General Anesthesia of Infant Mice by Isoflurane Inhalation for Medium-Duration Surgery

Hideo GOTOH, Yukiko MATSUMOTO, and Kenkichi IMAMURA

Model Animals Laboratory, Molecular Biology and Immunology Department, National Institute of Agrobiological Sciences, 2–1–2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan

Abstract: In this report we describe a means of general anesthesia for medium-duration (i.e., 20 to 60 min) surgery of infant mice. We tested isoflurane inhalation (2.0% isoflurane in air or oxygen during induction, and 1.5% after surgical anesthesia) anesthesia of 6- to 10-day-old C57BL/6J mice and obtained safe, effective, and reproducible results.

Key words: anesthesia, infant mouse, isoflurane

For medium-duration surgery of 6- to 10-day-old infant mice, such as the microinjection of biological materials into the seminiferous tubules [3], mice must be in a surgical plane of anesthesia for at least twenty minutes. Loss of animals due to anesthesia is one of the leading problems in this kind of experiment. The commonly used anesthesia for infant mice is hypothermia or ether inhalation [7]. However, hypothermia is highly controversial because the degree of analgesia achieved during hypothermia is not known, and its efficacy has not been established [2]. Ether inhalation also has the problem of substantial loss of animals because it is difficult to regulate the dose of ether [7].

In the present study, we used specific-pathogen-free 6- to 10-day-old infant C57BL/6J mice for injection of DNA solution into testicular seminiferous tubules. Animals were maintained on a cycle of 12 h of light and 12 h of darkness. A commercial mouse diet and water were provided. All procedures were approved by our Institutional Animal Care and Use Committee, and all animals were housed and cared for according to guidelines established by the Committee.

We assessed the safety and efficacy of inhalation anesthesia by isoflurane (Forane, Abbott, North Chicago, IL) delivered through a Univentor 400 anesthesia unit (Univentor, Zejtun, Malta) (Fig. 1). The anesthesia unit was designed to deliver isoflurane in air. Other kinds of gas such as halothane may be usable, but are not recommended by the manufacturer. The concentration of isoflurane was determined by the value displayed on the anesthesia unit. The specification of the anesthesia unit of gas concentration tolerance is +/− 0.15 units of the displayed % value. During induction, we used 2.0% isoflurane, then decreased the anesthetic concentration to 1.5% after surgical anesthesia had been achieved. Loss of reflex was detected by pricking the animal’s feet and legs with forceps. We occasionally had to transiently increase the concentration of isoflurane slightly during surgery to maintain a sufficient depth of anesthesia. After the animal was fully anesthetized, a twenty-minute surgery was performed to inject a DNA solution into testicular seminiferous
tubules. To correct intra-operative fluid loss, warmed 0.9% sodium chloride was administered by subcutaneous injection before surgery. To minimize heat loss, the animal was wrapped in cotton wool and aluminum foil, and was laid on a warm plate maintained at 38°C during and after completion of surgery. Initial recovery was designated as the time when reflex movements returned to the legs. The initial recovery was observed within a minute after surgery. Full recovery was specified as the time when the mice became ambulant. Mice were monitored until they were fully recovered and could be returned to their mothers. The infant mice were then assessed once more, at 24 h post-surgery.

In the initial studies, we mixed isoflurane with air according to the manufacturer’s protocol. For 6-day-old infant mice, 100% recovered fully from surgery, and 94% survived to 24 h post-surgery (Table 1). The one animal found dead the next day was considered to have died of surgery-associated causes because of a failed suture. After we changed the suturing procedure, there were no additional losses. The same protocol resulted in 100% survival at 24 h after surgery on 7- to 10-day-old infant mice (Table 1). In a second set of studies, we used a mixture of isoflurane and oxygen. A gas regulator was used to control the gas flow between 0 to 300 ml/min (Fig. 1). For the 6-, 7- and 10-day-old infant mice, 100% survival at 24 h after surgery was observed (Table 1).

Rapid recovery is another benefit of isoflurane inhalation anesthesia. We observed that reflex movements returned within a minute, and the righting reflex returned soon after, although we did not measure the exact time to the recovery of righting. In a study using isoflurane on 3-month-old rats, the righting reflex returned 7.5 ± 5.7 min after 0.5 h anesthesia when 1.6 minimum alveolar anesthetic concentration (MAC) was applied, and 0.9 ± 2.2 min after 2 h anesthesia when 0.4 MAC was applied [1]. Isoflurane MAC values for various inbred strains of 7- to 9-weeks-age mice have been determined [5]. The C57BL/6J strain which we used in this study was determined to have 1.30 ± 0.11 isoflurane MAC value. It is also known that neonatal animals usually require higher concentrations of anesthetic than adult animals [2]. Further investigations are needed to determine the MAC values and the recovery time from isoflurane inhalation anesthesia of infant mice.

The toxicity of inhalation anesthetics are reported to be different at different oxygen concentrations in the

Table 1. Anesthesia of infant mice by isoflurane inhalation

<table>
<thead>
<tr>
<th>Age of animals (days)</th>
<th>Carrier gas</th>
<th>No. of animals</th>
<th>Average body weight (g)</th>
<th>No. (%) of animals that fully recovered from anesthesia</th>
<th>No. (%) of animals alive 24 h post-surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Air</td>
<td>17</td>
<td>3.4</td>
<td>17 (100%)</td>
<td>16 (94%)</td>
</tr>
<tr>
<td>6</td>
<td>O₂</td>
<td>3</td>
<td>3.2</td>
<td>3 (100%)</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>7</td>
<td>Air</td>
<td>11</td>
<td>4.2</td>
<td>11 (100%)</td>
<td>11 (100%)</td>
</tr>
<tr>
<td>7</td>
<td>O₂</td>
<td>3</td>
<td>3.9</td>
<td>3 (100%)</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>8</td>
<td>Air</td>
<td>14</td>
<td>4.4</td>
<td>14 (100%)</td>
<td>14 (100%)</td>
</tr>
<tr>
<td>9</td>
<td>Air</td>
<td>19</td>
<td>5.1</td>
<td>19 (100%)</td>
<td>19 (100%)</td>
</tr>
<tr>
<td>10</td>
<td>Air</td>
<td>6</td>
<td>5.3</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>10</td>
<td>O₂</td>
<td>3</td>
<td>5.1</td>
<td>3 (100%)</td>
<td>3 (100%)</td>
</tr>
</tbody>
</table>

Fig. 1. Anesthetic apparatus, consisting of: (A) oxygen regulator, (B) oxygen flowmeter, (C) anesthesia unit, (D) face mask.
rat [6]. The percentage of rats experiencing injury to the liver varied inversely with the oxygen concentration when sevoflurane, isoflurane or halothane were used. However, use of 100% oxygen produced a significantly higher incidence of pulmonary injury for sevoflurane anesthesia. In the present study, we did not examine tissue injury. The appropriate oxygen concentration for isoflurane inhalation anesthesia with infant mice is unknown.

In this study, we found isoflurane inhalation anesthesia safely provided sufficient anesthesia for medium-duration surgery of infant mice. However, other anesthetics might also be suitable. For the rat, inhalation anesthesia by both I-653 (desflurane) and sevoflurane resulted in a more rapid awaking time than isoflurane [1]. However, the effects of these anesthetic procedures on infant mice have not been reported. Park et al. reported two effective anesthetizing techniques for retinectomy surgery (30–45 min) on 18 to 24 h old neonatal rats with an average weight of 6.31 ± 0.59 g [4]. Anesthesia with either halothane inhalation or subcutaneous injection of diluted Innovor®-Vet with oxygen supplementation was used and 100% of pups survived eye surgery with both anesthetics, and 97% of halothane and 100% of Innovor®-Vet anesthetized pups survived 7 days after surgery. These anesthetic procedures are also worth testing on infant mice.

References