Cell-mediated immune suppression due to alfathesin in short-term anaesthesia

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The immunosuppressive effects on cell-mediated immunity of alfathesin, when used as the sole agent for short-term anaesthesia, were investigated in ten women undergoing pregnancy termination. Cell-mediated immunity was evaluated by E rosette formation in peripheral blood (reflecting the percentage of T lymphocytes) and by proliferative responses to the mitogens concanavalin A and phyto-haemagglutinin in various concentrations. Measurements were made before alfathesin was administered, and again after 60 minutes, two days and seven days. Significant reductions in the percentage of E rosettes in peripheral blood and in mitogenic responses to concanavalin A were observed 60 minutes after administration of alfathesin. A model is proposed in which alfathesin binds to lymphocytic membrane receptors, thus affecting in vitro cell-mediated immune reactions.

Key words

ANAESTHETICS; INTRAVENOUS; alfathesin; immunity; cellular.

Transient immune suppression following the administration of various anaesthetic agents has been reported by several authors.¹⁻⁵ Halothane, which has been extensively studied, suppresses phyto-haemagglutinin (PHA)-induced transformation of human lymphocytes⁵ in a dose-dependent manner,^{2,5} whereas ketamine did not produce immunosuppression in burned children undergoing minor surgical procedures. Barbiturates, cyclopropane and nitrous oxide have been reported to have *in vitro* effects on lymphocyte proliferation similar to halothane.^{3,6}

Anaesthetics may interfere with the immune response in a number of ways.⁷ They may cause bone marrow depression, inhibit phagocytosis or macrophage motility, and decrease antibody production or cell-mediated immunity (CMI). The latter effect is of particular interest to the anaesthetist, since CMI is involved in the response to antigens following human organ allotransplantation or autotransplantation, and may also be associated with immunoprotection against the spread of malignant tumours.

CMI depends on the rapid cell division of T-lymphocytes which have appropriate receptors for a particular antigen. Anaesthetics capable of reducing the number of T-lymphocytes in peripheral blood or interfering with their cell division are likely to depress CMI, as reflected by E rosette formation with sheep red blood cells and the proliferative response to a mitogen such as PHA.

Alfathesin, a widely used steroid anaesthetic, is particularly suitable as an induction agent or as the sole anaesthetic agent for short-term procedures. It was found to be virtually devoid of hormonal effects when tested in mice, rats and rabbits⁸ and it did not produce changes in corticosteroid levels in human

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subjects.⁹ The present study was undertaken with the aim of investigating the direct effect of alfathesisin on CMI.

Methods

The subjects were ten women scheduled for elective pregnancy termination in the 2nd and 3rd months. Informed consent was obtained from each participant. All were healthy, had received no recent medication or blood transfusions, and had fasted for six hours prior to the procedure. None had been exposed recently to anaesthetic agents. No premedication was given. The women were anaesthetized with alfathesisin $0.05 \text{ ml} \cdot \text{kg}^{-1}$ given intravenously as the sole agent, and all spontaneously breathed room air. The average duration of anaesthesia was 5.6 minutes. Heparinized venous blood (10 ml) was withdrawn into sterile tubes immediately before anaesthesia, 60 minutes after induction, and again two days and seven days later.

Mononuclear (lymphocytic) cells were separated from whole peripheral blood on Ficoll-Isopaque gradients as described.¹⁰ Blood was diluted with an equal volume of phosphate buffered saline and added to the gradients without mixing. Following centrifugation at 1500 rpm for 30 minutes at room temperature, mononuclear cells were pipetted out from the middle layer. Cell viability, as determined by trypan-blue exclusion, was about 95 per cent.

Percentage of T-cells in peripheral blood was evaluated by assaying their capacity to spontaneously form E rosettes with sheep red blood cells (SRBC) without complement in five per cent human AB serum, as previously described.¹¹ The mixture of peripheral blood and SRBC was incubated at room temperature for ten minutes, centrifuged for 25 minutes at 1000 rpm and left overnight at 4°C. Percentages of rosetting cells were calculated from 200 mononuclear cells.

Mitogenic responses were assayed by measuring the incorporation of ^3H -methylthymidine (^3H -MT, Nuclear Center, Negev, Israel) into peripheral blood following addition of the mitogens phytohaemagglutinin (PHA, Burroughs-Wellcome, England) and concanavalin A (Con-A, Miles-Yeda, Israel).¹² Assays were performed in flat-bottomed microtitre plates (Costar 3596, Cambridge, Mass. USA), each well containing 1×10^5 mononuclear cells in 0.2 ml of medium 199 supplemented with ten per cent human AB serum. To this mixture PHA

$0.05 \mu\text{g} \cdot \text{ml}^{-1}$ or $0.5 \mu\text{g} \cdot \text{ml}^{-1}$ and Con-A $1 \mu\text{g} \cdot \text{ml}^{-1}$ or $10 \mu\text{g} \cdot \text{ml}^{-1}$ were added. The cells were cultured for 72 hrs at 37°C in five per cent CO_2 and 95 per cent air, pulsed for four hours with $2 \mu\text{Ci/well}$ of ^3H -MT, and harvested on paper filters (Whatman F) with a semiautomatic cell harvester (Yeda, Weizmann Institute, Israel). Cells undergoing the same procedure but without added mitogens were used as controls.

In addition, cells from an age- and sex-matched control were identically processed in each test for reproducibility assaying.

Radioactivity was measured in a Packard Scintillation Counter and recorded as counts per minute (cpm). The mitogenic stimulation index (SI) was calculated according to the following formula:

$$\text{SI} = \frac{\text{cpm of cells with mitogen}}{\text{cpm of control cells}}$$

Data were analyzed using a two-way analysis of variance (two-way ANOVA), which enables discrimination between treatment and patient variability¹³ and means were compared by the Least Significant Difference (LSD) Test.¹³ The level of significance was established at $p \leq 0.05$.

Results

Table I shows a reduction in T-cell percentage and in the mitogenic responses to PHA and Con-A at all concentrations 60 minutes after administration of the drug, with a tendency towards restoration of normal values two days later.

Statistical analysis of the data (Table II) shows that the changes in T-cell levels and Con-A mitogenic responses are significant, whereas the responses to PHA are not, despite the similarity in trend. Similar findings are reflected in the LSD test which shows significant differences between patients regarding their patterns of proliferative response as expressed by significant F values for patient variability.

Discussion

T-lymphocytes appear to play a role in certain pathological conditions such as neoplastic diseases, various infectious processes, rejection of transplanted organs and delayed hypersensitivity reactions.

Steroids depress lymphoid proliferative re-

TABLE I T-cell percentage and mitogenic responses in ten women undergoing pregnancy termination under alfathesin anaesthesia.*

	Units	Before anaesthesia	After anaesthesia		
			60 minutes	2 days	7 days
T-cells	%	76.5 ± 5.8	67.3 ± 5.8	74.5 ± 4.8	78.5 ± 4.5
PHA, 0.05 µg·ml ⁻¹	SI	115.2 ± 85.8	49.7 ± 39.7	108.0 ± 85.0	128.8 ± 92.4
PHA, 0.5 µg·ml ⁻¹	SI	134.4 ± 104.6	65.7 ± 50.1	117.3 ± 81.7	132.9 ± 107.4
Con-A, 1 µg·ml ⁻¹	SI	88.3 ± 67.5	34.0 ± 22.6	65.0 ± 43.6	67.2 ± 35.2
Con-A, 10 µg·ml ⁻¹	SI	53.1 ± 25.3	25.5 ± 19.7	64.1 ± 39.0	61.2 ± 34.0

*All values express mean ± 1SD.

PHA, phytohaemagglutinin.

Con-A, Concanavalin A.

TABLE II Two-way ANOVA analysis of T-cell percentage and stimulation indices after alfathesin anaesthesia

F-Value	Rosettes*	PHA† 0.05 µg·ml ⁻¹	PHA† 0.5 µg·ml ⁻¹	Con-A† 1.0 µg·ml ⁻¹	Con-A 10 µg·ml ⁻¹
Variability due to the drug F3/27	9.3	2.66	1.90	4.21	8.0
	‡	NS	NS	‡	‡
Variability due to the patients F9/27	1.3	2.40	2.82	3.90	6.3
	NS	‡	‡	‡	‡

*As assayed by percentage of mononuclear cells forming E-rosettes.

†Values expressed as mitogenic stimulation indices (SI).

‡Significant, $p \leq 0.05$.

NS Not significant.

PHA, phytohaemagglutinin.

Con-A, concanavalin A.

sponses.¹⁴ The increased levels of catecholamines and corticosteroids accompanying surgery may play a role in postoperative immunosuppression. The extent of hormonal change after surgery is proportional to surgical stress.³ Since only small alterations in cortisol levels have been found following minor surgical procedures, such as the one selected for the present investigation, the contribution of surgical stress to immunosuppression in such procedures may be considered negligible.^{9,15}

Alfathesin, the sole anaesthetic agent used in this study, does not affect corticosteroid levels.⁹ The effects on CMI, described above, should therefore probably be attributed to the direct action of the drug on T-lymphocytes, possibly mediated via attachment to membrane receptors. This could result in competitive inhibition for SRBC binding, thus transiently reducing E rosette formation. The

rapidity with which this effect takes place (60 min), and the quick recovery, do not support the assumption that a true reduction in circulating T-cells has occurred. It should be noted that direct short-term steroidal effects of alfathesin on steroid-sensitive lymphocytes are not precluded, although the drug does not affect the hormonal axis. In addition, a mild suppressive effect of some stress generation, which is associated with any medical procedure, cannot be completely excluded.

A mechanism of interaction with membranal receptors could also explain the effect of the drug on mitogenic responses. Separate receptors for PHA and Con-A are known to exist on lymphocyte membranes, and it is claimed that less mature T-cells have a higher density of Con-A surface receptors.¹⁶ A differential affinity of alfathesin for mitogen receptors might thus account for the drug's

more pronounced effect on responses to Con-A than to PHA. Alternatively, the lack of significant effect on PHA might be explained in terms of patient variability, since the number of cases was small and no experimental data can be forwarded to support the "membranal" hypothesis, since the cells were not exposed *in vitro* to alfathesin.

In conclusion, it appears that alfathesin may exert a short-lived suppressive effect which will probably not jeopardize host defence mechanisms of "normal" patients undergoing minor surgical procedures. However, such suppression might become significant in the immunocompromised individual, and should therefore be taken into account in patients undergoing radio- and chemotherapy, or with autoimmune diseases and viral infections associated with a defective T-cell system.

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Résumé

L'effet immunosuppressif de l'alfathesin, sur l'immunité par médiation cellulaire, quand celui-ci est employé comme agent unique de l'anesthésie de courte durée, fait l'objet de cet étude. L'immunité par médiation cellulaire a été évaluée par la formation de rosettes E dans le sang périphérique (ce qui reflète le pourcentage de lymphocytes T) et par la réponse proliférative aux mitogènes concanavaline A et phyto-agglutinine dans des concentrations variables. Les mesures ont été fait avant l'administration de l'alfathesin, et puis après 60 minutes, deux et, respectivement sept jours. Des réductions significatives dans le pourcentage des rosettes E dans le sang périphérique et dans la réponse mitogène a la concanavaline A ont été observées 60 minutes après l'administration de l'alfathesin. Un modèle est proposé dans lequel l'alfathesin se lie aux récepteurs lymphocytaires de la membrane, et, de cette manière affecte les réactions immunitaires par médiation cellulaire *in-vitro*.