Effect of anaesthetic agents on the phagocytic function of human polymorphonuclear leukocytes through analysis with a phagocytic plaque method

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Received 22 November 1994; returned for revision 1 December 1994; accepted by E. R. Pettipher 8 February 1995

Abstract. We examined the effects of the intravenous anaesthetic agents, thiamylal and ketamine, on the phagocytic function of polymorphonuclear leukocytes (PMN) in heparinized peripheral blood from healthy individuals using the phagocytic plaque method. Subclinical doses of thiamylal caused enhancement of the phagocytic activity of PMN and super-clinical doses of thiamylal inhibited phagocytic activity of PMN. Ketamine did not adversely affect phagocytic function at relevant therapeutic concentrations. The results presented in this manuscript indicate the phagocytic plaque method can provide a quantitative assessment of the phagocytic function of leukocytes. This method may prove useful in determining whether anaesthetic agents and other drugs adversely affect leukocyte function and hence help prevent the increased susceptibility to infection which can occur in anaesthetized patients. Conversely, the phagocytic plaque method may prove useful in the search for immune-enhancing drugs.

Key words: Thiamylal – Ketamine – Phagocytic plaque method – Phagocytosis – Polymorphonuclear leukocytes

Postoperative infection is a serious clinical problem, especially for immuno compromised patients with diabetes mellitus or patients treated with catheters and prosthetic devices.

In addition, anaesthesia might bring surgically treated patients to immuno compromised state by affecting the functional activity of phagocytes [1–4]. So far, however, no report has been available about the effects of intravenous anaesthetic agents on the phagocytic function of professional phagocytes, such as polymorphonuclear leukocytes (PMN). We examined the effects of the representative intravenous anaesthetic agents, thiamylal and ketamine, on the phagocytic function of PMN using the phagocytic plaque method recently devised by some of the present authors [5].

Materials and methods

Blood samples

Eight healthy volunteers aged 21–59 years (mean age, 40 years) were selected for the present study. Following institutional guidelines and with informed consent, 8 ml of heparinized blood samples were collected from each subject. The blood samples were used for the phagocytic plaque method described below.

Phagocytic plaque method

Figure 1 shows the preparation and performance of the phagocytic plaque method. S. aureus Cowan I organisms, suspended in saline and boiled, were adjusted to 1 x 10⁹ CFU/ml. Plastic dishes for tissue culture (60 mm diameter, Nunc, Roskilde, Denmark) were each filled with 2 ml of this bacterial suspension. The dishes were allowed to stand at room temperature for 1 hour. The fluid in the dish was then carefully removed with a Pasteur pipette without destroying the bacterial thin-layer formed at the bottom of the dish. Blood collected from a single donor (8 ml) was divided into eight 1 ml-samples. Phosphate-buffered saline (PBS) 1 ml was added to 1 of the 1 ml-blood samples and was used for control. Thiamylal was added to the remaining 7 blood samples in a series of concentrations as described below. Prior to blood collection, thiamylal was adjusted into 7 different concentrations with PBS. The concentrations of thiamylal were 3.13 μg/ml, 6.25 μg/ml, 12.5 μg/ml, 25.0 μg/ml, 50.0 μg/ml, and 100 μg/ml. After blood collection, solution of thiamylal 1 ml was added to a 1 ml-blood sample resulting in blood thiamylal concentrations of 1.56 μg/ml, 3.13 μg/ml, 6.25 μg/ml, 12.5 μg/ml, 25.0 μg/ml, 50.0 μg/ml, and 100 μg/ml.

The same procedure and concentrations were used for ketamine. These blood samples were pre-incubated at 37 °C for 1 hour, after which 1.5 ml of the diluted blood sample was laid on the bacterial thin-layer in the plastic dish. The dish was then incubated at 37 °C for 1 hour. The blood was carefully removed with a Pasteur pipette and the dish was filled with saline. The red color on the
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Floating cocci were removed with plenter.

Blood ml and PBS ml with intravenous anaesthetics were mixed and pre-incubated at 37°C for 30 min.

Figure 1. Phagocytic plaque method. Following the illustration, the dish was subjected to Giemsa's staining. Aseptic handling with special equipment was unnecessary.

Figure 2. A representative picture of phagocytic plaques in the control group. Magnification: ×100. The phagocytic activity was represented as blank spaces of bacterial thin-layer usually containing phagocytic leukocytes (plaque).

Figure 3. The picture of Fig. 2 processed by a computer. Black spaces correspond to bacterial thin-layers and white spaces to phagocytic plaques.

Results

The numbers of PMN in the diluted blood sample were 2200/µl to 4150/µl (mean ± SE. was 2950 ± 250/µl, N = 8). Each blood sample in a series of concentrations was obtained from same donor, then each number of PMN in a series of concentrations was equal. Therefore, we have not discussed the donor to donor variability.

Thiamylal inhibited phagocytic activity significantly in a dose-dependent manner to 11.0 ± 2.0% at 25.0 µg/ml, 8.6 ± 1.7% at 50.0 µg/ml, and 6.2 ± 1.1% at 100 µg/ml in comparison with the control group (14.1 ± 2.1%), with the exception of a transient increase at the low concentration of 3.13 µg/ml in comparison with the control group (18.3 ± 3.7%). Ketamine enhanced phagocytic activity at 100 µg/ml, significantly (17.3 ± 2.2%) in comparison with the control group (12.8 ± 1.6%). However, no significant effects were found at concentrations under 100 µg/ml (Figure 5).

The data were expressed as mean ± standard error (SE.). Repeated-measures analysis of variance (ANOVA) and Student-Newman-Keuls test were used for statistical analysis. P values <0.05 were considered to be statistically significant.
Our results also demonstrate that ketamine did not adversely affect phagocytic function at relevant therapeutic concentrations. The enhancement of phagocytosis observed at high doses is unlikely to be of clinical significance and may be related to ketamine's previously reported immune-enhancing properties [13].

In conclusion, the results presented in this manuscript indicate that the phagocytic plaque method can provide a quantitative assessment of the phagocytic function of leukocytes. This method may prove useful in determining whether anaesthetic agents and other drugs adversely affect leukocyte function and hence help prevent the increased susceptibility to infection which can occur in anaesthetized patients.

Conversely, the phagocytic plaque method may prove useful in the search for immune-enhancing drugs.

Acknowledgements. The authors greatly acknowledge Dr. Nobuhiko Yasuda for his advice and assistance.

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