Effects of i.v. anaesthetic agents on the chemotaxis of eosinophils in vitro

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Polymorphonuclear eosinophilic leucocytes (PME) participate in wound healing processes, the inflammatory response, bronchial asthma, allergies and defence against invading parasites. We have examined the effects of thiopental, methohexital, propofol, etomidate and ketamine on PME chemotaxis in vitro. PME were isolated from venous blood samples of 10 healthy volunteers using multi-stage Percoll gradient centrifugation. Eosinophilic chemotaxis was determined using a 48-well microchemotaxis chamber. Thiopental 150 µg ml⁻¹ and etomidate 0.32 µg ml⁻¹ caused significant (P<0.05) inhibition of PME chemotaxis. We conclude that thiopental and etomidate may have an adverse influence on wound healing processes and parasitic diseases. Further studies are recommended.

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While there are numerous scientific publications on the influence of anaesthetics on the function of polymorphonuclear neutrophilic leucocytes (PMN), effects on polymorphonuclear eosinophilic leucocytes (PME) are unknown. PME play an important role in the defence against parasitic diseases and are involved in the pathogenesis of bronchial asthma, allergic reactions and other hypersensitivity states. Furthermore, eosinophils modulate wound healing processes. Using a multi-well chamber, we have examined the influence of induction agents (thiopental, methohexital, propofol, etomidate and ketamine) on the chemotaxis of eosinophils in vitro.

Methods and results

Approval for the study was obtained from the Ethics Committee of the medical faculty of Justus-Liebig-University Giessen. Venous blood samples (ethylene-diaminetetraacetic-dipotassium 1.5 mg ml⁻¹) were obtained from 10 healthy male volunteers. Granulocytes were isolated using a modification of the method described by Hjorth, Jonsson and Vretblad.¹ Percoll 55% and Percoll 74% were produced by diluting isotonic Percoll (Sigma, Deisenhofen, Germany) with normal saline. Percoll 55% (4 ml) was added to a polystyrene test tube, underlaid with 4 ml of venous blood and centrifuged at 350 g for 20 min at 20°C. The band of granulocytes was removed and suspended in phosphate buffered saline 10 ml (PBS; Gibco, Karlsruhe, Germany). After centrifugation at 350 g for 10 min at 20°C, the supernatant fluid was decanted. The few erythrocytes contaminating the pellet were lysed by adding double-distilled water 2 ml. After 20 s, isotonicity was restored with 2.7% saline 1 ml (Merck, Darmstadt, Germany). After adding PBS 7 ml, the solution was centrifuged at 350 g for 10 min at 20°C and the supernatant fluid decanted.

Separation of eosinophils from neutrophils and basophils was performed using a modification of the Percoll gradient centrifugation method described by Cramer and colleagues.² The cell pellet was suspended in Percoll 4 ml (density 1.085 g ml⁻¹). The suspension was laid on a 1-ml cushion of high-density Percoll (density ≥1.1 g ml⁻¹) and overlaid with PBS 1 ml. After centrifugation at 1000 g for 20 min at 20°C, the eosinophils were found in a band formed at the Percoll–cushion interface. The purity of the eosinophil yield (virtually 100%) was evaluated microscopically and viability was confirmed as ≥95% by the Trypan blue exclusion test.³ After dilution with PBS containing glucose 0.99 g 100 ml⁻¹ (Merck, Darmstadt, Germany), a concentration of 0.4×10⁶ eosinophils ml⁻¹ was produced.

Eosinophilic chemotaxis was determined by a modification of the method described by Boyden⁴ using a 48-well microchemotaxis chamber (Neuroprobe, Cabin John, USA). A cellulose nitrate filter (SM 113, Sartorius,
Göttingen, Germany) was positioned between the upper and lower compartments. N-formylmethionylleucylphenylalanine 10⁻⁸ mol litre⁻¹ (Sigma, Deisenhofen, Germany) was the only chemotactic agent used. This was poured into the lower compartments. The upper compartments were filled with eosinophils and anaesthetic agents. The chemotaxis chamber was incubated for 1.5 h at 37°C. This incubation time had proved to be optimal in preliminary experiments. The filter was then removed, fixed in formaldehyde (37%; Merck, Darmstadt, Germany) and stained with Mayer’s haematoxylin solution (Sigma, Deisenhofen, Germany). The filter was then dehydrated with ethanol (pharmacy of Justus-Liebig-University, Giessen, Germany) and made transparent with xylene (Merck, Darmstadt, Germany). The filter was then positioned upside down. The number of eosinophils per five microscopic fields was determined (magnification ×400). The relative standard deviation (precision) of the technique was approximately 10%.

The following concentrations of anaesthetics and corresponding additives were tested: thiopental (Thiopental ‘Lentia’; Hormonchemie, Munich, Germany) 1.5, 15 and 150 µg ml⁻¹; methohexital (Brevimytal Natrium; Eli Lilly, Bad Homburg, Germany) 1.7, 17 and 170 µg ml⁻¹; propofol (Disopivan; Zeneca, Plankstadt, Germany) 0.5, 5 and 50 µg ml⁻¹; etomidate (Etomidat-Lipuro; B. Braun, Melsungen, Germany) 0.032, 0.32 and 3.2 µg ml⁻¹; and ketamine (Ketanest 10; Parke-Davis, Berlin, Germany) 0.236, 2.36 and 23.6 µg ml⁻¹.

Medium concentrations used (thiopental 15 µg ml⁻¹, methohexital 17 µg ml⁻¹, propofol 5 µg ml⁻¹, etomidate 0.32 µg ml⁻¹ and ketamine 2.36 µg ml⁻¹) are those found in serum after i.v. injection of clinically relevant doses. All tests were performed in duplicate. The Pearson Stephens test did not show normal distribution and therefore Friedman analysis of variance and a Miller test were performed. A probability of \( P \leq 0.05 \) was regarded as significant. The power of the study (1–\( \beta \)) was approximately 0.7. Because of the lack of normal distribution, medians are given instead of means. Results (median (IQR)) are shown in Table 1.

### Comment

Polymorphonuclear eosinophilic leucocytes (PME)⁵ are mobile phagocytes which develop from stem cells in the bone marrow within 2–6 days. They are released into the circulation where they stay for several hours. Eosinophils are then predominantly located in submucosal tissues thus forming part of the immunological response system at body surfaces. One of the main functions of PME is destruction of parasites. Attracted by chemotactic molecules, the eosinophil moves towards its target and ingests it. Several granula components interact in the elimination of the parasite (major basic protein, eosinophil cationic protein, eosinophil-derived neurotoxin and eosinophil peroxidase). If the target is too large to be incorporated, the aggressive contents of the granula are released, thus damaging the parasite in the extracellular space (‘frustrated phagocytosis’). In addition to elimination of parasites, eosinophils have several other important functions. PME produce cytokines which influence acute and chronic inflammatory responses (granulocyte–macrophage colony-stimulating factor, interleukin (IL)-1-alpha, IL-3, IL-5, IL-6, IL-8 and tumour necrosis factor-alpha). They modulate wound healing processes by migrating into wound sites and liberating transforming growth factor-alpha and -beta. Moreover, eosinophils synthesize several lipid mediators such as leukotriene C₄, lipoxins and platelet-activating factor. During asthmatic attacks, major basic protein and eosinophil peroxidase enhance bronchoconstriction.

In conclusion, both etomidate and thiopental inhibited PME chemotaxis in vitro. Theoretically, this may have various consequences in vivo. For example, both anaesthetics may impair defence against parasitic diseases, which would be relevant to anaesthetists in certain developing countries. Moreover, etomidate and thiopental may impair wound healing processes, which would be of general interest. However, as in vitro investigations have limitations, it would be premature to draw clinical conclusions from our study. For methohexital, propofol and ketamine, adverse effects may be of concern. However, due to the lack of normal distribution, these results cannot be compared to each other.

### Table 1 Influence of thiopental, methohexital, propofol, etomidate and ketamine on eosinophilic chemotaxis in vitro (number of eosinophils per five microscopic fields; \( n = 10; \text{median (IQR)} \)). *\( P \leq 0.05 \)

<table>
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<tr>
<th></th>
<th>Control</th>
<th>Thiopental 1.5 µg ml⁻¹</th>
<th>Thiopental 15 µg ml⁻¹</th>
<th>Thiopental 150 µg ml⁻¹</th>
<th>Methohexital 1.7 µg ml⁻¹</th>
<th>Methohexital 17 µg ml⁻¹</th>
<th>Methohexital 170 µg ml⁻¹</th>
<th>Propofol 0.5 µg ml⁻¹</th>
<th>Propofol 5 µg ml⁻¹</th>
<th>Propofol 50 µg ml⁻¹</th>
<th>Etomidate 0.032 µg ml⁻¹</th>
<th>Etomidate 0.32 µg ml⁻¹</th>
<th>Etomidate 3.2 µg ml⁻¹</th>
<th>Ketamine 0.236 µg ml⁻¹</th>
<th>Ketamine 2.36 µg ml⁻¹</th>
<th>Ketamine 23.6 µg ml⁻¹</th>
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\( \text{IQR} \) = interquartile range.
effects on eosinophilic chemotaxis cannot be ruled out totally as the statistical power of our study was limited. Additional investigations are warranted.

References