bacteria or yeast.22,23 The fact that an organism is ingested by a PMNL does not necessarily infer that killing will follow. These two processes are separate and mediated by different structural and physiologic components in the PMNL. Although Rosenbaum and Orkin, and Cullen failed to show that halothane inhibited phagocytosis, their studies do not demonstrate possible impairment of normally operative PMNL microbicidal systems. Additionally, halothane-exposed PMNL in the in vivo experiments by Cullen were separated by sedimentation and washing in nonhalothane tonometered buffer. It is likely that little, if any, halothane was present in the final reaction mixture, making the interpretation of such results difficult. In contrast to these studies, Bruce3 showed a ten-fold decrease in the number of bacteria phagocytosed by rat peritoneal neutrophils following halothane anesthesia. Cultures of peritoneal fluid in this study qualitatively demonstrated more bacterial colony forming units in the anesthetized mice compared to unanesthetized, suggesting reduced neutrophil bactericidal function in vivo.

Nunn et al. recently examined the effect of halothane on several parameters of human PMNL function. They found minimal inhibition in the phagocytosis of latex beads or in degranulation of primary and secondary (lysozyme containing) granules into phagocytic vacuoles between halothane treated and untreated PMNL. Oxygen consumption of PMNL decreased slightly when exposed to halothane concentrations from 1 to 3 per cent but the values remained within two standard deviations of control measurements. As previously mentioned, normal phagocytosis, but not bacterial killing of E. coli or K. pneumoniae by PMNL, can occur in the absence of important microbicidal systems like the myeloperoxidase mediated or NADPH oxidase generators of reactive oxidative species. Additionally, PMNL from patients with chronic granulomatous disease, which are unable to generate superoxide anions due to a defective NADPH enzyme, can degranulate normally and possess normal lysosomal enzymes.24,25 The absence of a significant inhibitory effect of PMNL O2 consumption after ex-

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posure to halothane reported by Nunn et al. does not negate the possibility that impairment of the PMNL microbialid systems may have occurred. For example, PMNL from patients who lack myeloperoxidase consume oxygen normally but are quite defective in killing bacteria.\(^2\) Furthermore, PMNL in the absence of atmospheric \(O_2\), a requirement for optimal microbialid activity, are able to phagocytose at rates equal to PMNL under an aerobic atmosphere.\(^2\)

In conclusion, the effects of clinical concentrations of halothane on the bactericidal ability of human PMNL were studied. The results indicate that a transient inhibition in PMNL bactericidal activity occurs after halothane exposure. Preliminary work from this laboratory suggests that a reduction in oxidative microbialid function in PMNL after treatment with halothane may be partly responsible for the inhibition of bacterial killing.

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References