Effects of intravenous anesthetics on bacterial elimination in human blood in vitro

Axel R. Heller, S. Heller, S. Blecken, R. Urbaschek, Thea Koch

Angaben zur Veröffentlichung / Publication details:

Heller, Axel R., S. Heller, S. Blecken, R. Urbaschek, and Thea Koch. 1998. "Effects of intravenous anesthetics on bacterial elimination in human blood in vitro." *Acta Anaesthesiologica Scandinavica* 42 (5): 518–26. https://doi.org/10.1111/j.1399-6576.1998.tb05160.x.

Effects of intravenous anesthetics on bacterial elimination in human blood *in vitro*

A. HELLER, S. HELLER, S. BLECKEN, R. URBASCHEK[1] and T. KOCH
Departments of Anesthesiology and Operative Intensive Care Medicine and [1]Medical Microbiology and Hygiene, Faculty of Clinical Medicine Mannheim, University of Heidelberg, Mannheim, Germany

Backround: Since anesthetics are widely used in critically ill patients, this study investigates anesthetic effects on neutrophil and monocyte function concerning bacterial elimination in human whole blood.
Methods: The effects of thiopental (20 and 200 μg/ml), propofol (5 and 50 μg/ml), midazolam (0.15 and 1.5 μg/ml) and ketamine (3 and 30 μg/ml) on elimination of *Escherichia (E.) coli* from whole blood were investigated *in vitro* after incubation for 1 h in both clinical (l) (n=10) and 10-fold higher (h) (n=11) concentrations. These data were compared to neutrophil and monocyte phagocytosis (l; n=6) and burst activity (l; n=10, h; n=11), measured by flow cytometry. To enable quantification of the clearance process, a defined number of 10^5 colony forming units of *E. coli* were added to the blood assays and bacterial growth was determined.
Results: All anesthetics delayed bacterial clearance from the blood in the 10-fold concentration ($P<0.05$). Thiopental (l+h) and propofol (h) suppressed neutrophil (59±3% and 38±6%) and monocytic (45±6% and 30±11%) oxidative burst ($P<0.01$). Phagocytosis was reduced even after propofol (l) in polymorphonuclear leukocytes (PMN) (34±9%; $P<0.05$) and monocytes (35±11%). Ketamine (h) prolonged bacterial elimination ($P<0.01$), which did correlate with inhibition of monocytic phagocytosis, by 26±14%. Midazolam application (h) resulted in an inhibition of PMN-respiratory burst by 19±6% ($P<0.05$) and impaired bacterial clearance ($P<0.05$).
Conclusion: Thiopental, propofol, midazolam and ketamine affect *E. coli* clearance and neutrophil and monocyte oxidative burst and phagocytosis *in vitro* only in high concentrations, while thiopental inhibited monocytic burst and propofol impaired PMN phagocytosis even in clinically used concentrations. These data suggest that i.v. anesthetics in concentrations recommended for general anesthesia seem to have minor influence on the investigated host defense mechanisms.

Key words: Anesthetics; neutrophils; monocyte; bacterial clearance; *E. coli;* respiratory burst; phagocytosis; flow cytometry; sepsis.

POSTOPERATIVE infections as well as nosocomial infections in critically ill patients still represent a severe problem. In the healthy organism, invading bacteria are rapidly eliminated by the complex system of humoral and cellular host defense. This homeostasis of invading bacteria and their elimination could be disturbed by anesthetic drugs. Patients receive intravenous anesthetics not only for short intervals in interventional surgery but also for long-term analgesia and sedation in intensive care units. In the latter case, anesthetics accumulate in the organism yielding high concentrations in blood and inflamed tissue. Because these patients often suffer from compromised immune functions, a further impairment of host defense mechanisms induced by anesthetics may increase the threat of sepsis and multiple organ failure (MOF).

The non-specific host defense is primarily based upon polymorphonuclear leukocytes (PMN) and mononuclear phagocytes. Over the last years evidence has accumulated that defects in PMN functions such as chemotaxis, adherence, phagocytosis, and bacterial killing may cause an increased incidence of sepsis and MOF (1). The bactericidal potential of the PMN, e.g. the generation of reactive oxygen intermediates and various proteolytic enzymes, represents a highly efficient system in maintaining the sterility of blood and tissue. It is known that different anesthetics cause depression of PMN functions such as phagocytosis (2, 3) and respiratory burst activity (4).

Although these effects of i.v. anesthetics have been studied by other investigators, the consequences of depressed neutrophil function on bacterial clearance in the blood are still unknown. Furthermore, anesthetic effects on other phagocytic cells such as monocytes have not been clarified. Most studies examine the effects of anesthetic agents on phagocytosis and burst activity of separated granulocytes by fluor-

escence microscopy (5) or chemiluminiscence methods (6). Leukocytes isolated from their typical serum environment containing immunoglobins and complement compounds which contribute to host defense in the organism, react in a different manner compared to native blood.

Therefore, the aim of this study was to investigate whether i.v. anesthetics impair bacterial elimination and granulocyte- and monocyte-dependent functions in human whole blood. In order to correlate the bactericidal capacity of the blood with cellular functions, the elimination of *E. coli* from the blood as well as PMN and monocyte phagocytosis and burst activity were determined after incubation of whole blood from healthy donors with propofol, ketamine, midazolam and thiopental.

Blood donors were treated according to the recommendations for biomedical research involving human subjects (Declaration of Helsinki). This study was approved by the ethics committee of the Faculty of Clinical Medicine Mannheim, University of Heidelberg.

Material and methods

Blood samples

Freshly drawn venous blood, 100 ml, from 21 healthy volunteers (19–35 years old), was heparinized with 10 U/ml (Liquemin 25000, Roche FRG).

Bacterial clearance

To determine the influence of different anesthetics on elimination of *E. coli* from the blood, the whole blood sample from each donor was divided into five 20-ml aliquots. These were incubated in a shaking water bath (frequency 60/min) at 37°C for 1 h either with propofol, ketamine, midazolam or thiopental in a clinical and a 10-fold dose, or saline which served as control, respectively (total assay volume 20.5 ml). After this preincubation with the anesthetics, samples were taken for control values and for the flow cytometrical determination of respiratory burst and phagocytosis capacity. Thereafter, a defined number of viable *E. coli* (10^5 colony forming units (CFU)) was added and specimens were taken in a 3-h follow-up period as described for the different groups. Bacterial growth was quantified by microbiological culture and allowed determination of bacterial elimination.

Control group (n=21)

Following a 60-min period after which 0.5 ml NaCl (0.9%) was added, *E. coli* suspended in 1 ml tryptic soy broth was administered into the blood in a final concentration of approximately 5000 CFU *E. coli* per ml. To study the time course of bacterial clearance, blood samples were aseptically taken for culture before and after addition of bacteria at 1, 15, 30, 45, 60, 90, 120, 150 and 180 min after carefully vortexing the assay. Leukocyte counts were determined before and 180 min after *E. coli* application. The procedure from the time point of bacterial injection to the end of the observation period was identical in all experimental groups.

Anesthetic groups (low concentration: n=10; 10-fold higher: n=11)

E. coli was added to the blood samples after the 60-min incubation period with clinical and 10 times higher concentrations of propofol (5 μg/ml; n=10 and 50 μg/ml; n=11 Disoprivan, Zeneca, FRG), ketamine (3 μg/ml; n=10 and 30 μg/ml; n=11 Ketanest, Parke Davis, FRG), midazolam (0.15 μg/ml; n=10 and 1.5 μg/ml; n=11 Dormicum, Roche, FRG) and thiopental (20 μg/ml; n=10 and 200 μg/ml; n=11 Trapanal, Byk Gulden, FRG). The appropriate concentration of anesthetics in the assay was achieved by dilution of each anesthetic in sterile saline to a total volume of 500 μl each. The further protocol was performed according to the control group.

Quantitative microbiology

Blood samples were chilled and prepared for bacterial culture immediately after collection. Blood was serially diluted 1:10 in sterile saline. An aliquot of 100 μl each was plated onto CLED (Cysteine Lactose Electrolyte Deficient)-agar plates, according to Sandys (7) in duplicate. The inoculated plates were incubated at 37°C for 24 h and bacterial counts were registrated as CFU per ml.

Bacterial inoculum

E. coli, an encapsulated, serum-resistant, non-hemolytic strain, freshly isolated from blood culture of a septicemic patient, was cultivated on blood agar plates. The grown colonies were scraped from the plates, carefully homogenized by vortexing in tryptic soy broth, serially diluted and then adjusted to a density of 10^5 CFU/ml. They were stored at −60°C until use. The amount of *E. coli* used was based on pilot experiments investigating the clearance of different numbers of *E. coli* (10^7, 10^6, 10^5) from the blood. Bacterial counts lower than 10^5 as well as amounts exceeding 10^6 were not suitable for the determination of differences in bacterial elimination kinetics between the miscellaneous treatments in this model. The reason for this is the rapid elimination of small numbers of *E. coli* on the one hand, and the prolonged clearance

of large numbers on the other hand, so that differences cannot be seen within the observation period. For this study, a dose was chosen which showed a well-reproducible elimination kinetics. The applied dose of 10^5 *E. coli* was eliminated during a time period between 150 and 210 min in control samples, and allowed registration of slight decreases as well as slight increases in elimination kinetics during the observation period. Serum resistance of the used strain was demonstrated in control experiments in which *E. coli* showed slightly increased numbers after incubation in serum at 37°C for 3 h.

Neutrophil and monocyte burst activity

The amount of intracellular oxygen radical production was determined in human blood, which was incubated for 1 h with the different anesthetics (n=10 each). A test kit (Bursttest, Orpegen Pharma, Heidelberg, FRG) for the determination of the oxidative burst of leukocytes in whole blood was used. This method is faster than those reported in previous studies (4) and does not require leukocyte isolation, which could lead to alteration of cell functions. Single-cell analysis was performed by flow cytometry. The method of the quantitative assay for monitoring the oxidative burst has been described in detail previously (8). Briefly, four assays (100 µl) of each sample were incubated (10 min, 37°C) with either phosphate-buffered saline (PBS, negative control), chemotactic peptide fMLP (low control), PMA (high control) or *E. coli* which had been opsonized with antibodies and complement from pooled sera. PMA, *E. coli* and fMLP stimulate production of reactive oxygen intermediates in descending order. After the oxidation step (incubation with substrate 10 min, 37°C) in which the non-fluorescent substrate, dihydrorhodamine 123 was taken up by the phagocytes and converted during the respiratory burst to a green fluorescent compound (rhodamine 123), the whole blood was lysed and fixed. To guarantee that no cell debris or dead cells or bacteria interfere with the measurement, DNA-staining was performed with propidiumiodide (red fluorescence, FL-3). From each sample, 15000 cells were measured with a laser flow cytometer (FACScan/Lysis II, Becton Dickinson, Heidelberg, FRG) using blue-green excitation light (488 nm Argon Laser). Photomultiplier gain setting was adjusted to register all intensities of cellular fluorescence within the scale of 1023 channel resolution (four decades). PMN cells and monocytes were identified by their characteristic size-to-granularity ratio (Fig. 1a). Only the selected cell population was considered for further analysis (multiple document interface for Windows 3.1 (WinMDI 2.0) by J. Trotter)). During data acquisition a "live" gate was set in the red fluorescence dot plot on those events which had at least the same DNA content as human diploid cells (Fig. 1b). Only these cells were examined. For characterization of the fluorescence distribution the geometric mean values (FL-1) were used, which indicate the amount of rhodamine 123 per cell (Fig. 1c).

Neutrophil and monocyte phagocytosis activity

To compare the data of bacterial elimination and burst activity with those of PMN and monocyte phagocytosis capacity, the quantity of intracellular *E. coli* in PMN and monocytes was measured after 1 h incubation with the different anesthetics in clinical concentrations (n=6). A test kit (Phagotest, Orpegen Pharma, Heidelberg, FRG) for the determination of the phagocytic activity of leukocytes in whole blood was used. Single-cell analysis was performed by flow cytometry. The method of the quantitative assay for investigating the phagocytic activity has been described in detail previously (9). Two assays of each sample (2×100 µl) were incubated with fluoresceine-isothiocyanate-(FITC)-marked opsonized *E. coli* either at 0°C (control) or at 37°C for 10 min. Cells of the phagocytic system have receptors for a complement component (C3b) and for the constant part of the immunoglobulin molecule (Fc) mediating the adhesion of the bacteria to the cell surface and subsequent engulfment. During the incubation period the green fluorescent FITC-marked *E. coli* were ingested in the 37°C assay. To exclude extracellular bacteria from measurement, they were quenched with a staining solution. The further steps such as lysis, fixation, DNA-staining and data acquisition by flow cytometer were performed in the same manner as in the burst assay. The phagocytosis activity of leukocytes was determined by the content of FITC-marked *E. coli* in the phagocytic cells, expressed as mean channel fluorescence (FL-1) per cell (Fig. 1d).

Statistics

Data are presented as arithmetic mean±standard error of means (SEM). To achieve Gaussian's distribution, the logarithm of the quantitative bacterial count was used for statistical comparison. Results of the oxidative burst activity are expressed as mean channel fluorescence per cell. Differences among the five groups were tested by an overall analysis of variance (ANOVA) for repeated measurements followed by Student-Newman-Keuls multiple comparison test. Significance was accepted at $P<0.05$.

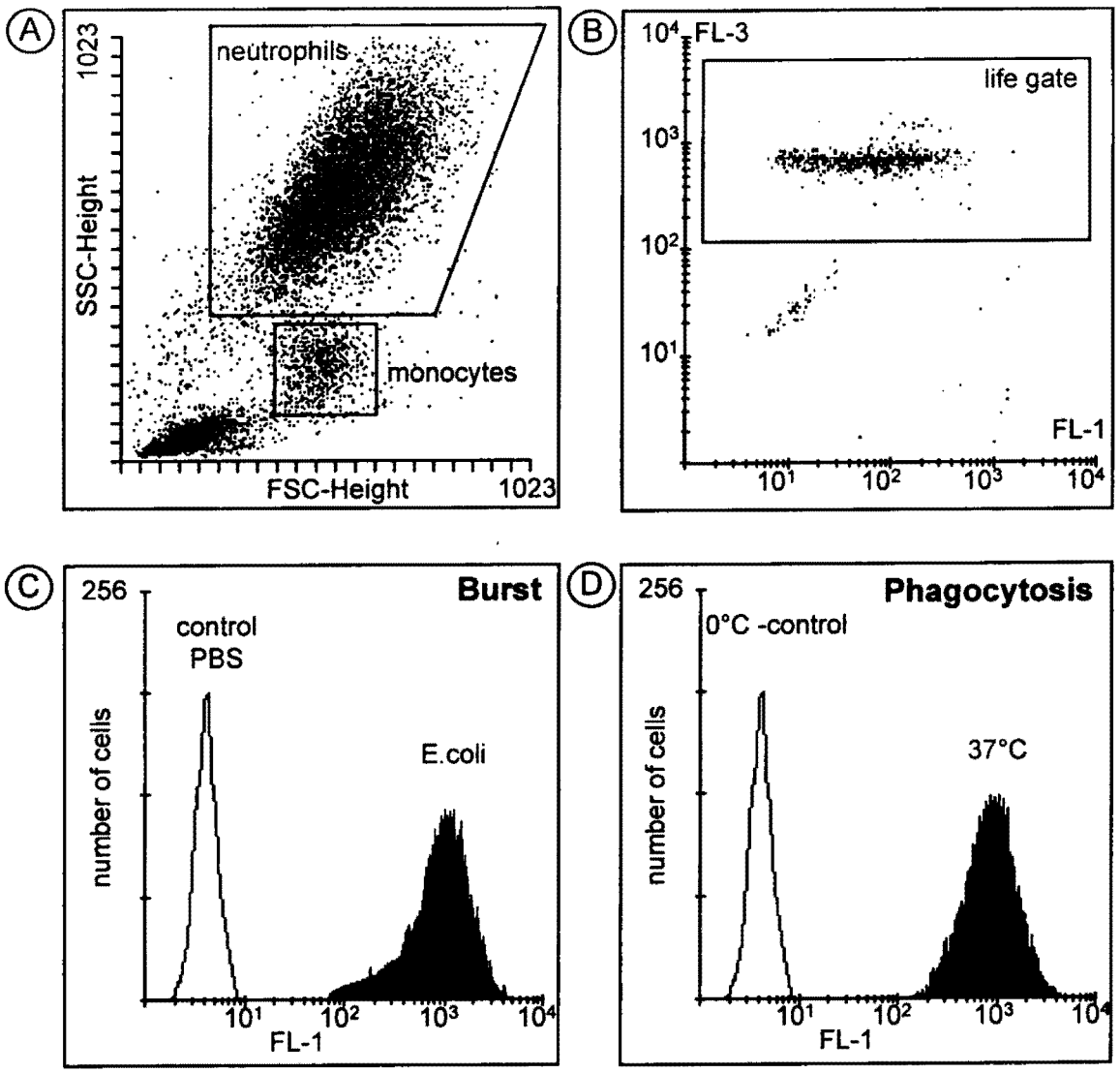

Fig. 1a–d. Flow cytometer adjustment for measurement of leukocyte burst activity and phagocytosis. Selection of appropriate cell population in forward scatter- (FSC, size of cells) vs. sideward scatter- (SSC; granularity of cells) dot plot (a). Exclusion of dead cells, debris and bacteria by setting a life gate (b) FL-1 green fluorescence (Rho 123 and FITC), FL-3 red fluorescence (DNA). Typical FL-1 histograms of the burst- (c) and phagocytosis-activity (d). Gates set on DNA-positive neutrophils or monocytes.

Results

Bacterial clearance

Bacterial cultures from the blood samples before addition of *E. coli* were sterile in all groups. The time course of bacterial clearance from the blood is shown in Fig. 2a, b. The elimination of bacteria is characterized by an exponential-like decrease of injected *E. coli* over the whole experimental period of 3 h in all groups. Mean bacterial counts detected 1 min after *E. coli* application of 5000 CFU/ml (n=10) decreased rapidly to 535±76 CFU/ml after 90 min and to 88±24 CFU/ml at 180 min in the control group. Compared to controls, clinical concentrations of anesthetics did not affect elimination of *E. coli* while the 10-fold concentration of all investigated anesthetics delayed the removal of bacteria from the blood. Mean values of viable *E. coli* ranged between 5000 in the first minute and 463±134 CFU/ml at 180 min after incubation with the higher concentrations of thiopental, and between 5000 and 282±135 CFU/ml after treatment with ketamine (30 μg/ml) respectively. Mean bacterial counts differed significantly from controls at 45 min, $P<0.05$, and from 60 min until the end of the observation period, $P<0.01$, after thiopental, and at 60, 90 and 120 min after ketamine, $P<0.01$. In the midazolam-group, *E. coli* counts ranged between 5000 and 208±85 CFU/ml (60 min, $P<0.01$; 90 and 120 min,

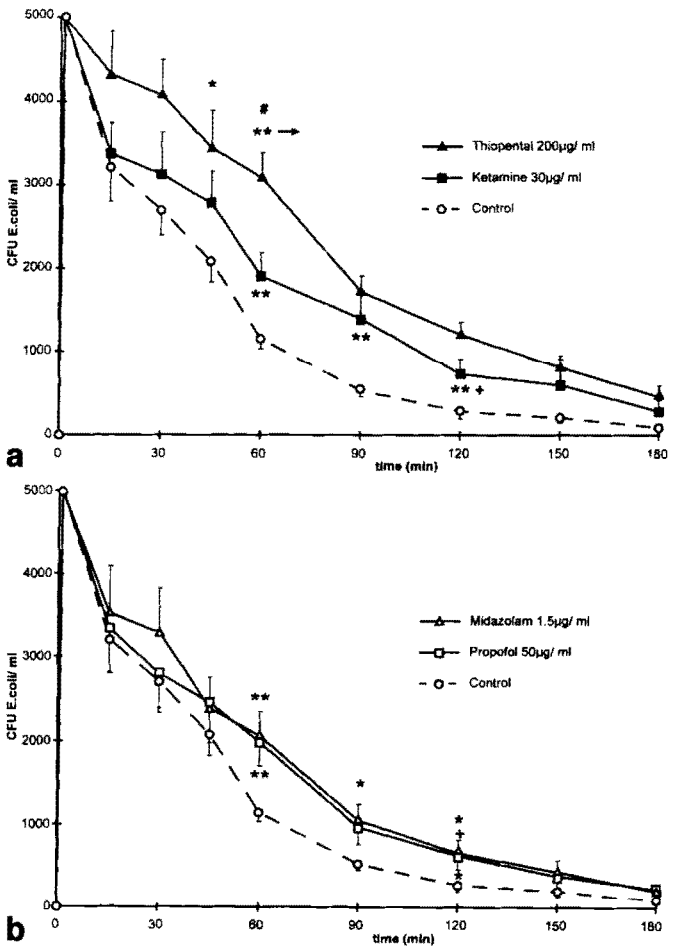

Fig. 2a. Time course of bacterial elimination from blood after addition of Escherichia coli *(5000 CFU/ml) in control assays and after incubation with ketamine 30 µg/ml and thiopental 200 µg/ml. Mean counts of CFU/ml are plotted against time in min. Mean values±SEM at each time point and significant differences between the groups are indicated (**P<0.01 vs. control; *P<0.05 vs. control; #P<0.05 vs. all other groups; +P<0.05 vs. thiopental). b. Time course of bacterial elimination from blood after injection of* Escherichia coli *(5000 CFU/ml) in control assay and after incubation with propofol 50 µg/ml and midazolam 1.5 µg/ml. Mean counts of CFU/ml±SEM are plotted against time in min. Significant differences between the groups are indicated (**P<0.01 vs. control; *P<0.05 vs. control; +P<0.05 vs. thiopental).*

P<0.05 vs. control). Propofol resulted in bacterial counts of 5000 CFU/ml at the beginning and 232±86 CFU/ml after 180 min. The counts differed significantly at 60 min (P<0.01) and 120 min (P<0.05). Comparison of data among the 10-fold concentrations of anesthetics indicated significantly higher bacterial counts after thiopental versus all other groups at 60 min (P<0.05) and at 120 min versus midazolam and ketamine (P<0.05).

PMN oxidative burst activity

Fig. 3 shows the inhibition of neutrophil oxygen radical production in a flow cytometric fluorescence diagram in anesthetic incubated assays. In contrast to the controls in which a characteristic radical production was determined, a concentration-dependent inhibition of respiratory burst activity was discovered in all anesthetic groups. For statistical comparison, oxidative radical generation of control blood was considered as 0% inhibition and alterations due to anesthetics were related to control values. A significant dose-dependent inhibition of oxidative burst activity was detected after incubation with thiopental of 59.4±2.8% (10-fold; P<0.01) and 29.6±3.7% (clinical concentration; P<0.01) respectively. In comparison with controls, a decrease in free oxygen radical production of 37.6±5.9% was found after propofol (50 µg/ml; P<0.01) and a reduction of 19.4±5.9% following midazolam incubation (1.5 µg/ml; P<0.05). The changes in oxidative burst induced by the low dose of propofol (16.5±8.3%) and midazolam (13.7±7.3%) did not reach statistical significance. The inhibition of PMN respiratory burst due to ketamine in both concentrations to 11.1±6.2% (30 µg/ml) and 12.2±5.0% (3 µg/ml), respectively, did not differ significantly from control values. Comparison among the anesthetic groups with 10-fold concentration exerted significant impairment of PMN burst activity after thiopental compared to all other anesthetics (P<0.01) and of propofol versus ketamine and midazolam (P<0.01).

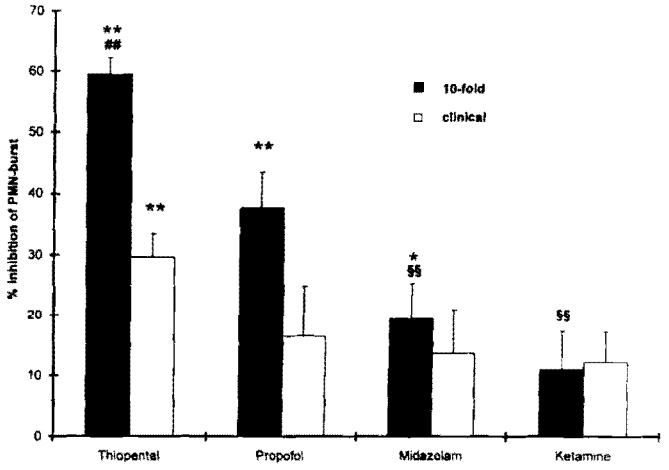

*Fig. 3. Inhibition of PMN respiratory burst activity in blood samples (n=10) incubated with different concentrations of thiopental (20 and 200 µg/ml), propofol (5 and 50 µg/ml), midazolam (0.15 and 1.5 µg/ml), and ketamine (3 and 30 µg/ml) related to control values. Results are presented as reduction of the relative mean channel fluorescence per cell. Mean values±SEM for both anesthetic concentrations and significant differences among the groups are indicated. (**P<0.01 vs. control; *P<0.05 vs. control; ##P<0.01 vs. all other groups; §§P<0.01 vs. propofol).*

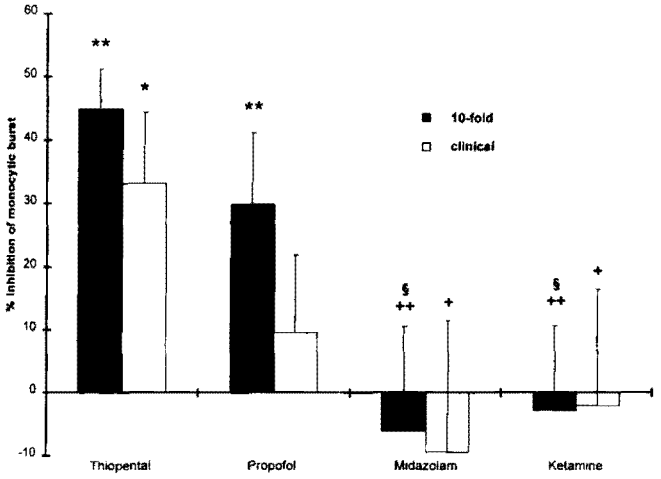

*Fig. 4. Inhibition of monocytic oxidative capacity in blood samples (n=10) incubated with different concentrations of thiopental (20 and 200 µg/ml), propofol (5 and 50 µg/ml), midazolam (0.15 and 1.5 µg/ml) and ketamine (3 and 30 µg/ml) related to control values. Results are presented as reduction of the relative mean channel fluorescence per cell. Mean values±SEM for both anesthetic concentrations and significant differences among the groups are indicated. (**P<0.01 vs. control; *P<0.05 vs. control; ++P<0.01 vs. thiopental; +P<0.05 vs. thiopental; §P<0.05 vs. propofol).*

Monocyte oxidative burst activity

The inhibition of monocytic oxidative capacity is shown in Fig. 4. Corresponding to PMN burst activity, thiopental and propofol showed dose-dependent impairment of monocyte free oxygen radical production compared to controls. At the high dosage, thiopental resulted in a reduction of 45.0±6.2% ($P<0.01$) and in the low dose of 33.3±11.1% ($P<0.05$). Propofol induced an inhibition of 30.0±11.2% ($P<0.01$) after high-dose incubation. Low doses of propofol (9.6±12.2%) as well as application of midazolam (high: −6.2±16.6; low: −9.4±20.9) or ketamine (high: −2.9±13.5; low: −2.1±18.4) in either concentration did not change monocytic oxygen radical production significantly. The comparison between the anesthetics indicated significantly higher inhibition of monocytic burst by thiopental than following ketamine or midazolam administration in clinical ($P<0.05$) as well as in 10-fold concentrations ($P<0.01$). High quantities of propofol also induced significantly enhanced inhibition of monocytic burst compared to midazolam and ketamine ($P<0.05$).

Phagocytic activity

To allow further insight into the mechanism of delayed bacterial elimination after incubation with anesthetics, we performed experiments (n=6) using another flow cytometric assay to determine the quantity of *E. coli* ingested by monocytes and neutrophils. Fig. 5 shows the inhibition of monocyte and neutrophil phagocytosis after stimulation with fluorescence-marked *E. coli* after incubation with anesthetics in clinical concentrations. These experiments resulted in a mean reduction of the phagocytic activity of about one-third (PMN 34.2±8.6%; $P<0.01$ and monocytes 34.5±11.2; n.s.) after propofol 5 µg/ml administration and a decrease of 17.1±4.0% (PMN) and 11.0±6.2% (monocytes) after thiopental (20 µg/ml). Midazolam (0.15 µg/ml) induced an inhibition of phagocytic activity of 12.6±8.0% (PMN) and 14.9±16.6% (monocytes). Although ketamine did not affect the PMN engulfment of *E. coli* (0.7±6.9%), it reduced the monocytic phagocytosis activity by 26.3±13.5% (n.s.). The PMN phagocytosis after propofol was inhibited to a significantly higher extent than after ketamine ($P<0.01$).

In additional experiments, it was tested whether the treatment with the doses of anesthetics used induce changes in pH or osmolality during the observation period. Changes in osmolality or pH itself may alter the reactivity of neutrophils and monocytes to exogenous stimuli. Blood pH and osmolality values were within the normal range over the observation period.

Discussion

Septic shock and multiple organ failure continue to be a major cause of death in severely injured patients (10). Observations of a transient but profound de-

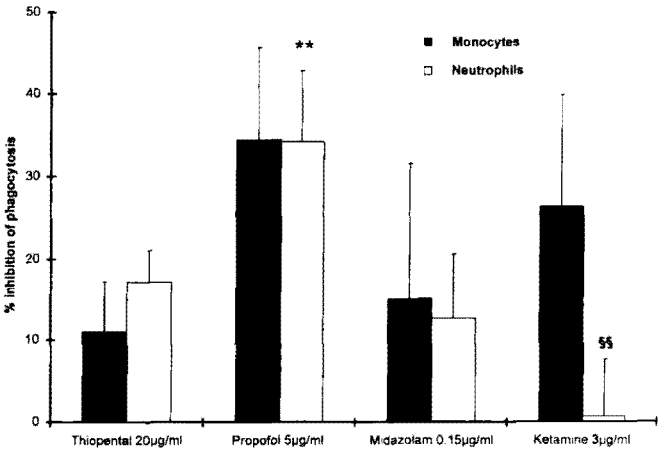

Fig. 5. Inhibition of phagocytosis of FITC-labeled E. coli *in monocytes and neutrophils (n=6) due to clinically used concentrations of thiopental (20 µg/ml), propofol (5 µg/ml), midazolam (0.15 µg/ml) and ketamine (3 µg/ml) treatment. Results are presented as reduction of the relative mean channel fluorescence per cell in the control assay. Mean values±SEM for both leukocyte populations and significant differences among the groups are indicated. (**P<0.01 vs. control; §§P<0.01 vs. propofol).*

pression of the mononuclear phagocyte system (MPS) following injury or surgical trauma implicate an essential role in the pathogenesis of septic events. Failure to eliminate inoculated exogenous or translocated endogenous bacteria due to host immunosuppression (11, 12) may contribute to the persistence of bacteria in blood and lymph nodes and to the development of septic complications. Leukocyte activation and subsequent phagocytosis and release of oxygen radicals contribute to host defense. Despite the frequent use of i.v. anesthetics in ICU patients, there is concern about depressing effects of several anesthetics (4, 13) on different leukocyte functions such as chemotaxis (14), locomotory functions (15, 16), phagocytosis (17) or free radical generation (18). Clinical studies can hardly evaluate whether these effects increase postoperative infection rates in patients (19) because of the difficulty in distinguishing the effects due to operative trauma from those induced by anesthetics that both influence immune system function. Various experimental studies provide evidence that several i.v. anesthetics reduce oxidative burst activity of neutrophils (4, 20). It is still unclear whether the impaired radical production predisposes the host to increased susceptibility to bacterial infections and to what extent radical production is required to sustain a normal immune response. Recent studies investigating effects of anesthetics on PMN functions have been performed using a variety of methods (3) and only rarely considered other cell populations. There are conflicting reports of the effects on different leukocyte functions.

Our interest was therefore focused on the question of whether or not there is a correlation between impaired neutrophil functions and removal of bacteria from the blood. We have studied the effects of thiopental, propofol, midazolam and ketamine on elimination of *E. coli* from human blood of healthy donors. The data of bacterial clearance from the blood was compared to the respiratory burst- and phagocytosis-activity of neutrophils and monocytes. In addition to concentrations found during general anesthesia, 10-fold higher concentrations of each drug were used in the current study to analyze dose-dependent effects. Since effects throughout non-physiologic pH or osmolality were excluded in additional assays, we conclude that inhibition of bacterial elimination can be attributed to the applied anesthetic agents or their solvents. To enable quantification of the clearance process, a defined number of *E. coli* (10^5 CFU) was added to each blood sample after incubation with the different anesthetics. To exclude other bacterial contamination, the sterility of each sample was checked before *E. coli* application. Our results demonstrate that thiopental, ketamine, midazolam and propofol impair bacterial elimination from the blood in high concentrations. This was shown by the delayed removal of injected *E. coli* from the blood compared to the saline-treated controls. These findings can be explained with the flow cytometric data. The cell function analysis clearly documents a significant suppression of PMN and monocytic oxidative burst activity after treatment with thiopental in a therapeutic and 10-fold higher dosage in parallel with the impaired blood clearance in high concentrations. Likewise, a reduced neutrophil burst activity was determined after propofol (50 µg/ ml) and midazolam (1.5 µg/ml) treatment, as well as monocyte burst impairment after propofol (50 µg/ml). Apart from respiratory burst, the phagocytosis activity of neutrophils and monocytes, was examined after low-dose incubation with anesthetics. A suppression of phagocytosis in both cell populations due to propofol was demonstrated in commonly used concentrations. The inhibition of phagocytosis in neutrophils and monocytes differed considerably after ketamine incubation, possibly pointing towards a receptor-specific disturbance of leukocyte functions. The application of ketamine in higher concentrations resulted in impaired bacterial removal which was associated with depression of monocytic phagocytosis activity, whereas PMN functions remained unaltered. The striking suppression of phagocytosis and radical production, especially after incubation with high concentrations of various i.v. anesthetics, substantiate the hypothesis that the prolongation of bacterial clearance from the blood after anesthetic treatment might be related to reduced PMN and monocyte phagocytosis and burst activity. The inhibitory effects of thiopental and propofol on phagocytosis and oxygen radical generation support previous studies which documented that thiopental and propofol inhibit human neutrophil chemotaxis and oxidative metabolism (4, 5). Most alterations of the investigated microbicidal functions occurred in the 10-fold concentration, which will not be reached during anesthesia in general. Nevertheless, on the one hand higher than commonly recommended concentrations potentially accumulate during long-term analgosedation or in septicemic patients with disturbance of micro- and macrocirculation. On the other hand, this study demonstrated functional alterations even in clinical concentrations, thus pointing towards concentration-dependent effects of the investigated anesthetics. The critical question of whether compromised immune functions induced by anesthetics predispose the host to an increased susceptibility to infection cannot, however, be answered finally from the current results. Clinical

studies from Duignan et al. (21) and Buffone et al. (22) have demonstrated that the reduced chemotaxis observed after surgery correlates with the development of postoperative infection and sepsis, whereas other investigators (23, 24) did not find any correlation between postoperative neutrophil dysfunction in terms of enzyme content (25), respiratory burst (26) and the incidence of bacterial infections.

Since bacterial infection and the development of sepsis is a multifactorial process that is influenced not only by leukocyte functions but also by a variety of humoral and cellular systems contributing to immune response, no final conclusion concerning the relationship between anesthetic treatment and risk of infection could be drawn. Summarizing our results, thiopental, ketamine, midazolam and propofol were shown to affect *E. coli* clearance, and neutrophil and monocyte oxidative burst and phagocytosis *in vitro* to a differing extent. After high concentrations of the investigated anesthetics, granulocyte- and monocyte-dependent bacterial elimination was impaired, which may affect host defense in critically ill patients. The ketamine-induced disturbance of bacterial removal without reduced neutrophil functions, but with impairment of monocytic phagocytosis, point towards cell-type or receptor-dependent mechanisms of anesthetic-induced immunosuppression. In contrast to thiopental which inhibited monocytic burst, and propofol which impaired PMN phagocytosis in commonly used concentrations, drug concentrations recommended for general anesthesia seem to have minor influence on the investigated host defense mechanisms. Further studies are required to elucidate the complex interrelationships between the treatment with i.v. anesthetics and host defense and to determine their clinical relevance.

Acknowledgments

The authors thank B. Kaschta, S. Thuy (Institut für Med. Mikrobiologie und Hygiene, Klinikum Mannheim) and J. Christophel (Zentrum für medizinische Forschung, Fakultät für Klinische Medizin Mannheim der Universität Heidelberg) for excellent technical assistance.

The study was supported by a grant from the Faculty of Clinical Medicine Mannheim.

References

1. Faist E, Schinkel C, Zimmer S. Update on the mechanisms of immune suppression of injury and immune modulation. *World J Surg* 1996: **20**: 454–459.
2. Graham EA. The influence of ether and ether anesthesia on bacteriolysis, agglutination and phagocytosis. *J Infect Dis* 1911: **8**: 147–175.
3. Stevenson GW, Hall SC, Rudnick S et al. The effect of anesthetic agents on the human immune response. *Anesthesiology* 1990: **72**: 542–552.
4. Heine J, Leuwer M, Scheinichen D et al. Flow cytometry evaluation of the *in vitro* influence of four i.v. anesthetics on respiratory burst of neutrophils. *Br J Anaesth* 1996: **77**: 387–392.
5. Krumholz W, Endrass J, Hempelmann G. Propofol inhibits phagocytosis and killing of *Staphylococcus aureus* and *Escherichia coli* by polymorphonuclear leukocytes *in vitro*. *Can J Anaesth* 1995: **41**: 446–49.
6. Weiss M, Mirow N, Birkhahn A et al. Benzodiazepins and their solvents influence neutrophil granulocyte function. *Br J Anaesth* 1993: **70**: 317–321.
7. Sandys GH. A new method of preventing swarming of *Proteus sp.* with a description of a new medium suitable for use in routine laboratory practice. *J Med Lab Technol* 1960: **17**: 224–233.
8. Emmendörfer A, Hecht M, Lohmann-Matthes M-L, Roesler J. A fast and easy method to determine the production of reactive intermediates by human and murine phagocytes using dihydrorhodamine 123. *J Immunol Methods* 1990: **131**: 269–275.
9. Rothe G, Kellermann W, Valet G. Flow cytometric parameters of neutrophil function as early indicators of sepsis- or trauma-related pulmonary or cardiovascular organ failure. *J Lab Clin Med* 1990: **115**: 52–61.
10. Parrillo JE. Pathogenetic mechanisms of septic shock. *N Engl J Med* 1993: **328**: 1471–1477.
11. Penn RL, Maca RD, Berg RD. Increased translocation of bacteria from the gastrointestinal tract of tumor bearing mice. *Infect Immun* 1985: **47**: 793–798.
12. Loegering DJ. Humoral factor depletion and reticuloendothelial depression during hemmorrhagic shock. *Am J Physiol* 1977: **232**: 283–287.
13. Krumholz W, Endrass J, Hempelmann G. Inhibition of phagocytosis and killing of bacteria by anaesthetic agents *in vitro*. *Br J Anaesth* 1995: **75**: 66–70.
14. Skoutelis A, Lianou P, Papageorgiou E et al. Effects of propofol and thiopentone on polymorphonuclear leukocyte functions *in vitro*. *Acta Anaesthesiol Scand* 1994: **38**: 858–862.
15. Hulse D, Kusel JR, O'Donnell NG, Wilkinson PC. Effects of anesthetics on membrane mobility and locomotor responses of human neutrophils. *Immunol Med Microbiol* 1994: **8**: 241–248.
16. Kress HG, Segmüller R. Comparative effects of intravenous anesthetics on human neutrophil motility *in vitro*. *Anaesthesist* 1987: **36**: 356–361.
17. Davidson JAH, Boom SJ, Pearsall FJ et al. Comparison of the effects of four i.v. anesthetic agents on polymorphonuclear leukocyte function. *Br J Anaesth* 1995: **74**: 315–318.
18. Krumholz W, Demel C, Jung S et al. The influence of intravenous anesthetics on polymorphonuclear leukocyte function. *Can J Anaesth* 1993: **40**: 770–774.
19. Pirttikangas CO, Salo M, Mansikka M et al. The influence of anesthetic technique upon the immune response to hysterectomy: A comparison of propofol infusion and isoflurane. *Anaesthesia* 1995: **50**: 1056–1061.
20. Krumholz W, Demel C, Jung S et al. The effects of thiopentone, etomidate, ketamine and midazolam on several bactericidal functions of polymorphonuclear leukocytes *in vitro*. *Eur J Anaesth* 1995: **12**: 141–146.
21. Duignan JP, Collins PB, Johnson AH, Boucier-Hayes D. The association of impaired neutrophil chemotaxis with post-operative surgical sepsis. *Br J Surg* 1986: **73**: 238–240.
22. Buffone V, Meakins JL, Christou NV. Neutrophil function in

surgical patients: relationship to adequate bacterial defenses. *Arch Surg* 1984: **119**: 39–43.
23. Kinnaert P, Van Geertruyden N, DePauw L et al. Modulation of inflammatory reactions by surgical trauma: lack of relationship with corticosteroid secretion. *World J Surg* 1989: **13**: 478–483.
24. Wakefield CH, Carey PD, Fould S et al. Polymorphonuclear leukocyte activation an early marker of the postsurgical sepsis response. *Arch Surg* 1993: **128**: 390–395.
25. Davies M, Sheppard K, Fletcher J. The effects of surgery on the activity of neutrophil granule proteins. *Br J Haematol* 1983: **53**: 5–13.
26. Utoh J, Yamamoto T, Utsunomiya T et al. Effect of surgery on neutrophil functions, superoxide and leukotriene production. *Br J Surg* 1988: **75**: 682–685.

Address:
Axel Heller
Dept. of Anesthesiology and Operative Intensive Care Medicine
University Hospital Carl Gustav Carus
Fetscherstrasse 74
D-01307 Dresden
Germany