Laboratory Report

Halothane and isoflurane enhance melanoma tumour metastasis in mice

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Purpose: To investigate the incidence of tumour metastasis from B16 melanoma tumour cells in experimental animals following exposure to equipotent concentrations of halothane or isoflurane, and to differentiate if exposure to one anaesthetic resulted in greater metastases than the other.

Methods: Experimental animals (C57Bl mice), were randomized to receive 1.3 MAC hours of halothane or isoflurane anaesthesia. The control group of animals received oxygen alone under identical conditions. Fifteen minutes after completion of anaesthesia, control and experimental groups were given $1x10^5$ B16 melanoma cells intravenously. After 21 days, all animals were autopsied, and the metastatic nodules in their lungs were counted. The difference in the numbers of metastatic nodules between control and experimental groups of animals was analyzed for significance by the Mann Whitney "U test".

Results: More metastases were observed in the animals exposed to halothane (37.28 ± 5.08, $P< 0.0001$), or isoflurane anaesthesia (28.24 ± 4.07, $P< 0.0014$) than in the control animals (12.22 ± 1.52).

Conclusion: Exposure to halothane or isoflurane anaesthesia increased the number of pulmonary metastases in C57Bl mice compared with the control groups but there was no difference in metastases among animals treated with halothane or isoflurane.

Objectif : Rechercher l'incidence des métastases de cellules tumorales mélanomateuses B16 sur des animaux de laboratoire après l'exposition à des concentrations équipotentes d'halothane et d'isoflurane, et vérifier si l'exposition à un anesthésique produisait un plus grand nombre de métastases qu'à l'autre.

Méthodes : Des animaux de laboratoires (souris 57Bl) étaient réparties aléatoirement pour recevoir 1,3 MAC heures d'anesthésie à l'halothane ou à l'isoflurane. Le groupe contrôle ne recevait que de l'oxygène dans des conditions expérimentales identiques. Quinze minutes après l'arrêt de l'anesthésie, les souris des groupes contrôle et expérimentaux recevaient $1x10^5$ de cellules mélanomateuses B16 par la voie intraveineuse. Après 21 jours, tous les animaux étaient autopsiés et les nodules pulmonaires métastatiques dénombrés. La signification statistique de la différence entre le nombre nodules métastatiques dénombrés entre le groupe contrôle et les groupes expérimentaux était déterminée avec le test U de Mann Whitney.

Résultats : On a observé plus de métastases chez les animaux exposés à l'halothane ($37,28\pm5,08$, $P<0,0001$) ou à l'isoflurane ($28,24\pm4,07$, $P<0,0014$) que dans le groupe contrôle ($12,22\pm1,52$).

Conclusion : L'exposition à l'halothane ou à l'isoflurane augmente le nombre de métastases pulmonaires chez de souris C57Bl comparativement au groupe contrôle mais il n'y a aucune différence entre les animaux anesthésiés que soit à l'halothane ou à l'isoflurane.

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DESPITE advances in early diagnosis, surgical intervention and medical therapy, tumour metastasis is a major cause of mortality among patients suffering from malignant disease.[1] The pathogenesis of metastasis is dependent on the responses of the host and the intrinsic properties of the tumour cells.[2-4] While a variety of factors is important in the pathogenesis of tumour dispersal and metastatic spread, an intact host immune system is of paramount importance in the elimination of tumour cells. In the perioperative period, integrity of the host immune response may be compromised by several factors including exposure to anaesthesia. Recent evidence suggests that exposure to anaesthesia may adversely affect both specific and non-specific components of the immune response.[5-7]

Previously, we have reported an anaesthesia induced depression of leucocyte function such as chemotactic migration[8-10] and phagocytic activity.[11] This depression of leucocyte function was variable with different anaesthetic agents, and appeared to correlate with their lipid solubility. Likewise, an anaesthesia induced depression of lymphoproliferation[12] and leucocytotoxicity[13] was also reported. Since anaesthesia is essential for surgery, different anaesthetic agents may contribute to a variable degree towards tumour metastasis by a suppression of immune response. Therefore, the effects of equipotent concentrations of halothane and isoflurane on pulmonary metastasis from B16 melanoma tumour in an animal model have been investigated.

Materials and methods

Animals

Following study approval by the Institutional Animal Investigation Committee, adult mice of C 57 Bl strain were obtained from the Jackson Laboratory, Bar Harbor, Maine, USA and were utilized during the study.

Tumour Cell Line

Transplantable B16 melanoma tumour cells (syngeneic to C 57 Bl mice) were utilized in this study. The B16 melanoma tumour was grown subcutaneously in C 57 Bl mice. The tumour was dissected aseptically and the cells were grown in medium RPMI 1640 supplemented with fetal calf serum (FCS) 10%, L-glutamine 1%, penicillin 1% and streptomycin 1% in plastic tissue culture flasks in a humidified atmosphere of 5% CO_2 in air at 37°C. The stock cells were frozen in liquid nitrogen. At the time of tumour inoculation, cells from frozen stocks were revived and maintained until the fifth passage *in vitro*. Cells were trypsinized with EGTA (5 mM) and harvested during the exponential phase of growth. Single cell suspension was obtained by passing the cells through a 20 gauge needle and cell viability ascertained by trypan blue dye exclusion test, was > 95%. The final cell suspension containing 1×10^6 cells·ml^{-1} was prepared in pyrogen free saline.

Experimental Protocol

Adult mice of C 57 Bl strain were randomly divided into two groups. One group of animals received either halothane 1% or isoflurane 1.5% (approximately 1.3 MAC) for one hour while the control group received oxygen alone for a similar period under identical conditions. Briefly, halothane or isoflurane was vaporized in a continuous stream of 100% oxygen by use of calibrated Fluotec and Isotec Mark III Ohmeda vaporizers. The anaesthetic vapor was delivered into a perspex box that had an anaesthetic entrance port at the base and a sampling port near the top. The animals were placed on an iron grid inside the anaesthetic box, which prevented them from eating the soda-lime at the base of the box for CO_2 absorption. During anaesthesia, the ambient temperature inside the anaesthetic box was kept constant by providing heat from a radiant lamp.

Experiments were repeated three times with exposure to halothane and isoflurane respectively. In the three experiments with halothane, there were 10 animals in each of the control and the anaesthesia groups. In the experiments with isoflurane, there were 10 animals in the control and the anaesthesia group in the first experiment. In the subsequent two experiments with isoflurane there were 10 animals in the anaesthesia groups, and five animals in each of the control groups. Fifteen minutes after exposure to anaesthesia with halothane or isoflurane, the control and the anesthetized groups of animals were given 1×10^5 B16 melanoma cells through the tail vein. Twenty-one days after tumour inoculation, all animals were sacrificed and their lungs were fixed with Bouins saline solution by an intratracheal injection. The numbers of tumour nodules in the lungs of each animal were counted under a dissecting microscope. The difference in the number of metastatic nodules in the lungs of the control animals and the animals exposed to different anaesthetic agents was analyzed for significance by the Mann Whitney U-test.

Results

There was no animal morbidity or mortality associated with halothane or isoflurane anaesthesia alone. However, one animal died immediately after intravenous tumour challenge following isoflurane anaesthesia. Primary melanoma B16 tumours were readily

observed in the lungs of all animals 21 days following inoculation of melanoma cells. The mean number of tumours was higher (37.28±5.08) in animals that had 1.3 MAC hours of halothane anaesthesia than in the control group (P <0.0001). Similarly, an increase in the number of metastatic tumour nodules (28.24±4.07) was observed after exposure to isoflurane anaesthesia (P <0.0014), compared with the control group of animals (12.22±1.52) that had no anaesthesia (Figure 1) The number of tumours in the halothane groups was 24% higher than in the isoflurane groups but these differences did not achieve statistical significance (P < 0.0819).

Discussion

Tumour metastasis following surgery is a major cause of morbidity and mortality in patients suffering from malignancy. The results of the present study demonstrate that anaesthetic exposure to equipotent concentrations of halothane or isoflurane (1.3 MAC hours) increased the number of pulmonary metastatic nodules from B16 melanoma tumour in C57 Bl mice. These data are in accord with the earlier observations of enhanced post-operative progression of spontaneous lung metastasis from B16 melanoma and 3LL Lewis lung tumour following surgery under anaesthesia with thiopentone, ketamine, or halothane.[14] Likewise, thiopentone given on the day of tumour cell inoculation, increased the growth rate of 3-methyl-cholanthrene induced syngeneic murine fibrosarcoma in C 57 Bl/6J mice.[15] Anaesthesia with halothane 1–4%, and thiopentone was also shown to cause reactivation of 20 to 22% of the tumours.[16] In all of the above investigations, neither the concentration of the anaesthetic agents used, nor the severity of surgical stress was compared. In addition, the effects of surgical intervention were not delineated from those of anaesthesia. The present investigation has studied the influence of equipotent concentrations of anaesthetic agents alone without surgical intervention, and has demonstrated that anaesthesia alone can increase the incidence of metastases in an animal model. The concentrations of anaesthetic agents used in this investigation are within the clinical range and suggest that anaesthetic exposure at these concentrations can facilitate tumour metastasis. Although the duration of anaesthetic exposure, and the concentration of anaesthetic agents used in the present study were kept constant, it is possible that a longer duration of anaesthetic exposure, or the use of higher concentrations of anaesthetic agents, may have revealed differences in pulmonary metastases with different anaesthetic agents. Indeed, after exposure to anaesthesia for only 1.3 MAC hours, the number of tumours in the halothane group appears to be higher than those in the isoflurane group but the difference was not statistically significant.

The number of tumour cells (tumour burden) used in the present study was determined as being optimal on the basis of our earlier investigation where tumour growth was studied by varying the numbers of tumour cells used in the inoculum.[17] The interactions between tumour burden and the host defense systems are important in the pathogenesis of tumour metastases. It has been demonstrated that the incidence of spontaneous metastases is associated with a diminished immune-responsiveness of the host to the tumour.[18] The ability of peripheral blood lymphocytes to proliferate in response to a mitogen or an alloantigen is a recognized correlate of an intact cell-mediated immune response. Likewise, lymphocytotoxicity and T_4 helper/T_8 suppressor ratios are used to assess the competence of cell-

TABLE Effect of anaesthesia with halothane and isoflurane on tumour metastases

Groups	*Experiments*	*Animals*	*Tumours*	*Significance*
	n	n	(Mean±SD)	*P*
Control	6	50	12.22±1.52	
Isoflurane	3	30	28.24±4.07	<0.0014
Halothane	3	30	37.28±5.08	<0.0001

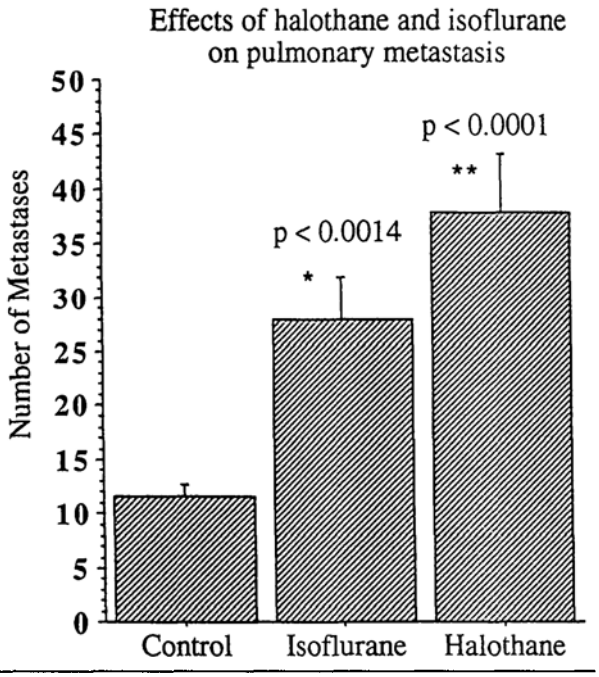

FIGURE Effect of halothane and isoflurane on pulmonary metastasis.

*P < 0.0014. **P < 0.0001.

mediated immunity. Lymphocytes with an ability to destroy malignant cells, have been classed as Natural Killer cells (NK cells), and are thought to form a primary defense against the development of tumours. An inhibition of NK cell cytotoxicity following exposure to halothane, enflurane, and nitrous oxide has been demonstrated previously in vitro.[19-20] Anaesthetic agents have also been shown to cause a depression of mitogen and alloantigen induced lymphoproliferative responses.[21-25] These studies suggest that an anaesthesia induced suppression of immune response may have contributed to an enhanced incidence of metastatic activity in the present study.

Both surgery and anaesthesia have been shown to depress lymphocyte function,[26] and the severity of this depression has been further correlated with the degree of surgical stress.[27] In an earlier study we investigated the ability of host leucocytes to destroy tumour cells (tumour type-specific leucocytotoxicity) and observed it to be depressed for up to seven days after mastectomy with halothane anaesthesia.[13] A similar effect was also reported following surgery for Wilm's tumour with general anaesthesia.[28] These studies tend to suggest that anaesthesia may affect different types of tumours in animals as well as in humans by a suppression of the immune response. The results of the present study, showing an increase in the incidence of metastases following exposure to anaesthesia, may have clinical relevance in terms of the contribution of anaesthesia in the pathogenesis of tumour dispersal and metastasis.

The mechanism whereby anaesthesia increases the incidence of metastatic nodules remains to be identified. Future studies should investigate the effects of different anaesthetic agents on the various cell populations, signal transduction, cytokines, and modulation of proto-oncogene expression that enhance the incidence of tumour metastasis. An understanding of these mechanisms may decrease morbidity and mortality from tumour growth and its metastatic dispersal.

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