

Comparative effects of volatile anaesthetic agents and nitrous oxide on human leucocyte chemotaxis *in vitro*

G.C. Moudgil MB BS MSC FFARCS FRCP(C),
J. Gordon BSc,
J.B. Forrest MB PHD FFARCS FRCP(C)

Infection following surgery is not uncommon. Human leucocytes play a vital role in the body's defense against infection. In order to decrease perioperative morbidity and mortality from infection, it is important to define the comparative effects of different anaesthetic agents on the leucocyte function. Therefore, the effect of equipotent concentrations (MAC 1) of isoflurane, enflurane, halothane, methoxyflurane and 70 per cent nitrous oxide, on the leucocyte chemotactic migration was investigated in vitro. The chemotactic migration of neutrophils and monocytes, with and without equilibration with MAC 1 concentrations of different volatile anaesthetics and 70 per cent nitrous oxide, was compared by using a modification of Boyden's method. Chemotactic migration of both cell types was unaffected by isoflurane, but a significant depression of chemotactic migration was observed with enflurane, halothane, methoxyflurane and nitrous oxide ($p < 0.05$). The severity of depression of migration was maximal with nitrous oxide, followed by methoxyflurane, halothane and enflurane in order. It is concluded that equipotent concentrations of various anaesthetic agents produce different degrees of depression of leucocyte chemotactic migration in vitro.

Key words

BLOOD: leucocytes, chemotaxis; ANAESTHETICS, VOLATILE: isoflurane, enflurane, halothane, methoxyflurane; ANAESTHETICS, GASES: nitrous oxide.

From the Department of Anaesthesia, McMaster University, Hamilton, Ontario.

Address correspondence to: G.C. Moudgil, Department of Anaesthesia, McMaster University, 1200 Main Street West, Hamilton, Ontario, Canada, L8N 3Z5.

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In recent years experimental evidence of depression of immunocompetence by anaesthetic agents has appeared in the literature. Reviews of the published data^{1,2} suggest that anaesthetics may compromise the host defense mechanisms by adversely affecting both the specific and the non-specific components of the normal immune response. Among the human host defenses, the role of leucocytes is of considerable interest since these cells form the first line of defense against infection. In an earlier *in vitro* study,³ local anaesthetics, intravenous induction agents and some volatile anaesthetics caused a dose dependent depression of human leucocyte migration towards chemical attractants. Stanley *et al.*⁴ and Moudgil *et al.*⁵ in their subsequent investigations observed that surgery and anaesthesia caused a depression of leucocyte migration in the perioperative period. Similarly, suppression of leucocyte migration, with halothane anaesthesia has also been reported in experimental animals.⁶ In contrast, *in vitro* studies by Duncan and Cullen⁷ and Nunn *et al.*⁸ failed to reveal any depression of leucocyte migration by thiopentone or halothane.

To date, investigations of the effects of anaesthesia on leucocyte locomotion have not compared the influence of equipotent concentrations of different volatile anaesthetic agents on the ability of leucocytes to migrate towards an antigenic or chemical stimulus. With the advent of newer anaesthetic compounds possessing safer pharmacological and physiological properties, it is desirable that the comparative effects of these agents on host defense systems be investigated. Since leucocyte chemotaxis is an important early feature of the defense against invading organisms, in the present study the

comparative effects of isoflurane, enflurane, halothane, methoxyflurane and nitrous oxide on leucocyte migration towards the chemical attractant casein have been investigated. Such an assessment of the effect of these commonly used anaesthetic agents, on leucocyte function, provides new information on the relative safety of these agents.

Methods

Separation of monocytes and neutrophils

Heparinised peripheral venous blood samples (20 ml) were obtained from healthy volunteers. Erythrocyte sedimentation was facilitated by adding 2 mls of Dextran '75' in 0.9 per cent saline for a period of 60 to 90 minutes at room temperature. The leucocyte-rich supernatant was aspirated and layered gently on a Hypaque/Ficoll gradient. Following centrifugation at 800 g for ten minutes, predominantly monocyte- and neutrophil-rich cell fractions were obtained respectively from the supernatant/Hypaque interface and the cell pellet at the bottom of the centrifuge tube. Both cell types were washed twice in Gey's balanced salt solution at pH 7.2 and were resuspended in it to give a final count of approximately 10^6 cells·ml⁻¹. The cell viability was ascertained by "Trypan Blue dye exclusion test."

Equilibration with volatile agents

One MAC concentrations of isoflurane (1.15 per cent), enflurane (1.68 per cent), halothane (0.75 per cent) and methoxyflurane (0.16 per cent) were vaporised in five per cent CO₂ in air using their agent specific vapourisers. Separate experiments were also done with 70 per cent N₂O in O₂. The vapour concentration delivered was analysed by a Riken 18 Interferometer. Each anaesthetic vapour was delivered through a series of three-way stop cocks connected by tubing to glass pipettes, into the tubes containing test-cell suspensions and Gey's solution containing varying concentrations of chemo-attractant casein (0.5 and 2.0 mg·ml⁻¹). Equilibration with the anaesthetic vapour was achieved by bubbling the vapour through all tubes for 30 minutes, this time period having been found in a previous study to achieve complete equilibration that was well maintained in a sealed environment.³ Control cell suspensions and the different

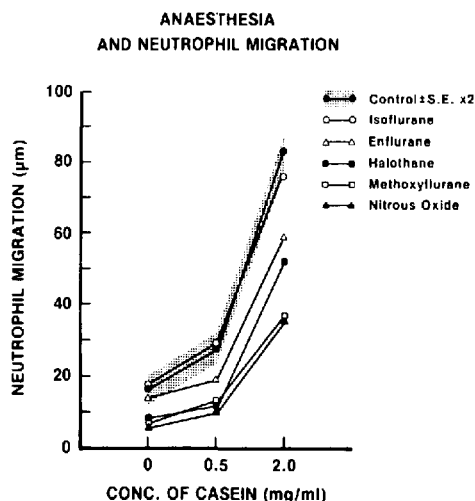


FIGURE 1 Effect of volatile anaesthetic agents (MAC 1), and 70 per cent nitrous oxide on the neutrophil chemotactic migration towards different concentrations of casein. SE for all tests were similar and in no test greater than those shown for the control tests.

concentrations of casein solutions were treated with five per cent CO₂ in air in an identical manner. Following equilibration with the anaesthetic vapour or five per cent CO₂ in air, cell viability was ascertained as before and the test cells were transferred immediately into chemotaxis chambers and subsequently placed in a sealed desiccator previously flushed with anaesthetic vapour for ten minutes. Control cell suspensions were placed in another desiccator flushed with five per cent CO₂ in air. Both systems were incubated at 37°C for the duration of the test. The effect of each volatile anaesthetic agent and 70 per cent N₂O in O₂, on neutrophil and monocyte cell chemotaxis was investigated six times.

Measurement of chemotaxis

A modification of Boyden's method was used to measure the leucocyte chemotactic migration. Cells were placed above a micropore filter (Millipore, Bedford, Mass.) attached to the lower end of a sawn off tuberculin syringe. The micropore filter pore sizes for neutrophil and monocyte migration were 3 µm and 8 µm respectively. The lower chamber

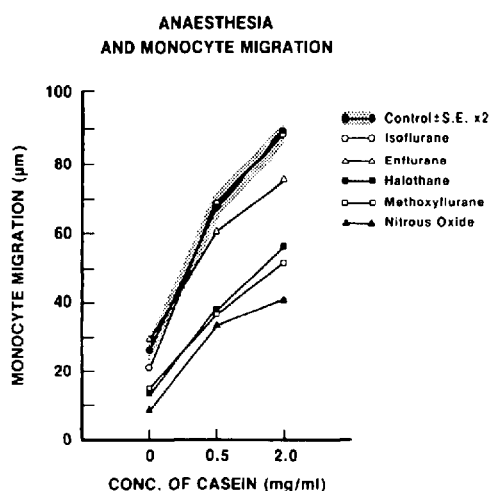


FIGURE 2 Effect of volatile anaesthetic agents (MAC 1), and 70 per cent nitrous oxide on the monocyte chemotactic migration towards different concentrations of casein. SE for all tests were similar and in no test greater than those shown for the control tests.

contained different concentrations of casein in Gey's solution equilibrated with each anaesthetic agent under investigation. Since the cell and casein suspensions were equilibrated with the same concentration of anaesthetic vapour, no drug gradient was present across the micropore filter but only the gradient of the chemotactic agent casein.

A minimum of two chambers were used at each concentration of casein for assessment of chemotactic migration. The tests were incubated at 37°C for 90 minutes or 120 minutes for neutrophil or monocyte migration respectively. The cell migration was then halted by fixation in ethanol so that the leading cells did not entirely migrate across the filter. Subsequently the filters were stained with haematoxylin and rendered transparent by immersion in xylene for ten minutes. The filters were mounted on a glass slide and examined in a light microscope. Cell migration from the filter top of the "leading front" of the cells through the micropore filter towards the gradient of chemotactic agent casein was measured by the fine adjustment focus control of the microscope. The mean of five readings of the "cell fronts" was calculated on each

filter. The significance of the difference in migration between the control cells and the cells treated with different anaesthetic agents was assessed by Students' *t* test. A value of $p < 0.05$ was considered significant.

Results

Both the neutrophil and the monocyte cell fractions showed a dose-dependent increase in migration towards different concentrations of the chemotactic agent casein, the chemotactic migration being maximal in response to casein $2 \text{ mg} \cdot \text{kg}^{-1}$. This migration was decreased following exposure to different volatile anaesthetics as well as 70 per cent nitrous oxide in O_2 . Although both cell types were treated with equipotent concentrations (MAC 1) of different volatile anaesthetics, the depression of chemotactic activity was not identical but varied with the agent. With the exception of isoflurane, chemotactic migration of neutrophils and monocytes was decreased to varying degrees by all the other anaesthetics.

Figures 1 and 2 show the effects of equipotent concentrations of isoflurane, enflurane, halothane, methoxyflurane and 70 per cent nitrous oxide on control neutrophil and monocyte chemotactic migration respectively in response to different concentrations of casein. Chemotactic migration of both cell types was unaffected by isoflurane, but enflurane, halothane, methoxyflurane and nitrous oxide each caused a significant depression of chemotactic migration ($p < 0.05$). Halothane, methoxyflurane and nitrous oxide caused a significant depression of "random locomotion" (migration in the absence of a chemotactic stimulus) as well as "stimulated locomotion" (migration in response to casein), compared with "random" and "stimulated" locomotion of untreated (no drug exposure) control cells.

Figures 3 and 4 illustrate the comparative effects of the different volatile anaesthetics and 70 per cent nitrous oxide on neutrophil and monocyte migration following maximal stimulation with casein ($2 \text{ mg} \cdot \text{kg}^{-1}$). The chemotactic migration of neutrophils following exposure to one MAC concentrations of isoflurane, enflurane, halothane, methoxyflurane and 70 per cent nitrous oxide was reduced to 92, 68, 50, 42 and 45 per cent of the control activity respectively. Similarly, monocyte migration was

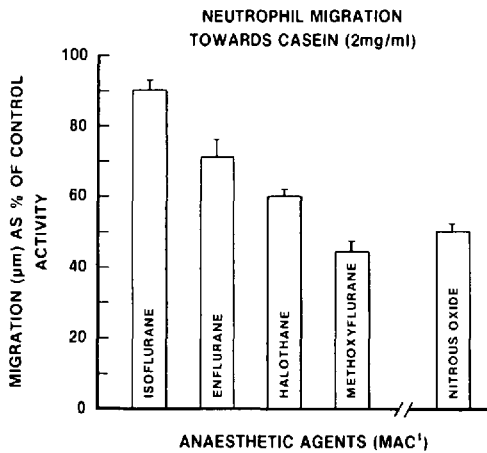


FIGURE 3 Effect of equipotent concentrations of volatile anaesthetics (MAC 1) and 70 per cent nitrous oxide on neutrophil migration towards casein ($2 \text{ mg} \cdot \text{kg}^{-1}$). Distance migrated expressed as per cent of control activity \pm SE.

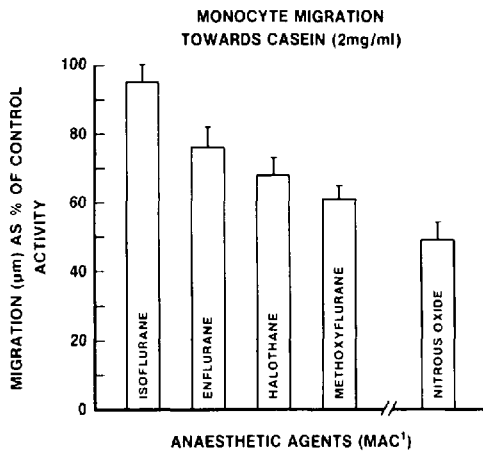


FIGURE 4 Effect of equipotent concentrations of volatile anaesthetics (MAC 1) and 70 per cent nitrous oxide on monocyte migration towards casein ($2 \text{ mg} \cdot \text{kg}^{-1}$). Distance migrated expressed as per cent of control activity \pm SE.

reduced to 95, 76, 68, 61 and 49 per cent of the control activity with the respective agents. Leucocyte chemotactic migration was significantly depressed with all anaesthetic agents with the exception of isoflurane.

Discussion

The migration of human leucocytes towards inflamed or injured tissues is an important immunological defense mechanism against infection. Any impairment of the leucocyte responsiveness to chemotactic stimulation is likely to enhance the incidence of perioperative infection. To date, evidence regarding the effects of anaesthetic agents on leucocyte chemotactic migration remains contradictory. Studies showing a depression of chemotactic migration,³⁻⁶ as well as unaltered or stimulated migration due to different anaesthetic agents^{7,8} have been reported. However, in all these studies, the effects of differing concentrations of various anaesthetic agents were investigated. Therefore, in this study, the effects of equipotent concentrations (MAC 1) of various volatile agents and 70 per cent nitrous oxide on the human leucocyte chemotactic migration towards different concentrations of the chemotactic agent casein, have been investigated.

Chemotactic factors may exert their effect on

cells directly or may act only in the presence of serum, plasma or other biological fluids. A great majority of directly acting leucocyte chemotactic agents are polypeptides or proteins whereas indirectly acting substances activate humoral enzyme systems and liberate chemotactic products. The latter may belong to a wide variety of molecular classes including proteins, polysaccharides or lipids, and may be derived from a wide variety of biological sources. In this study, the chemotactic agent casein, which is a macromolecular protein complex of α , β , and κ -casein, was used because of its ability to directly stimulate the directional migration of cells by combining directly with the receptor sites on the responding cells.

In the present study, we observed that both the neutrophils and the monocytes appropriately showed enhanced chemotactic migration in a dose dependent manner, in response to stimulation with different concentrations of the chemotactic agent, casein. Following exposure to equipotent concentrations (MAC 1) of isoflurane, enflurane, halothane and methoxyflurane, the chemotactic migration was decreased by different degrees with the various anaesthetic agents. The chemotactic migration of both the neutrophils and the monocytes was not affected by isoflurane, but equipotent concentrations of all other agents caused a significant

depression of migration of both the cell types. Exposure of 70 per cent nitrous oxide also caused a similar depression of chemotactic migration. The severity of this depression was maximal with nitrous oxide, followed by methoxyflurane, halothane and enflurane, in decreasing order.

These findings are in accord with our earlier study³ showing a depression of chemotactic migration by halothane (one and two per cent) and methoxyflurane (one per cent). Similarly, the depression of chemotactic migration observed in this study corroborates the depression of neutrophil migration with 80 per cent nitrous oxide observed by Nunn *et al.* in a recent study.⁹ It is interesting, however, that equipotent concentrations of various anaesthetics caused different degrees of depression of chemotactic migration. The mechanism(s) whereby anaesthetics cause a defect of chemotactic migration remain to be defined.

Intrinsic defects of leucocyte chemotactic migration may operate at different levels, and the evidence concerning different mechanisms involved is sparse at present. Defects of chemotactic migration may result from failure of chemotactic recognition, changes in cell membrane configuration and receptor sites, failure to mobilize energy sources, and a disruption or paralysis of microtubules and microfilaments comprising the cellular locomotor assembly. Anaesthetic agents may affect one or several of these steps.

Amongst the parameters that correlate best with anaesthetic potency is that of lipid solubility.^{10,11} Mullins postulated that anaesthesia occurs when absorption of anaesthetic molecules expands the volume of a hydrophobic region beyond a critical amount. Inhalational anaesthetic agents increase the lateral pressure of lipid monolayers in a manner that parallels their potency.^{13,14} Insertion of anaesthetic molecules into lipid membranes results in membrane expansion and thereby reduces stability of ionic channels.¹⁵ Such an expansion is also likely to distort the receptor sites for chemotactic agents and thus result in decreased migration. Indeed, anaesthetic agents have been shown to induce a configurational change by combining with the hydrophobic regions of protein molecules.¹⁶

In the present study, of all the volatile anaesthetic agents investigated, maximal depression of chemotactic migration was produced by methoxyflurane, an agent which also has the highest lipid solubility.

The severity of depression of chemotactic migration observed with isoflurane, enflurane and halothane, was also in step with their lipid solubilities.

Thus, it would appear that as lipid solubility increases, the chemotactic migration decreases. However, the depression observed with nitrous oxide does not relate to its lipid solubility and would suggest a different mechanism of its action on chemotactic migration.

The energy requirements for chemotactic migration are provided by anaerobic glycolysis and oxidative phosphorylation. Since halothane, methoxyflurane, ethrane and diethyl ether act upon the mitochondrial respiratory chain to inhibit NADH oxidation,¹⁷ it is possible that the depression of chemotactic migration observed may have resulted from an anaesthetic-induced interference with subcellular oxygen availability or utilization.

Leucocyte migration requires an intact contractile locomotor assembly comprised of microfilaments and microtubules. Agents like cytochalasin B, that have been claimed to paralyze contractile microfilament systems, were observed also to inhibit chemotactic migration of human and rabbit neutrophils.^{18,19} Similarly, drugs which caused the disruption of microtubules in human neutrophils, also inhibited neutrophil motility and chemotactic migration.^{20,21} Inhalational anaesthetics at two to four MAC concentrations also have been shown to cause a dispersion of microtubules in heliozoa.²² A similar dispersion of microfilaments has also been described in fibroblasts treated with local anaesthetics.²³ Since agents causing dispersion of microfilaments and microtubules also caused depression of chemotactic migration, it is conceivable that anaesthetic agents induce depression of chemotactic migration by a disruption of the contractile locomotor assembly. In addition, various investigations indicate that in the neutrophil the effects of chemotactic factors are mediated by variations in the levels of free intracellular calcium.²⁴⁻²⁶ Since anaesthetic agents have been shown to alter the cation permeability of liposomes in a dose-dependent manner,^{27,28} a possibility exists that the depression of chemotactic migration may be induced by alterations in cation fluxes.

Thus, there are several possible mechanisms whereby anaesthetic agents could cause a depression of chemotactic migration. By investigating the mechanisms of anaesthetic-induced depression of

chemotactic migration, we may gain better insight into the anaesthetic action at cellular levels. Also, the clinical relevance of depression of chemotactic migration in terms of morbidity and mortality needs further clarification. A better understanding of both quantitative and qualitative defects of leucocyte function by different anaesthetic agents should help in a more appropriate selection of anaesthetic agents and techniques and thereby result in decreased morbidity and mortality from infection in the perioperative period.

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Résumé

L'infection post-chirurgicale demeure un problème qu'on n'a pas complètement éliminé. Dans la défense de l'organisme contre l'infection, les leucocytes jouent un rôle essentiel et il est donc important de connaître les effets respectifs des différents agents anesthésiques sur la fonction leucocytaire. Dans cette étude, nous avons observé in vitro les effets sur la chimiotaxie des leucocytes de concentration comparable d'isoflurane, d'enflurane, d'halothane, de méthoxyflurane (1 MAC) et de protoxyde d'azote à 70 pour cent.

On a utilisé une modification de la méthode de Boyden pour comparer la chimiotaxie des neutrophiles et monocytes avec et sans agent anesthésique. L'isoflurane n'a pas modifié l'activité chimiotaxique de l'un et l'autre type de cellules contrairement à l'enflurane, l'halothane, le méthoxyflurane et le protoxyde d'azote ($p < 0.05$). Par ordre d'importance, le protoxyde d'azote s'est montré plus dépresseur suivi du méthoxyflurane, de l'halothane et de l'enflurane. On conclut que des concentrations comparables de divers agents anesthésiques dépriment l'activité chimiotaxique des leucocytes à des degrés divers lorsqu'observés in vitro.