Date:

Title: Effect of Intravenous Anesthetics on Leukocyte Migration

Authors: Diana Mathieu, Ph.D. and Alix Mathieu, M.D.

Affilation: Department of Anesthesia, University of Cincinnati College of Medicine Cincinnati, Ohio 45267

Introduction. Chemotaxis, the directed migration of cells, such as polymorphonuclear leukocytes (PMN) towards a locus of inflammation or infection, is an early and significant event in host defense against infection. Clinicians involved in the care of patients during the peri-operative period should therefore be interested in the effects of anesthetic drugs on chemotaxis. The present study was undertaken to determine the in vitro effects on PMN chemotaxis of the new narcotic analgesics, butorphanol and nalbuphine, and to compare these results with 3 well-known narcotics: morphine, meperidine and sublimaze. We also studied the effect of 3 induction agents on chemotaxis.

Methods. The effect of intravenous anesthetics, narcotics and induction agents on the chemotaxis of polymorphonuclear cells was evaluated in 20 individuals, 12 females and 8 males. Chemotaxis was measured in vitro by the radial migration of PMN cells beneath agarose gel. The cells were aseptically separated from whole blood by Ficoll-Hypaque density gradient centrifugation followed by dextran sedimentation of the PMN-rich cell pallets. Cell viability was determined by trypan blue dye exclusion. Approximately 1.4 x 10^6 cells were delivered into each of 5-8 replicate wells of a petri dish containing 10% horse serum (the chemoattractant) in agarose gel and a given concentration of drug, previously determined to be non-toxic to cells. A plate with horse serum alone represented the control. Plates were incubated for 18 hours at 37°C in a humidified chamber with 5% CO_2 and 95% air, after which the gels were fixed in glutaraldehyde, removed and radial migrations determined with an occulometer. The net migration diameter expressed in mm was calculated by subtracting the standard well diameter (2.5 mm), from the observed migration diameter. For any plate, the mean migration $\pm$ standard error is calculated and compared statistically (paired t test) to the positive control. The following drugs were studied: 5 narcotics, i.e. morphine, meperidine, butor phanol, nalbuphine, sublimaze and 3 induction agents, i.e. diazepam, inapsine and methohexital.

Results. In vivo analgesic concentrations of the 5 narcotics investigated were found to have mild or no inhibitory or stimulating effect on PMN migration, the latter more frequently observed with nalbuphine and butorphanol, with variable statistical significance. Inhibition of migration was generally noted at concentrations of drugs ranging from 0.1- 10 mM, and between 0.001-0.1 mM for sublimaze (Table I). Except for morphine and nalbuphine, when used at high doses, i.e. 3 mg/kg, these inhibitory effects have little relevance clinically. The molar concentration of drugs giving 50% inhibition of PMN migration was extrapolated for each drug to determine whether these numbers correlated with the drug's analgesic potency. From these studies, nalbuphine and morphine behaved similarly reaching 50% inhibition at 1.4 and 1.3 mM respectively. Butorphanol and meperidine were more inhibitory with 50% inhibition points calculated at 0.17 and 0.09 mM respectively. At the highest concentration tested, sublimaze inhibited 23% so that a 50% point could not be determined. Thus the new drugs affect PMN locomotion similarly to the other 3 narcotics, i.e. clinical concentrations have minimal or no effect on directed cell locomotion. In the present study, we also found significant inhibition of chemotaxis with diazepam at concentrations from 4- 200 ug/ml. Inapsine displayed only minimal inhibition of chemotaxis, which was statistically significant only at the highest concentration. Methohexital at 5-1250 ug/ml showed consistently a dose-dependent increase in PMN locomotion. (Table 2) The significance of our data is difficult to evaluate in relation to Stanley et al's findings in vivo. The latter reported significant depression of chemotaxis in patients under general anesthesia, before the start of surgery; however, patients had received a combination of drugs. The mechanism of action of these drugs on chemotaxis remains to be established by further studies.

Reference.

1. Stanley TH, Hill GE, Portas MR et al: Neutrophil chemotaxis during and after general anesthesia and operation. Anesth Analg 55: 668-673, 1976.

Table I: Summary of Effects of Narcotics on PMN Migration

Drug	Effect
Butorphanol:	+ significant inhibition at 0.5-1 mM no or minimal effect 5 x 10^{-4} -5 x 10^{-2} mM
Nalbuphine:	significant inhibition at 5 mM minimal effect 5 x 10^{-4} - 5 x 10^{-1} mM
Sublimaze:	inhibition at 1.8-3.5 x 10-2 mM minimal effect 3.5 x 10^{-5}- 3.5 x 10^{-3}
Morphine:	significant inhibition at 5 mM no or minimal effect 5 x 10^{-4} -5 x 10^{-1}
Meperidine:	significant inhibition at .35-35 mM minimal effect 3.5 x 10^{-3}- 3.5 x 10^{-2}

Table 2: Summary of Effect of Induction Agents on PMN Migration

Drug	Effect
Diazepam:	significant inhibition 75-200 ug/ml slight inhibition 4-40 ug/ml
Inapsine:	inhibition 18-357 ug/ml
Methohexital:	stimulation 10-1250 ug/ml

Stimulation and inhibition: an increased or decreased net migration of cells, respectively.
Significant: statistically significant variation compared to control with $p > 0.05$.
Minimal effect: no action or a slight increase or decrease ($\pm$10%) on migration.