Neutrophil mobility during anaesthesia in children. A trial of ascorbate premedication

A. J. CHARLTON, B. A. HARVEY, D. J. HATCH and J. F. SOOTHILL
Department of Anaesthesia, The Hospital for Sick Children and Department of Immunology, Institute of Child Health, London, UK

Contributions to the concurrent series of 20 children who were studied were made by G. BURGESS, R. S. KIRK, K. MITRA, A. R. HODGSON, and J. F. SOOTHILL.

In 20 healthy children undergoing elective surgery, mobility of neutrophils, both unstimulated and stimulated by endotoxin, was studied using a millipore filter system with microscopic determination of leading front migration. Paired samples were incubated with $10^{-2}$ mol l$^{-1}$ calcium ascorbate and ten children also received 10 mg kg$^{-1}$ ascorbic acid before premedication. Stimulation of mobility was reduced after the opioid premedication ($P<0.05$) in the ascorbate group only, but not significantly during anaesthesia and surgery.

A few individuals showed persisting abnormally low values. No effect of ascorbate in vivo or in vitro was demonstrated. There were no infections.

Results were evaluated with the paired and unpaired Wilcoxon rank-sum tests, applying the Bonferroni corrections for multiple comparisons. The level of significance chosen was $P<0.005$.

RESULTS

Samples before premedication gave values of neutrophil mobility comparable with our previous control series in adults and children. The concurrent series of 15 healthy adults who were not anaesthetised gave a mean ± s.d. of $110 ± 14$ μm for stimulated preparations, $52 ± 15$ μm when unstimulated, and $58 ± 11$ μm for net stimulation (endotoxin-stimulated minus unstimulated mobility).

Our two randomised groups did not differ significantly.

Stimulated mobility was reduced slightly in both groups after premedication from a mean of 103 to 97

PATIENTS AND METHODS

Twenty children were studied while undergoing general anaesthesia for a variety of elective procedures (Table I). All were free from cardiorespiratory, infective or inflammatory illness and were not receiving any medication before the study. The parents gave informed consent and the study was approved by the hospital ethical committee. Patients were allocated randomly to receive either 10 mg kg$^{-1}$ ascorbic acid i.v. or the equivalent volume of saline, before premedication. Neither patient, parent, anaesthetists nor laboratory staff were told the allocation.

On the morning of surgery an indwelling peripheral venous cannula was placed, through which first a blood sample was taken into preservative free heparin, and then ascorbic acid or saline given. Premedication with papaveretum 0.4 mg kg$^{-1}$ (maximum 15 mg) and hyoscine 0.008 mg kg$^{-1}$ (maximum 0.3 mg) followed, by intramuscular injection, timed for 90 min before surgery. A second blood sample was taken on arrival in the anaesthetic room.

Anaesthesia was induced with thiopentone 4–5 mg kg$^{-1}$ and maintained with nitrous oxide, oxygen and halothane. Seventeen children were intubated using suxamethonium 1–2 mg kg$^{-1}$ i.v. A third blood sample was taken about 5–10 min after induction but before surgical stimulation. Eight children then received tubocurarine 0.5 mg kg$^{-1}$ and were ventilated (Table I). A fourth sample was taken at the end of surgery before terminating anaesthesia or giving atropine and neostigmine. Two cases (8 and 10, Table I) also received local anaesthesia. No case required blood transfusion during surgery. All were followed up for 7 days for evidence of infection.

Neutrophils for mobility measurement were separated by dextran sedimentation from 4-ml samples of heparinised blood. They were washed, then resuspended in Hank’s solution at a concentration of $2\times10^8$ ml$^{-1}$ and placed above 3 μm millipore membranes and incubated for 2 h. The migration of the leading front of neutrophils was measured microscopically as the mean of five readings on each of duplicate membranes. The chemotactic factor used was E. coli endotoxin-activated serum (12). Neutrophil mobility was also measured with $10^{-2}$ mol l$^{-1}$ calcium ascorbate in the cell suspension. Control studies with cells from healthy adults were performed in parallel.

Statistical methods

Results were evaluated with the paired and unpaired Wilcoxon rank-sum tests, applying the Bonferroni corrections for multiple comparisons. The level of significance chosen was $P<0.005$.
Table 1
Patient and operation details.

| Case no. | Age (years) | Weight (kg) | Operation                | Duration (min) | Ventilation |
|----------|-------------|-------------|--------------------------|----------------|-------------|
|          |             |             | Ascorbate group           |                |             |
| 1        | 8           | 25          | Cataracts                | 44             | C           |
| 2        | 13          | 28          | Examination, eyes        | 23             | C           |
| 3        | 8           | 37          | Dacrorhinoctostomy       | 79             | C           |
| 4        | 5           | 27          | Squint                   | 85             | S           |
| 5        | 14          | 58          | Rib biopsy               | 40             | C           |
| 6        | 10          | 36          | Hypospadias              | 20             | S           |
| 7        | 11          | 31          | Tonsilleotomy            | 24             | S           |
| 8        | 6           | 25          | Hypospadias              | 135            | S           |
| 9        | 15          | 54          | Squint                   | 78             | S           |
| 10       | 10          | 60          | Hypospadias              | 98             | S           |
| Mean     | 10.4        | 38          |                          | 63             |             |
|          |             |             | Control group            |                |             |
| 11       | 8           | 32          | Fundoplication           | 92             | C           |
| 12       | 11          | 48          | Cataract                 | 65             | C           |
| 13       | 8           | 17          | Change plaster           | 26             | S           |
| 14       | 15          | 46          | Change plaster           | 17             | S           |
| 15       | 6           | 24          | Pyeloplasty              | 80             | C           |
| 16       | 7           | 22          | Adenotonsillectomy       | 38             | S           |
| 17       | 7           | 17          | Excision accessory digit | 57             | S           |
| 18       | 18          | 52          | Pulete repair            | 104            | C           |
| 19       | 12          | 45          | Squint                   | 67             | S           |
| 20       | 12          | 43          | Myelogram                | 102            | S           |
| Mean     | 10.4        | 35          |                          | 65             |             |

G = controlled ventilation; S = spontaneous ventilation. Duration = interval between Samples II and IV.

344 A. J. CHARLTON ET AL.

µm in the ascorbate group and from 97 to 89 µm in the controls (n.s.) (Table 2). In two patients, stimulated mobility fell to abnormally low levels (32.2 and 36.3 µm).

The net stimulation values fell in both groups after premedication (Table 2), with a drop in mean value in the ascorbate group from 60.9 to 53.2 µm (P < 0.05).

During anaesthesia and surgery (Samples III and IV), group values were not significantly different from their initial levels. Results varied widely (Fig. 1 and 2).

Stimulated mobility values which were clearly abnormal (< 70 µm) were found in only three children after induction of anaesthesia, but in two these persisted throughout surgery.

In vitro ascorbic acid did not produce a significant rise in mobility in any group. There were no differences between the in vitro ascorbate and placebo groups. No postoperative infections occurred.

DISCUSSION

Our study demonstrated a significant depression of neutrophil mobility after premedication, an observation that does not seem to have been reported previously, but we failed to find the large reduction described in adults during anaesthesia (1). The effects seen during anaesthesia were less than we had expected from pilot studies in children, but in these we did not control for anaesthetic technique and used a shorter incubation.

Direct comparison of our data with those of other series is made difficult by the many differences in technique of mobility measurement between reported studies. Aspects of our techniques may be sub-optimal for the detection of anaesthetic drug effects (4). In particular, we made no attempt to preserve anaesthetic concentrations during incubation, but in the context of postoperative infection it was felt relevant to use a test capable of demonstrating effects on mobility outlasting the exposure to anaesthetic agents. Anderson et al. (13) suggested that the various chemotactic agents may produce stimulation of mobility by different metabolic routes within the cell, so our results might have altered with an alternative chemoattractant. We did, however, demonstrate that the neutrophil mobility values were not significantly different from their initial levels. Results varied widely (Fig. 1 and 2).

Stimulated mobility values which were clearly abnormal (< 70 µm) were found in only three children after induction of anaesthesia, but in two these persisted throughout surgery.

In vitro ascorbic acid did not produce a significant rise in mobility in any group. There were no differences between the in vitro ascorbate and placebo groups. No postoperative infections occurred.

Table 2

| Sample | Neutrophil mobility (µm) |
|--------|--------------------------|
|        | I (25–65)                | II (24–60) | III (27–61) | IV (23–61) |
| Random | 42                       | 43         | 44          | 43          |
| Stimulated | 103 (91–120)           | 97 (56–117)| 99 (61–132)| 94 (60–125)|
| Net stimulation | 61 (44–85)                | 53 (27–85)* | 55 (22–88) | 51 (15–90) |
| Stimulation + in vitro ascorbate | 101 (74–127) | 98 (55–124) | 96 (76–124) | 95 (57–129) |
| B Random | 46 (30–69)               | 43 (24–59) | 44 (22–59) | 50 (16–73) |
| Stimulated | 97 (73–115)             | 89 (59–109)| 90 (53–130)| 103 (62–120)|
| Net stimulation | 51 (30–78)                | 46 (23–61) | 46 (23–71) | 53 (40–80) |
| Stimulation + in vitro ascorbate | 100 (75–115) | 88 (57–109) | 93 (57–121) | 101 (50–122) |

* = P < 0.05 for the change from Sample I. Net stimulation = stimulated minus unstimulated values.
phil mobility of some apparently normal children remained depressed during anaesthesia and surgery.

Although in vitro ascorbic acid stimulated neutrophils from patients with primary defects of mobility (8–10), and there have been reports of this in healthy subjects (8, 11), we found no increase in our patients. Furthermore, in vivo pre-treatment with ascorbic acid did not appear to protect against this type of neutrophil depression. The only significant change found in our study was a reduction in mobility in the ascorbate group in Sample 11. Ascorbate has previously been reported to increase mobility in healthy subjects 1 h after intravenous injection (14), so it seems unlikely that the reduction we observed was due to the ascorbate.

In vitro exposure of neutrophils to anaesthetic agents can demonstrate pure drug effects, but the influence of in vivo administration occurs against a background of the complex and variable endocrine stress response to anaesthesia and surgery. It is not known whether the hormonal changes which occur during anaesthesia in children (15) are associated with the neutrophil depression found in this study, but the need for a further investigation is suggested.

ACKNOWLEDGEMENTS
The authors would like to thank their colleagues in the anaesthetic department at the Hospital for Sick Children for their help with this study, and Mrs Belinda Kay for her assistance in preparing the manuscript.

REFERENCES
1. Stanley T H, Hill G E, Portas M R, Hogan N A, Hill H R. Neutrophil chemotaxis during and after general anaesthesia and operation. Anesth Analg 1976: 55: 668-673.
2. Moudgil G C, Pandya A R, Ludlow D J. Influence of anaesthesia
and surgery on neutrophil chemotaxis. Can Anaesth Soc J 1981: 28: 232-238.
3. Nunn J F, O'Morain C. Nitrous oxide decreases motility of human neutrophils in vitro. Anesthesiology 1982: 56: 45-48.
4. Moudgil G C, Allan R B, Russell R J, Wilkinson P C. Inhibition by anaesthetic agents of human leucocyte locomotion towards chemical attractants. Br J Anaesth 1977: 49: 97-104.
5. Duncan P G, Cullen B F. Neutrophil chemotaxis and anaesthesia. Br J Anaesth 1977: 49: 345-349.
6. Nunn J F, Sturrock J E, Jones A J et al. Halothane does not inhibit human neutrophil function in vitro. Br J Anaesth 1979: 51: 1101-1108.
7. Kuo K N, Lloyd Roberts G C, Orme I M, Soothill J F. Immunodeficiency and infantile bone and joint infection. Arch Dis Child 1975: 50: 51-56.
8. Boxer L A, Watanabe A M, Rister M, Besch H R, Allen J, Bashner R L. Correction of leucocyte function in Chediak-Higashi syndrome by ascorbate. N Engl J Med 1976: 295: 1041-1045.
9. Anderson R, Theron A. Effects of ascorbate on leucocytes. Part III. In vitro and in vivo stimulation of abnormal neutrophil motility by ascorbate. S Afr Med J 1979: 56: 429-433.
10. Hayward A R, Harvey B A M, Leonard J, Greenwood M C, Wood C B, Soothill J F. Delayed separation of the umbilical cord, widespread infections and defective neutrophil mobility. Lancet 1979: i: 1099-1101.
11. Anderson R, Oosthuizen R, Maritz R, Theron A, Van Rensburg A J. The effects of increasing weekly doses of ascorbate on certain cellular and humoral immune functions in normal volunteers. Am J Clin Nutr 1980: 33: 71-76.
12. Aggett P J, Harries J T, Harvey B A, Soothill J F. An inherited defect of neutrophil mobility in Schwachman syndrome. J Pediatr 1979: 94: 391-394.
13. Anderson R, Glover A, Robson A R. The effect of chemotactic factors and agents which influence neutrophil movement on aerobic glycolysis and hexose monophosphate shunt activity. Immunology 1978: 35: 141-149.
14. Anderson R. Ascorbate-mediated stimulation of neutrophil mobility and lymphocyte transformation by inhibition of the peroxidase/H2O2/halide system in vitro and in vivo. Am J Clin Nutr 1981: 34: 1906-1911.
15. Lindahl S G E, Charlton A J, Norden N F. Endocrine response to surgery in children after premedication with midazolam or papaveretum. Eur J Anaesthesiol 1985: 2: 369-377.

Address:
A. J. Charlton, MB, ChB, FFARCS
Department of Anaesthesia
Manchester Royal Infirmary
Oxford Road
Manchester M13 9WL
UK