EFFECT OF HALOTHANE AND NITROUS OXIDE ANAESTHESIA ON NATURAL KILLER LYMPHOCYTES FROM PATIENTS WITH BENIGN AND MALIGNANT BREAST DISEASE

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Natural killer (NK) cells are a lymphocyte subset which can recognize and kill tumour cells without prior processing of tumour cell specific antigen. Hence, they are widely believed to defend against the development of primary tumours, and the metastatic spread of established tumours in the host (Herberman and Ortaldo, 1981). This phenomenon is known as "natural cytotoxicity."

Many facets of the host immunological response are known to be depressed following anaesthesia and surgery (Walton, 1979) and this could possibly be implicated in the dissemination of tumour cells following surgery for malignant disease.

The aim of the present study was to determine the effects of halothane and nitrous oxide anaesthesia on the capacity of human natural killer lymphocytes to kill the tumour cell line K562.

PATIENTS AND METHODS

Patients

Twenty female patients undergoing surgery for benign and malignant breast disease were studied after giving informed consent. Ten patients (mean age 43 yr, range 35–60 yr) underwent excision biopsy of breast for benign breast disease and 10 patients (mean age 54 yr, range 36–73 yr) underwent segmental or total mastectomy for breast cancer. Whole blood was taken from an antecubital vein before surgery, heparinized and stored at 4 °C overnight before study.

SUMMARY

The effect of halothane and nitrous oxide on the capacity of natural killer (NK) lymphocytes from female patients with benign and malignant breast disease to kill the tumour cell line K562, was studied in vitro. There was no depression of activity of NK lymphocytes when exposed to 2% halothane and 66% nitrous oxide either alone or in combination. However, NK lymphocyte activity was depressed at higher concentrations of halothane and the decrease in activity was significant (P < 0.01) when 4% halothane was used. These findings suggest that exposure to clinically-used concentrations of halothane and nitrous oxide does not interfere with the NK lymphocyte response of the host.

Anaesthesia

Equal volumes of whole blood and RPMI 1640 culture medium with 10% fetal calf serum (Flow Laboratories, Irvine, Scotland) were mixed. One hundred microlitre of this mixture was placed into round-bottomed micro test plates (Falcon Products, Becton Dickinson, Ca., USA). The test plates were then placed in sealed 1-litre chambers and exposed to air (control) or flushed with an anaesthetic mixture delivered from a Boyle machine at a fresh gas flow of 9 litre min⁻¹ for 5 min of either 2% halothane + 98% oxygen, or 66% nitrous oxide + 34% oxygen or 2% halothane plus 66% nitrous oxide in oxygen. Each chamber was incubated at 37 °C for 2 h before assessment of cytotoxicity.

In a further part of the study, heparinized whole blood from five patients (two patients suffering from benign disease and three from breast cancer,
mean age 42 yr, range 34–53 yr) was diluted with RPMI 1640 (+10% FCS) and exposed to increasing concentrations of halothane (2, 3 and 4%) in oxygen for 2 h at 37 °C before assessment of cytotoxicity.

Assay of cytotoxicity

The whole blood natural cytotoxicity assay (Rees and Platts, 1983) was used to test NK cell function rather than using separated lymphocytes since it is more representative of the in vivo situation.

The leukaemic cell line K562 was used as the target cell. Cells were labelled with chromium 51-labelled Na\textsubscript{2}CrO\textsubscript{4} 100 μCi (Radiochemical Centre, Amersham, Bucks), washed in RPMI (10% FCS) and resuspended at 10⁶ cells ml\textsuperscript{-1}. One hundred microlitre of labelled K562 cells was added to 0.1-ml volumes of whole blood (half dilution) after exposure to either air (control) or the anaesthetic agent under study. Each test was performed in triplicate.

The test wells were incubated at 37 °C in a 5% carbon dioxide–95% air atmosphere for 6 h. Then 0.1 ml of supernatant was removed into separate wells. The test wells were dried in an oven, sprayed with Nobecutane (Astra Pharmaceuticals, Kings Langley, Herts) and individual wells counted in a Wilj gamma spectrophotometer. The release of chromium 51 is directly proportional to tumour target cell killing and was calculated as follows:

\[
\% \text{ chromium 51 release} = \frac{2 \times (\text{half SN})}{(\text{Cells} + \text{half SN}) + (\text{half SN})} \times 100
\]

where SN = supernatant.

Percentage cytotoxicity was then calculated as follows:

\[
\% \text{ cytotoxicity} = \frac{\text{test release} - \text{spontaneous release}}{100 - \text{spontaneous release}} \times 100
\]

Spontaneous release represented the chromium 51 released from K562 target cells incubated in culture medium alone.

Statistical analysis

Paired and unpaired Student t tests were used, as appropriate.

RESULTS

There was no significant difference between the mean percentage cytotoxicity of the 10 patients with benign breast disease and the 10 patients with carcinoma of the breast (table I).

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<th>Table I. Percentage cytotoxicity of whole blood (one-half dilution) to K562 exposed to air (control). Differences between groups not significant (unpaired t test)</th>
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<td>Cytotoxicity (%)</td>
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Fig. 1. Percent cytotoxicity of whole blood (one-half dilution) to K562 cells after exposure to halothane and nitrous oxide (patients with benign breast disease).
In the group of patients with benign breast disease (fig. 1), exposure of their blood to 2% halothane + 98% oxygen, 66% nitrous oxide + 34% oxygen or 2% halothane, 66% nitrous oxide + 32% oxygen did not alter NK cell cytotoxicity to K562 tumour cells when compared with cytotoxicity of blood exposed to air alone.

Similarly, in the patients with breast cancer (fig. 2), NK cytotoxicity of whole blood to K562 tumour cells was not significantly different when incubated in 2% halothane + 98% oxygen, 66% nitrous oxide + 34% oxygen or 2% halothane, 66% nitrous oxide + 32% oxygen or air alone.

However, NK cell cytotoxicity from the additional five patients was depressed (fig. 3) after exposure to 3% halothane + 97% oxygen for 2 h, and this depression became statistically significant (P < 0.01) when 4% halothane + 96% oxygen was used. This appears to demonstrate a dose-dependent inhibition of natural killer lymphocyte cytotoxicity after exposure to increasing concentrations of halothane.

**DISCUSSION**

Depression of the lymphocyte-mediated immune response has been reported following surgical operations performed under general anaesthesia. Studies have concluded that the depression of lymphocyte function (Riddle, 1967) was related to the degree of surgical stress (Cullen and Van Belle, 1975) and that this was more important than exposure to anaesthetic agents. However, lymphocyte transformation to mitogens was reduced following induction of anaesthesia (Espanol, Todd and Soothill, 1974) and there was also a reduction in circulating T lymphocytes and inhibition of the mixed lymphocyte culture reaction (Slade et al., 1975).

Halothane, *in vitro*, inhibits lymphocyte transformations (Cullen, Sample and Chretien, 1972), cell division (Nunn, Lovis and Kimball, 1971) and motility (Nunn, Sharp and Kimball, 1970), and both halothane and nitrous oxide inhibit antibody-dependent cellular cytotoxicity (Cullen, Duncan and Ray-Keil, 1976) mediated by K lymphocytes.

The NK lymphocyte, because of its unique ability to recognize and kill tumour cells, without
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processing tumour specific antigen, is thought to form a primary immune defence mechanism against the development of tumours and has a secondary role in defence against tumour metastases. Natural killer lymphocyte cytotoxicity in patients with breast cancer was found to be depressed 3 days after mastectomy (Uchida, Kolb and Micksche, 1982); however, cytotoxicity was not assayed following the induction of anaesthesia or during the surgical procedure. A further study showed that the induction of anaesthesia with inhalation anaesthetic agents (nitrous oxide and halothane) or i.v. anaesthetic agents (etomidate and fentanyl) did not affect the NK activity of patients with benign and malignant abdominal conditions. NK activity was increased during the surgical procedure and for 48 h following operation as the result of an increase in circulating NK lymphocytes (Griffith et al., 1984).

In this study we have shown that exposure to clinically used concentrations of nitrous oxide and halothane either alone, or in combination, had no effect on the ability of NK lymphocytes to kill the tumour cell line K562. This investigation was designed to simulate the maximum time required for a mastectomy and it is reassuring that this particular limb of the immune response was found to be intact after exposure to clinical concentrations of the anaesthetics.

When the concentration of halothane was increased to 3% and 4%, marked inhibition of natural cytotoxicity to tumour cells was shown which was significant after exposure to 4% halothane (P < 0.01, Student’s paired t test). However, it is unlikely that this concentration of halothane would be used for a prolonged period in clinical practice.

The mechanism of inhibition of natural killer lymphocyte activity after exposure to high concentrations of halothane is unknown, but the local anaesthetics lignocaine and procaine are known to inhibit NK cell activity in a dose-dependent manner (Takagi et al., 1983) probably by changing the cell membrane which will then affect recognition and binding of target cells.

Anaesthetic agents are known to cause destruction or depolymerization of the microtubular structure of cells in vitro (Hinkley and Telser, 1974). In addition, one of the theories of the mechanism of general anaesthesia may be its effect on membrane proteins and on ion transport across the membrane (Mullins, 1954). This membrane effect could be the mechanism behind the decrease in NK activity seen after exposure to high concentrations of halothane in vitro. Th evidence from this study, however, shows that NK lymphocyte activity from patients with benign and malignant breast disease is not adversely affected by exposure to clinically-used concentrations of the inhalation anaesthetic agents halothane and nitrous oxide.

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REFERENCES


