Alterations in Influenza Virus Pulmonary Pathology Induced by Diethyl Ether, Halothane, Enflurane, and Pentobarbital Anesthesia in Mice

P. R. Knight, M.D., Ph.D.,* E. Bedows, Ph.D.,† M. L. Nahrwold, M.D.,‡ H. F. Maassab, Ph.D.§
C. W. Smitka, M.S.,¶ M. T. Busch, M.S.**

Three-week-old CD-1 mice infected with the PR-8 (mouse-adapted) strain of influenza virus while exposed to enflurane demonstrated a decrease in virus titers from the lungs of infected animals, less abnormality of lung histology, and an increase in survival in animals as compared with those receiving the other anesthetics tested. Greater than 90% mortality occurred in groups of mice which inhaled aerosolized virus and received no anesthesia, pentobarbital, diethyl ether, or halothane anesthesia 96 h following infection. Infected mice anesthetized with enflurane 96 h post-infection had significantly lower mortality rate (68%) when compared with the other groups.

Halothane-anesthetized mice receiving intranasal influenza virus during anesthesia demonstrated increased survival and a delay in the mean day of death when compared with animals receiving either diethyl ether or pentobarbital anesthesia. Animals receiving enflurane during virus inoculation had an even lower mortality rate and a later mean day of death when compared with infected animals receiving any of the other three anesthetics.

Examination of lungs from animals infected during anesthesia demonstrated influenza virus titers significantly less in the animals that received enflurane anesthesia when compared with the other groups. Histologic sections of lungs revealed extensive spread of the disease process into the alveoli and interstitium of the lungs of animals infected while receiving pentobarbital or diethyl ether anesthesia. Animals infected during halothane demonstrated pathologic characteristics similar to pentobarbital- and diethyl-ether-treated groups; however, the changes were not as extensive. Mice infected during exposure to enflurane revealed only a mild bronchopneumonia. (Key words: Anesthesiology, intravenous: pentobarbital. Anesthesia, volatile: diethyl ether; enflurane; halothane. Lung: pneumonia. Viruses: influenza.)

Most anesthesiologists, through training and empirical personal experience, do not electively give anesthesia to patients with respiratory tract infections. Experimental justification for these practices lacks substantiation as documentation on the effects of anesthesia on viral pathogenesis in humans is scant. Several investigators have studied virus infection and anesthesia in laboratory animals. Diethyl ether and pentobarbital anesthesia have been used to facilitate induction of respiratory tract infections by influenza virus in mice and ferrets.1,2 Furthermore, mice infected with murine hepatitis virus have a more severe pathology following exposure to halothane.3 However, administration of diethyl ether to canines infected by distemper virus in-
creases survival. Similarly, mice infected with either Eastern or Western equine encephalitis virus or St. Louis encephalitis virus are protected by diethyl ether anesthesia.

Patients may undergo anesthesia and surgery while incubating a virus infection prior to displaying symptoms. In addition, patients needing emergency surgery while infected require anesthesia regardless of their condition. There is significant cost involved in having a patient admitted to the hospital and subsequently being sent home because of an upper respiratory tract infection. For these reasons, more should be known about the effects of anesthesia on respiratory tract infections.

The virus infection most commonly seen clinically in humans is an upper respiratory tract infection with or without lower respiratory tract involvement. Earlier results suggest that patients with respiratory tract infections who undergo anesthesia and surgery have increased morbidity. However, these reports were poorly documented and the infecting agents (i.e., virus vs. bacteria) were not determined. Moreover, several of the anesthetic agents used in these cases, namely diethyl ether and cyclopropane, do not enjoy significant clinical use in this country today and have been replaced by the newer agents, halothane and enflurane.

Viral infections of the respiratory system in mice are used as a model to evaluate human influenza vaccines and to determine the pathogenicity of influenza virus strains. Influenza virus infection in mice involves both the upper and lower respiratory tracts and the severity of pneumonia and mortality depends upon the concentration of the virus to which the animal is exposed. In the following report we examine the effects of commonly used anesthetics on influenza virus pathogenicity in mice.

**Materials and Methods**

**ANIMALS**

Male, 3-week-old, pathogen-free, CD-1 (ICR), barrier raised mice were purchased from the Charles River Breeding Laboratories Inc., Wilmington, Massachusetts. A total of 610 animals were used in the subsequent studies. The animals were housed in stainless steel cages and fed Purina Lab Chow® and water ad lib. All cages were washed and autoclaved prior to use.

**EXPOSURE OF MICE TO ANESTHESIA**

The mice were anesthetized in polyethylene cages. Anesthetic vapor was delivered to the cage by directing 5 l/min of O₂ and air mixture through a Dräger or Ethane® vaporizer, or a Boyles Bottle. Exhaust was vented to the outside. Anesthetic concentrations were determined by a GOW MAC gas chromatograph. During any single experiment, animals were randomly divided into separate groups and every anesthetic was tested. In the first set of experiments, mice were randomly selected from virus-infected or noninfected populations and anesthetized at various times throughout the experiment for one hour with 1 MAC enflurane, halothane, or diethyl ether. Concentrations of the agents were determined previously to give the greatest degree of anesthesia in mice without mortality. Pentobarbital was given 2 mg/kg intraperitoneally. This resulted in a sleep time of approximately one hour.

In the second set of experiments where mice were inoculated intranasally, animals were exposed to the anesthetic for 15–20 min immediately prior to instillation of the virus onto the external nares. Animals were allowed to recover after virus instillation. Animals were selected randomly in regard to the type of anesthesia and whether they were to be inoculated with virus.

**VIRUS**

The mouse-adapted PR-8 strain of influenza virus was grown in the allantoic fluid of embryonated eggs after serial passage in mice to increase virulence to that animal. Embryonated eggs were obtained from Omega Chicks (Lansing, Michigan). Using an established procedure, influenza virus infectivity was assayed. Briefly, log dilutions of control or lung homogenates were added in a volume of 0.1 ml to the allantoic sac of 9- to 11-day-old embryonated chicken eggs. Eggs were maintained at 35°C. After 48 h, the allantoic fluid was harvested and tested at each dilution for hemagglutination activity. Eggs were scored positive for infectivity at a given virus dilution when the allantoic fluid demonstrated positive hemagglutination of chicken erythrocytes. Hemagglutination of influenza virus was determined using the microtiter technique. Finally, the dose required to infect 50% of the embryonated eggs (EID₅₀) was determined by the method of Reed and Muench.
min period. In a separate series of experiments, anesthetized animals underwent intranasal instillation of the virus. Virus was diluted in Hanks-balanced salt solution (HBSS), pH 7.4. While mice were anesthetized, 0.05 ml of virus inoculum was given intranasally drop-wise into both external nares using a 1-ml syringe with a 26-gauge needle. There were no animals in this group which did not receive anesthesia since awake mice do not tolerate this method of inoculation.

**Mouse Necropsy**

The pathogenicity of influenza virus infection in mice following anesthesia was compared in two ways. Animals were killed at 24, 48, 72, and 96 h following exposure to either diethyl ether, pentobarbital, halothane, or enflurane. Six mice were necropsied at each sampling period. The mice were killed by cervical dislocation and immediately necropsied. In three animals per sampling period, the lungs were removed under aseptic conditions, minced with scissors, and stored at −70°C. A 20% (wt/vol) lung suspension was prepared by homogenizing in HBSS. This suspension was clarified by centrifugation at 500 × g for 10 min at 4°C, and infectious virus assayed in embryonated eggs as described above. At the time that mouse lungs were removed for infectious virus assay, replicate mice were killed and lungs were inflated and fixed in 10% formalin. After fixation, lung tissues were embedded in paraffin, cut to a thickness of 6 μm, stained with hematoxylin and eosin, and viewed by light microscopy. All pathologic examinations were coded and the examiner was blind with respect to virus infection and anesthetic exposure of the histologic sections.

Lung sections were analyzed using a standard grading protocol for grading the severity of the histopathology. This consisted of 0 for no pathology, +1 for minimal bronchial thickening, +2 for bronchial epithelial sloughing with intraluminal exudate but only minimal alveolar involvement, +3 for extension of the disease into alveoli just adjacent to the affected bronchi, and +4 for extensive involvement of the alveoli.

**Statistics**

All data were analyzed statistically using the Michigan Interactive Data Analysis System. This computerized data system allows the analysis of both analytical and categorical variables. A two-way analysis of variance and the chi-square test were used to determine if the measured variables were statistically different. Specific comparisons also were made using the Student’s t test for unpaired data. Significance was achieved at P < 0.05. All data were expressed as mean ± SD.

**Results**

**Mortality of Mice Exposed to Aerosolized Virus Prior to Anesthesia**

In the first part of the study, inoculation of influenza virus was performed by exposing awake animals for 20 min to a small particle aerosol containing 10^5 EID₉₀/ml of infectious influenza virus particles as described in the Methods section. Mice then were anesthetized for one hour at either 0, 24, 48, 72, or 96 h post-infection. Initial symptoms of pneumonia, such as lethargy and tachypnea, first appeared 96 h following inoculation of the virus. Animals also demonstrated mortality starting at 96 h post-infection. In both unanesthetized and anesthetized mice, the mean day of death was 7.3 ± 1.0 days post-infection, regardless of the anesthetic used or the time the animal was exposed to the anesthetic. There was a cumulative mortality rate greater than 90% in unanesthetized controls as well as in mice anesthetized 96 h following infection with diethyl ether, pentobarbital, or halothane (fig. 1). Mice anesthetized with enflurane 96 h post-infection had less than a 70% cumulative mortality rate, statistically less (P < 0.01) than the other groups (fig. 1). Furthermore, animals which received enflurane anesthesia at 24, 48, or 72 h following virus exposure had less cumulative mortality than groups exposed to other anesthetics at these times; however, this did not achieve statistical significance. There was no statistical difference in the onset of symptoms or mean day of death when mice received no anesthesia, diethyl ether, pentobarbital, halothane, or enflurane anesthesia at 0, 24, 48, 72, or 96 h following virus exposure, i.e., only the cumulative mortality was reduced following enflurane exposure after 96 h post-infection.

**Mortality of Mice Infected with Virus Intranasally While Anesthetized**

In the second part of the study, mice were exposed to anesthesia and infected by instilling drops containing infectious influenza virus directly into the external nares while they were anesthetized. Using this method of inoculation, there was a significant difference in the onset of symptoms and mortality between the groups of mice receiving the different anesthetics.

Figure 2 demonstrates the cumulative mortality when mice were inoculated with an influenza virus titer of 10^5 EID₉₀/ml. The highest mortality (84%) occurred in the group of animals who received pentobarbital anesthesia during inoculation. The group receiving diethyl ether anesthesia demonstrated a 71% mortality, while halothane and enflurane treated groups had 58% and 37% mortalities, respectively. The onset of symptoms of pneumonia and the mean day of death of the animals...
COMPARISON OF PULMONARY INVOLVEMENT FOLLOWING VIRUS INOCULATION DURING ANESTHESIA

The histopathologic changes first occurred approximately 24 h prior to the onset of symptoms, approximately 48–72 h post-infection. The group of mice exposed to either diethyl ether or pentobarbital (fig. 4A) displayed acute bronchopneumonia with purulent infiltrates into the alveoli and lung interstitium. There were areas of necrosis and intraluminal sloughing of the bronchial epithelium. The interstitial purulent inflammatory component was dominant over the intraalveolar purulent exudate (+4 histopathology). Slides of lungs from

who received either diethyl ether or pentobarbital occurred significantly earlier than the animals receiving halothane anesthesia ($P < 0.05$) (mean day of death = 4.2 ± 1.7 days vs. 5.7 ± 1.3 days). Animals anesthetized with enflurane developed symptoms and mortality (mean day of death = 6.4 ± 1.3 days) significantly later than the groups of animals that had received pentobarbital or diethyl ether ($P < 0.01$).

Examining mortality of mice as a function of virus concentration (fig. 3), demonstrated that the different anesthetic treatment groups had different dose-response curves. Animals receiving enflurane had the lowest mortality regardless of the virus concentration ($P < 0.05$). Mortality occurred only at virus concentrations greater than $10^4 \text{ EID}_{50}/\text{ml}$ in mice anesthetized with enflurane. Mice anesthetized with halothane, diethyl ether, and pentobarbital demonstrated mortality at all virus concentrations except one ($10^2 \text{ EID}_{50}/\text{ml}$ concentration).

![Graph showing cumulative mortality of three-week-old CD-1 mice exposed to an aerosol of $10^5 \text{ EID}_{50}/\text{ml}$ of influenza virus for 20 min. The mice then received no further treatment (Δ —), or were given halothane (○ —), diethyl ether (● —), pentobarbital (□ —), or enflurane anesthesia (■ —), 96 h after infection. The numeral in parenthesis is the number of animals in the group. The group receiving enflurane had significantly less total mortality ($P < .01$) than the other groups.](image1)

![Graph showing cumulative mortality of three-week-old CD-1 mice inoculated intranasally with $10^5 \text{ EID}_{50}/\text{ml}$ of influenza virus during pentobarbital (○ —), diethyl ether (● —), halothane (□ —), or enflurane (■ —) anesthesia. There were 19 animals per group ($n = 19$). Enflurane has significantly less total mortality ($P < .01$) than all other groups. Halothane had significantly less ($P < .05$) total mortality when compared with the groups receiving either pentobarbital or diethyl ether anesthesia.](image2)
infected animals exposed to halothane were similar, except there was less spreading of the disease process from the bronchi to the interstitium and alveoli (+3 histopathology). Infected mice exposed to enflurane demonstrated significantly less pathology. Focal bronchial inflammation with minimal alveolar involvement (+2 histopathology) (fig. 4B) were the maximal present. Slides of lungs from animals exposed to the anesthetics alone revealed no pathologic changes (+0 histopathology).

The minced lungs from mice, which were infected while anesthetized, were assayed for the presence of infectious influenza virus (table 1). Peak influenza virus titers occurred prior to the appearance of the severe histopathologic changes, 48 to 72 h post-infection. Lungs from infected mice anesthetized with diethyl ether or pentobarbital had influenza infectious virus titers in excess of $10^8$ EID$_{50}$/ml. Lungs from infected animals exposed to halothane demonstrated virus titers of $10^7$ to $10^8$ EID$_{50}$/ml. Lungs from mice exposed to enflurane during inoculation of influenza displayed virus titers of $3 \times 10^5$ EID$_{50}$/ml.

**Discussion**

Halothane and enflurane, at concentrations used in this study, inhibit measles virus replication in tissue culture. $^{11,12}$ Furthermore, diethyl ether anesthesia increases survival in dogs infected by canine distemper virus$^5$ and in mice infected with several different arboviruses. $^5$ These viruses are large enveloped RNA-containing particles similar to influenza virus, although there are significant differences in replication. In this study, we show in vivo modification of influenza virus infection in mice by anesthetics. The degree and direction of modification depends on the anesthetic agent and the method used to infect the animals.

Enflurane-anesthetized mice have decreased severity of influenza virus pneumonia regardless of whether the virus is inoculated by aerosolized small particle mist or by direct instillation of the virus intranasally when compared with infected animals receiving either diethyl ether, pentobarbital, or halothane anesthesia. This decrease in severity is manifested by a decrease in mortality, a delay in the mean day of death, a decrease of infectious virus titer in the lung, and a decrease in histopathology of lungs in animals receiving enflurane during virus inoculation. The increase in survival is greater when the virus is instilled into the external nares of the anesthetized animals than when the animal is infected by aerosolized virus and exposed to anesthesia subsequently. Similarly, the mean day of death of the infected animals receiving enflurane was not different from the other groups when the virus was inoculated by small particle aerosol in awake animals.

Mice infected with influenza virus intranasally during halothane anesthesia have lower mortality, a later mean

![FIG. 3. Total mortality of three-week-old CD-1 mice inoculated with different virus concentrations ($10^5$ EID$_{50}$/ml–$10^8$ EID$_{50}$/ml) during pentobarbital (—— ○ ——), diethyl ether (—— ● ——), halothane (—— □ ——), or enflurane (—— ■ ——) anesthesia. There were 16 animals per group (n = 16). Enflurane has significantly less total mortality ($P < 0.05$) than all other groups at all virus concentrations. Halothane had significantly less ($P < 0.5$) total mortality at the $10^8$ EID$_{50}$/ml dose when compared with the groups receiving either pentobarbital or diethyl ether anesthesia.

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>Virus Titer*</th>
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<tbody>
<tr>
<td>Pentobarbital</td>
<td>$1.27 \pm 0.50 \times 10^9$</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>$6.86 \pm 0.47 \times 10^8$</td>
</tr>
<tr>
<td>Halothane</td>
<td>$3.00 \pm 1.14 \times 10^5$</td>
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<tr>
<td>Enflurane</td>
<td>$2.17 \pm 0.56 \times 10^5$</td>
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* Given as 50% egg infectious dose (EID$_{50}$).
† $P < 0.0001$ when group compared with either the pentobarbital- or diethyl-ether-anesthetized animals.
day of death, and a small decrease in histopathology when compared with groups of animals receiving either pentobarbital or diethyl ether anesthesia. However, this is not the case in mice anesthetized with halothane following exposure of influenza virus by a small particle aerosol. Using this mode of infection, there is no decrease in mortality when the group receiving halothane is compared with either unanesthetized control animals or groups of mice receiving diethyl ether or pentobarbital.

The mechanism by which these anesthetics alter virus pathology is speculative. Henry and Port,\textsuperscript{15} investigating a small particle suspension of benzo[a]pyrene-ferric oxide instilled into the trachea of the hamsters during anesthesia, found differences depending on the anesthetic agent used. This agent causes bronchopneumonia and is carcinogenic. Mortality secondary to bronchopneumonia as well as tumor growth was greatest in the barbiturate- (methohexital) treated group and least in the group receiving the halogenated anesthetic (methoxyflurane). Animals exposed to diethyl ether exhibited an intermediate survival rate. Animals that received the carcinogen during diethyl ether anesthesia had the most severe bronchopneumonia changes by microscopic examination of the histopathology. The methoxyflurane-anesthetized animals which were exposed to the carcinogen produced a slower onset of deaths from tumors and exhibited a lower tumor incidence when compared with the other groups. Thus, the results in bronchopneumonia severity secondary to benzo[a]pyrene small particle instillation parallel the results reported here of bronchopneumonia secondary to influenza virus instillation with similar anesthesia.

Although differential effects of various anesthetics on respiratory mechanics suggest a possible mechanism of differential virulence via deposition of virus particles, the site of deposition of virus particles along the respiratory tract does not appear to influence virus pathogenicity.\textsuperscript{8} However, diethyl ether can produce severe airway irritation and increased mucous production. Defects or impairment of the mucociliary blanket mechanism increases the pathogenicity of influenza virus in the pulmonary tract.\textsuperscript{8} Furthermore, diethyl ether anesthesia has been shown to damage epithelial tight junction using horseradish peroxidase tracer.\textsuperscript{14} The epithelial tight junctions of the bronchial lining may be differentially affected by the anesthetics allowing increased bronchial permeability to foreign substances. Neither
halothane nor pentobarbital alters these junctions\textsuperscript{14} (enflurane has not been studied).

Humoral immunity appears to be the predominant defense mechanism to influenza virus.\textsuperscript{8} It is unlikely that the anesthetics used here produce significant differential changes in this type of immunity. Influenza virus infection reduces the chemotactic, ingestive, and killing ability of lung phagocytes which may contribute to bacterial superinfection during influenza pneumonia.\textsuperscript{8} Interactions between lung mono- and polymorphonucleocytes, influenza virus, and different anesthetics may be fruitful avenues for research.

The reasons for differences in mortality resulting from varying methods of virus infection in the group of mice receiving halothane is not obvious. The two methods of infecting mice, aerosol \textit{vs.} direct intranasal administration during diethyl ether anesthesia, result in a similar distribution of influenza virus particles in the pulmonary tract.\textsuperscript{8} Furthermore, pathology in the respiratory tract at a given concentration of virus is also similar regardless of inoculation method.\textsuperscript{8}

Another explanation for differences in mortality observed between the two methods of inoculation is that the virus itself is in a different point of its replicative cycle. At zero hours post-infection (fig. 2), no virus particles are being synthesized, while at 96 h post-infection (fig. 1), extensive virus replication and assembly are occurring.

We have presented data that indicate in an animal model system that a specific anesthetic may alter the morbidity and mortality rates of a viral upper respiratory tract infection. It is hoped that further work in this area will answer the questions that these studies have raised.

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