Does chronic occupational exposure to volatile anesthetic agents influence the rate of neutrophil apoptosis?

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Purpose: The purpose of this preliminary investigation was to determine whether the rate of neutrophil apoptosis in health care workers is influenced by exposure to volatile anesthetic agents.

Methods: Percentage neutrophil apoptosis (Annexin-V FITC assay) was measured in health care workers (n = 20) and unexposed volunteers (n = 10). For the health care workers, time weighted personal exposure monitoring to N₂O, sevoflurane and isoflurane was carried out.

Results: The sevoflurane and isoflurane concentrations to which health care workers were exposed were less than recommended levels in all 20 cases. Percent apoptosis was less at 24 (but not at one and 12) hr culture in health care workers {50.5 (9.7)%; P = 0.008} than in unexposed volunteers {57.3 (5.1)%}.

Conclusion: Inhibition of neutrophil apoptosis at 24 hr culture was demonstrated in health care workers chronically exposed to volatile anesthetic agents. Exposure was well below recommended levels in the both scavenged and unscavenged work areas in which the study was carried out. Further study is required to assess the effect of greater degrees of chronic exposure to volatile anesthetic agents on neutrophil apoptosis.

Objectif : Vérifier, lors d'une étude préliminaire, si le taux d'apoptose des polynucléaires neutrophiles chez les travailleurs de la santé est influencée par l'exposition aux agents anesthésiques volatils.

Méthode : Le pourcentage d'apoptose neutrophile (dosage Annexin-V FITC) a été mesuré chez des travailleurs de la santé (n = 20), et chez des volontaires non exposés aux anesthésiques (n = 10). On a procédé au monitoring pondéré du temps d'exposition individuel au N₂O, au sévoflurane et à l'isoflurane.

Résultats : Les concentrations de sévoflurane et d'isoflurane auxquelles les travailleurs ont été exposés étaient plus faibles, dans tous les cas (20), que les niveaux recommandés. Le pourcentage d'apoptose a été moindre à 24 h de culture, mais non pas à une et 12 h, chez les travailleurs de la santé {50,5 (9,7) %; P = 0,008} comparés aux volontaires non exposés {57,3 (5,1) %}.

Conclusion : L'inhibition de l'apoptose neutrophile à 24 h de culture a été démontré chez les travailleurs de la santé exposés régulièrement aux anesthésiques volatils. L'exposition était bien en deçà des niveaux recommandés dans les environnements de travail stériles ou non où l'étude a été menée. Une étude complémentaire devra évaluer l'effet d'une exposition régulière à de plus fortes concentrations d'anesthésiques volatils sur l'apoptose neutrophile.

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HEALTH care workers (HCW) who work in unscavenged operating theatres or in recovery rooms may be chronically exposed to low concentrations of volatile anesthetic agents. It has been reported that such chronic exposure influences immune status.¹ Chronic exposure to volatile anesthetic agents has been implicated in increased incidence of carcinogenesis and teratogenesis, both processes in which apoptosis has a fundamental role.² Apoptosis is a process of genetically regulated cell deletion, which is integral to normal and pathological processes including embryological development and cell proliferation.^{3,4}

It is hypothesized that chronic occupational exposure of health care workers to low concentrations of volatile anesthetic agents may inhibit the rate of neutrophil apoptosis. To test this hypothesis, we compared the rate of neutrophil apoptosis in unexposed volunteers and HCWs who work in scavenged and in unscavenged areas.

Methods

With institutional ethical approval, and informed consent from each, 30 subjects were studied: healthy unexposed volunteers ($n = 10$), HCWs who work in scavenged operating theatres (scavenged group) ($n = 12$) and in unscavenged operating theatres or recovery areas (unscavenged group) ($n = 8$). Volunteers and

HCWs were excluded if they had undergone general anesthesia within the previous three months.

Personal gas exposure monitors were placed on the shoulders or cap of each HCW for one day from 8:30 am to 2:00 pm. This monitoring was carried out using charcoal (volatile anesthetic agents) and Dräger diffusion (nitrous oxide) sampling tubes followed by thermal desorption and quantitative infra-red analysis. The latter procedures were carried out in a National Measurement Accreditation Scheme (NAMAS) accredited laboratory.⁵

Heparinised venous blood (9 ml) was withdrawn from each volunteer and from the HCWs at 2:00 pm from personnel who had been working in the operating theatre or recovery area since 8:30 am. Neutrophils were isolated by sequential sedimentation in 6% Dextran (mol.wt. 520,000, Sigma, UK) in sodium chloride 0.9% for 45 min, centrifugation in Ficoll-Paque (Pharmacia LKB Biotechnology, Piscataway, NJ) at 300 g for 30 min to pellet granulocytes and remaining erythrocytes, and centrifugation of the resuspended pellet over an 81% isotonic Percoll (Sigma, UK) gradient at 350 g for 15 min to pellet erythrocytes. The diffuse layer at the interface containing neutrophils was harvested, washed, resuspended in medium and counted. Preparations of isolated neutrophils were maintained in RPMI1640 supplemented with 10% autologous platelet poor plasma, and L-glutamine,

FIGURE The percentage apoptosis was assessed by dual staining with Annexin V-FITC and propidium iodide at one, 12 and 24 hr culture. Data are reported as the percentage of Annexin V-FITC positive and propidium iodide negative cells.

Propidium iodide stains the cell when cell membrane has been damaged. Thus, cells which are stained with only Annexin V indicates apoptotic condition in the early phase where the cell membrane is still intact.

The percentage in the upper left quadrant indicates the percentage of Annexin V-FITC positive viable neutrophils.

TABLE I Time weighted average exposures (Mean $\pm$ SD) and recommended exposure limits

	Scavenged group (n = 12)	Unscavenged group (n = 8)	Recommend
Sevoflurane	9.0 $\pm$ 5.6 mg·m ⁻³ 1.1 $\pm$ 0.7 ppm	6.6 $\pm$ 12.2 mg·m ⁻³ 0.8 $\pm$ 1.5 ppm	No assigned limit
Isoflurane	11.5 $\pm$ 2.5 mg·m ⁻³ 0.2 $\pm$ 0.3 ppm	2.2 $\pm$ 1.1 mg·m ⁻³ 0.3 $\pm$ 0.2 ppm	380 mg·m ⁻³ (UK, IRISH) 50 ppm (UK, IRISH)
Nitrous oxide	71.1 $\pm$ 66.9 mg·m ⁻³ 39.5 $\pm$ 37.2 ppm	46.8 $\pm$ 28.9 mg·m ⁻³ 26.0 $\pm$ 16.1 ppm	90 mg·m ⁻³ (ACGIH) 50 ppm (ACGIH)

TABLE II Percentage neutrophil apoptosis. Data are mean $\pm$ SD.
* $P < 0.05$ compared to volunteers.

Duration in culture (hr)	Health care workers (%)	Unexposed volunteers (%)
	(n = 20)	(n = 10)
1	5.40 $\pm$ 1.9	6.48 $\pm$ 2.1
12	35.06 $\pm$ 6.8	35.6 $\pm$ 8.6
24	50.5 $\pm$ 9.7*	57.3 $\pm$ 5.1

penicillin, streptomycin and amphotericin B, at a concentration of $1 \times 10^6 \cdot \text{ml}^{-1}$ in polypropylene tubes at 37°C in a humidified CO₂ incubator. After 1, 12 and 24 hr in culture, neutrophils ($0.5 \times 10^6 \cdot \text{ml}^{-1}$ cells) were dual stained with propidium iodide (sigma, UK) (final concentration 10 $\mu\text{g} \cdot \text{ml}^{-1}$) and Annexin V-FITC (Bender MedSystems, Austria) (final concentration 0.6 $\mu\text{g} \cdot \text{ml}^{-1}$).⁶ Neutrophils were analysed on a Becton Dickinson FACScan flow cytometer equipped with Cell Quest (Figure). Ten thousand events were collected while gating on physical parameters to exclude cell debris.

Data relating to rate of apoptosis were analyzed with unpaired, one tail Student's *t* tests.

Results

The age and weight of unexposed volunteers and HCWs were 32.3 (9.7) yr, 38.8 (8.5) yr respectively and 74.6 (11.4) kg, 65.3 (8.4) kg respectively. The proportion of male patients was greater in the unexposed volunteers (8 / 10) than in HCWs (4 / 20).

The study periods were selected as representative of a normal working day, therefore no attempt was made to delineate the proportion of each study period during which subject was or was not exposed to anesthetic.

The results of personal gas monitoring are shown in Table I. Time weighted exposures to anesthetic gases were similar in the scavenged and unscavenged groups.

Rates of neutrophil apoptosis in HCWs and unexposed volunteers were similar after one and 12 hr culture (Table II). At 24 hr culture, compared with unexposed volunteers {57.3 $\pm$ 5.1%}, percent apoptosis was less in HCWs {50.5 $\pm$ 9.7%; $P = 0.008$ }.

Discussion

In this study, we demonstrated a reduction of the rate of neutrophil apoptosis only at 24 hr in culture, in comparing HCWs who are occupationally exposed to volatile anesthetic agents with unexposed volunteers. These findings must be interpreted in the light of the small number of subjects studied and the relatively low degrees of exposure to which the HCWs were subjected. Several other investigators have reported degrees of environmental contamination with anesthetic gases that are far in excess of those reported in this study, and of the occupational exposure standards.¹ A power analysis was not performed in this study because these data are preliminary and represent an attempt to identify whether a full investigation of the effects of volatile agents on apoptosis is warranted. Furthermore, it is not clear why the moderate effect seen at 24 hr in culture was not present earlier.

Recently, the role of apoptosis in many physiological and pathophysiological processes has been demonstrated.^{3,4} Normally, apoptosis of neutrophils is responsible in part for termination of the inflammatory response to tissue injury (such as surgery). Although the functional state of neutrophils, in which apoptosis has been inhibited, has not been defined, it is possible that an augmented or prolonged inflammatory response may result. One possibility is if apoptosis is inhibited, neutrophils may follow the alternative death pathway, necrosis, which would be associated with a greater inflammatory response.

These preliminary results suggest that chronic occupational exposure to volatile anesthetic agent may influence neutrophil apoptosis. Further investigation is warranted to assess the effect of chronic exposure to greater concentrations of anesthetic gases and the

implication of such an effects on regulation of the inflammatory response.

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