Preliminary Communication

Anaesthesia by intravenous emulsified isoflurane in mice

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An emulsion of isoflurane in Intralipid™ for intravenous (iv) injection was formulated and its anaesthetic properties determined in mice. The major advantage of iv delivery of volatile agents is to accelerate the induction of anaesthesia by circumventing the anaesthetic circuitry and the lung's functional residual capacity. Isoflurane was added to Intralipid™ in varying concentrations. The ED_{50} ($n = 34$) and LD_{50} ($n = 20$) were determined by a single iv bolus injection. Anaesthesia was also induced and maintained for 30 min ($n = 5$) by continuous infusion and the time to emergence was measured. The ED_{50} and LD_{50} were 0.7 ± 0.2 μl and 2.4 ± 0.2 μl of isoflurane equivalent respectively. An average infusion rate of 1.6 ± 0.4 $\mu l \cdot min^{-1}$ of isoflurane equivalent was required for maintenance following which the average emergence time was 193 ± 35 secs. The only negative effect was local skin ulceration with an inadvertent interstitial injection. We conclude that iv induction and maintenance with emulsified isoflurane in Intralipid™ can be carried out with safety and reproducibility in the mouse. Further larger animal studies are warranted assessing the haemodynamic, toxicological, physiochemical and pharmacokinetic characteristics of these and other similar preparations.

Une émulsion d'isoflurane dans l'Intralipid™ pour injection intraveineuse est préparée et ses propriétés anesthésiques sont déterminées chez la souris. L'avantage principal de l'administration iv d'agents volatils est d'accélérer la vitesse d'induction de l'anesthésie en court-circuitant le circuit anesthésique et la capacité résiduelle fonctionnelle pulmonaire. De l'isoflurane est ajouté à l'Intralipid™ en variant les concentrations. L'ED_{50} ($n = 34$) et la LD_{50} ($n = 20$) sont déterminées pour une injection unique en bolus. De plus, l'anesthésie est induite et entretenue pendant 30 min ($n = 5$) par perfusion continue et le temps de récupération mesuré. L'ED_{50} et la LD_{50} sont respectivement de 0,7 ± 0,2 μl et de 2,4 ± 0,2 μl d'équivalent isoflurane. En moyenne, une vitesse de perfusion de 1,6 ± 0,4 $\mu l \cdot min^{-1}$ est nécessaire pour l'entretien; par la suite, le temps moyen de récupération est de 193 ± 35 sec. Le seul effet négatif consiste en une ulcération locale au site d'une injection interstitielle accidentelle. Nous concluons que l'induction et l'entretien iv avec une émulsion d'isoflurane dans l'Intralipid™ peuvent-être réalisés avec sécurité et reproducibilité chez la souris. Des études ultérieures chez des animaux plus gros sont justifiées pour évaluer les caractéristiques hémodynamiques, toxicologiques, physicochimiques et pharmacocinétiques de ce type de préparation.

Key words
ANAESTHETIC TECHNIQUES: general, intravenous;
ANAESTHETICS, VOLATILE: isoflurane.

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Intravenous (*iv*) administration of volatile anaesthetics has many advantages over inhalational delivery. Induction of anaesthesia would be rapid as equilibration with the anaesthetic circuitry and the lung's functional residual capacity is circumvented. Capital and maintenance costs of anaesthetic equipment could be reduced as the need for an agent specific vaporiser would be eliminated. This would be particularly advantageous with the newer inhalational anaesthetics. Other volatile agents previously thought of as unacceptable for inhalational delivery due to airway irritation at high concentrations or low vapour pressure may prove better suited to iv delivery. The amount of agent used could be minimized if a closed circuit breathing system were also incorporated further

reducing cost and atmospheric pollution with fluorocarbons. Anaesthesia could be deepened rapidly by bolus delivery of the agent or lightened by opening the closed breathing system. Finally, this method of volatile agent delivery creates a research tool for further elucidating mechanisms of uptake and distribution of anaesthetic agents.

Previous reports of the *iv* use of volatile anaesthetics, either accidentally in humans[1,2] or in experimental animals,[3-5] have often resulted in death or severe morbidity. This appears to have been due to relative overdosing at various tissue levels with subsequent direct toxic sequellae. Krantz *et al.* successfully gave methoxyflurane emulsified in fat to test animals[6] although subsequent thrombophlebitis at the injection site developed and he concluded that the agent was not suitable for use in man.[7] Biber *et al.* gave halothane emulsified in Intralipid™ *iv* to rats[8] and dogs.[9] No untoward events were noted nor were any microvascular changes seen; it was concluded that this formulation might prove useful for experimental animals requiring anaesthesia. This pilot study attempts to discern if the emulsion of isoflurane in Intralipid™ is an effective and safe *iv* agent and to determine its basic pharmacological characteristics.

Methods

Following approval by the University of British Columbia animal ethics committee the following experiments were conducted:

ED_{50} and LD_{50} studies

Isoflurane emulsions of one, two and three percent by volume were prepared by adding pure isoflurane to Intralipid™ in a gas-tight glass container and shaking for three minutes. The effective dose for induction of general anaesthesia in 50% of the animals (ED_{50}) was determined by injecting 34 CD-1 male mice with a single bolus of between 0.07 and 0.15 ml of a 1 or 2% mixture via their tail veins. The loss of the forepaw righting reflex (FRR) was accepted as successful induction of general anaesthesia. The time to return of the righting reflex was also recorded. To determine the lethal dose for 50% of the test animals (LD_{50}) 20 additional mice were given a single bolus injection of between 0.07 and 0.15 ml of a 3% preparation. No mouse was subjected to more than one injection. The ED_{50} mice and the survivors of the LD_{50} experiment were observed for four weeks afterwards for general wellbeing.

Continuous infusion study

Five CD-1 male mice had their tail veins cannulated with either a 27ga butterfly or a 24ga over the needle cannula (Jelco®). Anaesthesia was induced by delivery of two

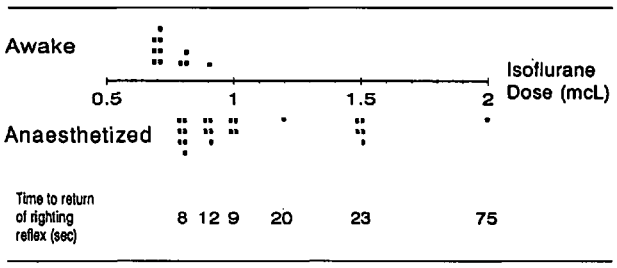

FIGURE 1 Each "▪" represents one mouse receiving a single bolus dose plotted along the X axis. The mouse either remained awake or became anaesthetized by the bolus (see text).

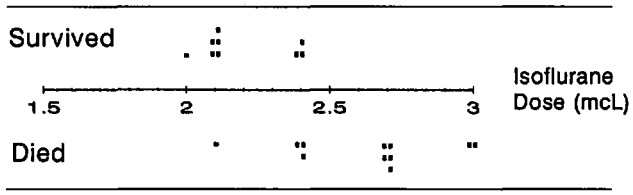

FIGURE 2 Each "▪" represents one mouse which received a single bolus dose plotted along the X axis. The mouse either died or survived the dose (see text).

times the above derived ED_{50} dose over 5–15 secs via a Bard infusion pump using a 10% vol:vol emulsion that was prepared in a similar manner as described above. The pump rate was then reduced and titrated empirically to maintain spontaneous respiration as well as to eliminate the FRR. No airway manipulation nor enrichment of FIO_2 was instituted. Following 30 minutes the infusion was discontinued and the time to return of the FRR was noted. These animals were observed for four weeks.

Statistics

The ED_{50} and LD_{50} data were analyzed by a probit analysis (NCSS software package). This analysis generated a dose percentile nomogram and a quantal dose response curve with standard error for the two experiments. The continuous infusion data is expressed as the mean ± standard deviation.

Results

ED_{50} and LD_{50} studies

Average mouse weights were 40 ± 4 g and 37 ± 2 g in the ED_{50} and LD_{50} studies respectively. Induction of the anaesthetic state was approximately three seconds after the bolus injection. No excitatory phenomena were observed and spontaneous respiration was maintained. The ED_{50} and LD_{50} data are depicted in Figures 1 and 2 respectively. The abscissa shows the volume of pure isoflurane (volume given × volume percent of total isoflurane in the emulsion). The ordinate is nominal with the mice either losing their FRR or retaining it for the

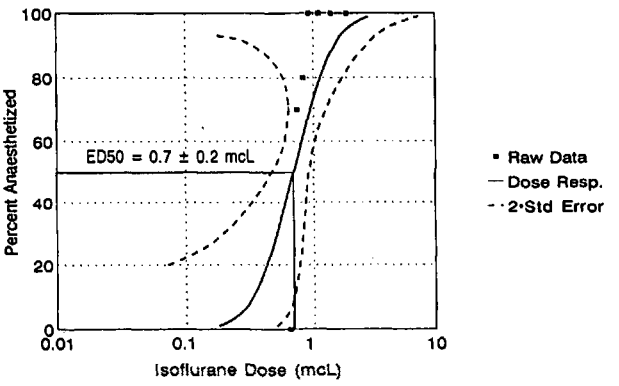

FIGURE 3 Data shown in Figure 1 is plotted according to the dose received on the X axis and the percent anaesthetized at that dose on the Y axis. A dose response curve with 2 · standard error is shown.

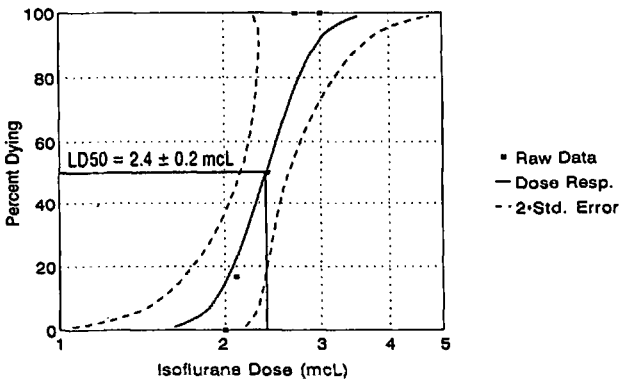

FIGURE 4 Data shown in Figure 2 is plotted according to the dose received on the X axis and the percent dying at that dose on the Y axis. A dose response curve of 2 · standard error is shown.

ED_{50} study or with the mice either dying or surviving in the LD_{50} study.

The mice tested at each dose were grouped as a percentage that were either anaesthetized (ED_{50} study) or died (LD_{50} study) at that dose. These values appear as points labelled "raw data" in Figures 3 and 4. Quantal dose response curves were then constructed utilizing a dose percentile nomogram generated by the probit analysis of this grouped data. These curves and two times their standard error also appear in Figures 3 and 4.

In the surviving animals those with a successful *iv* injection fed well and gained weight for four weeks following the experiment. Fifteen animals suffered an inadvertent interstitial injection during the experiment and developed skin ulceration over the area of extravasation. When an interstitial injection occurred the animal was eliminated from the study.

Continuous infusion study

Average mouse weight was 36 ± 2 g. An average infusion rate of 1.6 ± 0.4 $\mu l \cdot min^{-1}$ of isoflurane equivalent was required to maintain anaesthesia. The time to return of the FRR following 30 min of anaesthesia was 193 ± 35 sec. Loss of ataxia on spontaneous ambulation was seen between 320–540 secs following discontinuation of the infusion.

Discussion

This study demonstrates that induction and maintenance of anaesthesia is possible with an *iv* lipid emulsion of isoflurane. The characteristics of induction are that of rapid loss of consciousness as shown by the loss of FRR with preservation of spontaneous respiration. The loss of FRR has been previously shown in rats to parallel the abolition of movement following a standardized painful stimulus to the tail.[11] Recovery from a single induction bolus is fast likely owing to the rapid redistribution as well as near immediate elimination of the drug through the lungs. The emulsion showed no gross separation over a four-week period. Our dose calculations assume that all liquid isoflurane added to the sealed glass container remained in the liquid phase of the fat emulsion. We feel that this is a valid assumption within the experimental error of our study given that the calculated fat:gas solubility coefficient for isoflurane is 63:1 based on a fat:blood coefficient of 45:1 and a blood:gas coefficient of 1.4:1.[10] The therapeutic index (LD_{50}/ED_{50}) was typically narrow at 3.2; this compares with a therapeutic index for inhalationally delivered isoflurane of 15.3 for the rat.[11] Kissen's therapeutic index is wider probably because of their use of controlled ventilation and that the ED_{50} was achieved as a steady state over time and not in a bolus fashion as with our experimentation. The depth of anaesthesia in the infusion experiment could be altered rapidly by changing the rate of infusion. One death occurred in an infusion experiment animal when a bolus was delivered in an attempt to increase the depth of anaesthesia.

This investigation served only as a biological assay of the feasibility of emulsified volatile agents. Further large-animal studies are required to demonstrate the pharmacodynamic, pharmacokinetic and toxicological properties of this preparation. It was not within the mandate of this study to quantify the *in vivo* or *in vitro* physicochemical nature of the emulsion. Moreover, the haemodynamic consequences of our experimentation remain unsolved awaiting future study. In conclusion this pilot study demonstrates the feasibility of *iv* emulsions of volatile anaesthetics and may offer new avenues for the development of a more ideal *iv* anaesthetic agent.

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