# Dose-dependent influence of barbiturates but not of propofol on human leukocyte phagocytosis of viable *Staphylococcus aureus*

Annette Ploppa, MD; Ralph-Thomas Kiefer, MD; Boris Nohé, MD; Helene A. Haeberle, MD; Hans-Jürgen Dieterich, MD; Klaus E. Unertl, MD, PhD; Wolfgang A. Krueger, MD, PhD

***Objective:*** **Deep sedation with barbiturates or propofol is a standard therapy for patients with critically elevated intracranial pressure. Such patients are prone to infectious complications, especially to pneumonias, which are most commonly caused by *Staphylococcus aureus*. Although various immunomodulatory effects of barbiturates have been described *in vitro*, their influence on the phagocytosis of viable *S. aureus* has yet to be investigated. Therefore, we examined the effects of thiopentone, methohexitone, and propofol on the phagocytosis of viable *S. aureus*.**

***Design:*** **Laboratory study.**

***Setting:*** **University laboratory.**

***Patients:*** **Ten healthy volunteers aged 32.5 ± 7 yrs.**

***Interventions:*** **Blood sampling.**

***Measurements and Main Results:*** **Whole blood samples were preincubated with different concentrations of thiopentone, methohexitone, and propofol, which is an isopropylphenol derivate. After viable *S. aureus* was added, phagocytosis was stopped at different time points. Leukocytes were then stained with monoclonal antibodies for flow cytometric analysis of granulocyte recruitment (ratio of ingesting granulocytes) and phagocytosis activity (fluorescence intensity of ingested bacteria). Both barbiturates inhibited granulocyte recruitment and phagocytosis activity in a dose-dependent manner, whereas propofol did not affect any of the investigated variables. At concentrations higher than $7.6 \times 10^{-3}$ M (for thiopentone, $p < .008$) and $1.1 \times 10^{-3}$ M (for methohexitone, $p < .04$), granulocyte recruitment and phagocytosis activity were significantly inhibited. The calculated inhibitory concentrations (IC50) of thiopentone for granulocyte recruitment and for phagocytosis activity were $1.3 \times 10^{-2}$ M and $1.1 \times 10^{-2}$ M, respectively. The corresponding values for methohexitone were $3.6 \times 10^{-3}$ M and $1.1 \times 10^{-3}$ M.**

***Conclusions:*** **Our *in vitro* model points at substantially different effects of barbiturates and propofol on phagocytosis of *S. aureus*, which is one of the most important pathogens in patients who need neuroprotective therapy. The inhibitory effects of both barbiturates demonstrate a strong dose-dependency, with more pronounced effects for methohexitone. Impairment of phagocytosis activity was more pronounced than granulocyte recruitment. (Crit Care Med 2006; 34:478–483)**

Deep sedation with barbiturates or propofol is a well-established standard therapy for patients with critically elevated intracranial pressure. Since immunosuppressive side effects of barbiturates have been reported *in vitro*, there is growing clinical concern about potentially increased risks of infectious complications from barbiturates. Clinical studies remain inconclusive, since the disposition for infectious complications in severely brain traumatized patients is multifactorial and accompanied by a variety of confounding factors (1–4). Nonetheless, the specific immunomodulatory effects of drugs are relevant but difficult to define in a clinical setting.

*In vitro*, barbiturates have been shown to affect various components of the immune response, such as cytokine release, chemotaxis, respiratory burst, and phagocytosis (5). These studies, however, report inconsistent results, especially for leukocyte phagocytosis (6, 7). The application of various stimuli, such as latex beads, heat-inactivated bacteria, or *Escherichia coli*, as well as the application of different models might contribute to these differences. Gram-negative bacteria like *E. coli* and Gram-positive pathogens like *Staphylococcus aureus* activate different mechanisms of bacterial ingestion. Therefore, data on phagocytosis cannot be extrapolated from one pathogen to an other.

Furthermore, there is still a lack of clinically relevant models, such as phagocytosis of viable bacteria, and there are also no studies systematically investigating dose-effect relationships. Due to the diversity of the existing studies, conclusive interpretations and comparisons of the inhibitory potency of barbiturates are still very difficult.

Clinically, *S. aureus* represents one of the most important pathogens in critically ill patients and most particularly in the subset of brain-injured patients (8–10). An increased disposition for pulmonary infections caused by *S. aureus* has been reported repeatedly, mirrored by a significantly higher incidence than in other trauma patterns (8). We therefore selected *S. aureus* to investigate the potential side effects of different sedatives at increasing concentrations.

The aim of this study was to investigate whether the reported immunomodulatory effects of barbiturates affect phagocytosis of live *S. aureus* in a comparable manner. We hypothesized that the effects on *S. aureus* phagocytosis are dose-dependent and differ in terms of inhibitory potency depending on whether

From the Department of Anesthesiology and Intensive Care Medicine, Tübingen University Hospital, Tübingen, Germany.

Supported by institutional scientific budgets.

The authors declare that they have no competing interests.



**DOI: 10.1097/01.CCM.0000199067.71968.6E**

thiopentone or methohexitone is used. To test this hypothesis, we investigated the effects of increasing concentrations of barbiturates and of propofol as the reference substance in an *in vitro* whole blood model. We chose granulocyte recruitment (ratio of ingesting granulocytes) and phagocytosis activity (fluorescence intensity of ingested bacteria) of human leukocytes as key variables of bacterial elimination.

## MATERIALS AND METHODS

*Blood Samples and Study Subjects.* The study protocol was in compliance with the Helsinki Declaration and approved by the institutional review board. Written informed consent was obtained from each volunteer.

Phagocytosis assays were performed as previously described with slight modifications (11). In brief, 10-mL blood samples were taken from ten healthy volunteers aged 32.5 ± 7 yrs and heparinized with 5 units/mL heparin sodium (Vetren 200 Altana, Konstanz, Germany). Then 100 μL of each sample was used for leukocyte counts using trypan blue solution with 3% acetic acid for the lysis of red blood cells. The remaining blood was divided into volumes of 2 mL and mixed with different concentrations of anesthetics or with isotonic saline as control. Blood samples were incubated with the different concentrations of anesthetics for 30 mins at 37°C.

*Anesthetics.* We decided to study a wide range of anesthetic concentrations that had been reported in clinical settings. For all anesthetics, the lowest concentration was selected according to plasma levels described in the literature for patients with raised intracranial pressure treated by deep barbiturate sedation (12–15). For propofol we chose plasma levels obtained during sedation of ICU patients and in patients with elevated intracranial pressure (16, 17). Then, concentrations exceeding these values by a factor of ten and 100 were chosen.

Preliminary results showed no effect of thiopentone at concentrations corresponding to the clinical concentration and its ten-fold, whereas for methohexitone inhibitory effects were already observed at ten times the clinical concentration. We therefore decided to investigate higher concentrations of thiopentone, beginning at the 100-fold clinical concentration, to be able to compare the dose-dependent effects of the two barbiturates.

Different concentrations of thiopentone (Trapanal, Altana, Konstanz, Germany), methohexitone (Brevimythal 660, Lilly, Gieβen, Germany), and propofol (Disoprivan 1%, Astra Zeneca, Wedel, Germany) were diluted in isotonic saline to a total volume of 100 μL for each sample. Final concentrations of anesthetics in whole blood samples were $7.6 \times 10^{-3}$ M, $9.5 \times 10^{-3}$ M, and $1.9 \times 10^{-2}$ M for thiopentone; $1.1 \times 10^{-4}$ M, $1.1 \times 10^{-3}$ M, and $1.1 \times 10^{-2}$ M for methohexitone, and $1 \times 10^{-5}$ M, $1 \times 10^{-4}$ M and $1 \times 10^{-3}$ M for propofol. The pH was adjusted to 7.4 in all experiments. Eight independent experiments were carried out for each anesthetic concentration.

*Bacteria. S. aureus* (ATCC 25923) was grown in tryptone soy broth (Oxoid, Wesel, Germany) for 3 hrs and centrifuged at 375 × *g* for 5 mins. Pellets were resuspended in phosphate-buffered saline (without calcium or magnesium; Sigma, Deisenhofen, Germany) and then washed twice. For the detection of intracellular bacteria by flow cytometry, pellets were incubated with calcein AM (calcein AM special packing, Molecular Probes, Eugene, OR) for 50 mins at 37°C. Stained bacteria were washed twice with phosphate-buffered saline and adjusted to final concentrations of $2 \times 10^9$ colony-forming units/mL. Bacterial counts were performed by plating appropriate dilutions of bacterial suspensions on mannitol salt agar (Oxoid, Wesel, Germany); colony-forming units were counted after 24 hrs of incubation at 37°C. Repeated assays were performed to ascertain stable fluorescence intensity and bacterial viability during all experiments.

*Phagocytosis.* For each assay, bacteria were adjusted to a constant ratio of 25:1 (bacteria/leukocytes) based on whole blood leukocyte counts. The culture medium was RPMI (RPMI 1640, Sigma) containing 10% autologous serum for preopsonization of bacteria for faster phagocytosis. Diluted bacteria and anesthetic-treated whole blood samples were incubated separately for 30 mins at 37°C. Bacteria were then added to the blood samples and incubated at 37°C for 2.5, 5, 10, 20, and 40 mins before phagocytosis was stopped by adding N-ethyl maleimide (10 mM, Sigma). Between these steps, samples were stored on ice.

Granulocytes were then stained with the monoclonal antibody directed against CD13 (myeloid cell CD13 RPE, DAKO, Glostrup, DK) for flow cytometric detection, followed by lysis of erythrocytes (FACS Lysing Solution, Becton Dickinson, Heidelberg, Germany). Samples were centrifuged (375 × *g* for 5 mins), resuspended in phosphate-buffered saline, and kept on ice until flow cytometric analysis.

*Flow Cytometry.* Flow cytometric analysis was performed using a FACS SORT system (Becton Dickinson). Lymphocytes and cell debris were excluded from the analysis using a threshold on low angle forward scatter. Free

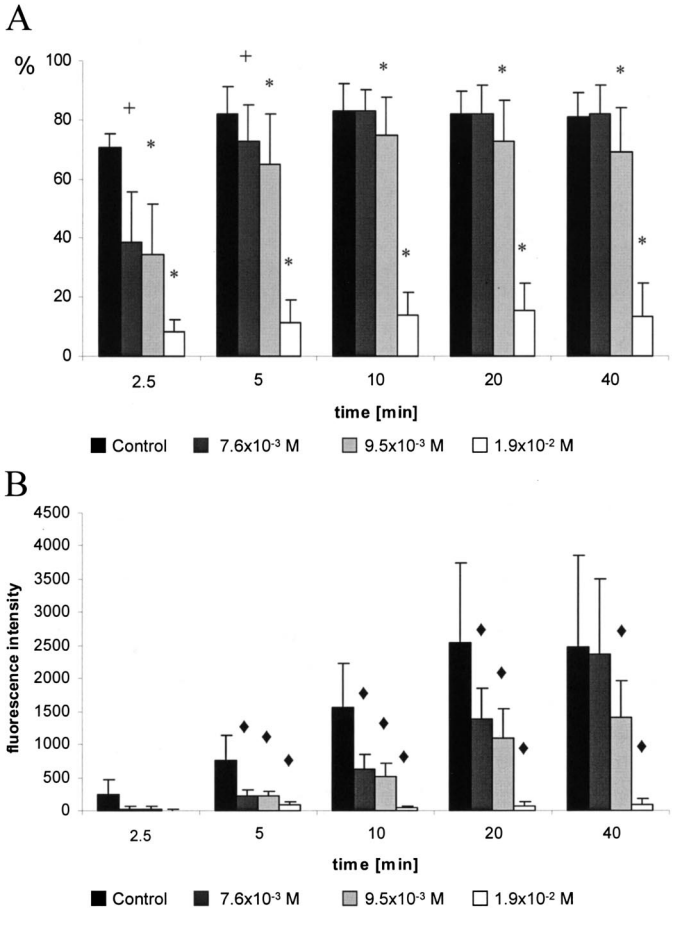


**Figure 1.** Effects of different concentrations of thiopentone on (*A*) granulocyte recruitment and (*B*) phagocytosis activity. Results are presented as mean ± SD. Significant inhibition refers to the control at each time point (+$p < .002$; *$p < 0.008$; *diamonds*, $p < .04$).

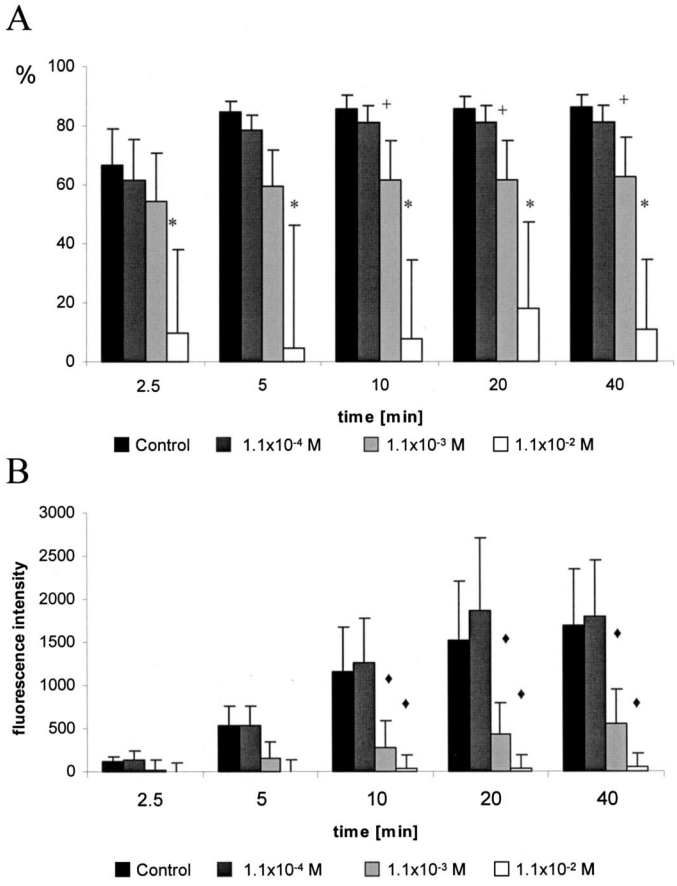


**Figure 2.** Effects of different concentrations of methohexitone on (*A*) granulocyte recruitment and (*B*) phagocytosis activity. Results are presented as mean ± SD. Significant inhibition refers to the control at each time point (+$p < .045$; *$p < .0002$, *diamonds*, $p < .0001$).

bacteria were also excluded by this threshold because of their small size. Granulocytes were differentiated from monocytes by their fluorescence signal and their scatter properties. To define the cutoff for positive fluorescence of the monoclonal antibody, we used an isotype control (mouse immunoglobulin IgG-1-RPE, Caltag, San Francisco, CA). Likewise, unstained bacteria were used as control for calcein AM stained bacteria to exclude autofluorescence phenomena from the bacteria.

*Statistical Analysis.* Phagocytosis was determined as a) granulocyte recruitment − the ratio of granulocytes ingesting bacteria to the total number of granulocytes; and b) phagocytosis activity − fluorescence intensity of intracellular bacteria as a semiquantitative measurement of bacterial uptake. At least 10,000 granulocytes were analyzed for each data point. Data are reported as mean ± SD.

Statistical analysis was performed with the Kolmogorov-Smirnov test, which confirmed normal distribution, and analysis of variance. Blood donors were included as random factors, and time was entered as fixed point for the analysis of variation. Student's *t*-test was applied to compare differences between the groups at different time points; $p < .05$ was considered significant. Where appropriate, an adjustment of $\alpha$ according to Bonferroni Holm was applied.

To determine inhibitory concentrations for barbiturates, concentration-response curves were fitted for the data at 40 mins following a logistic function from the Hill equation: $y = amin + (amax - amin) \cdot [1 - xn/(\times 50n + xn)]$, where amax and amin were defined as the maximum and minimum obtained, n is the Hill coefficient, and $\times 50$ is the half-maximal inhibitory concentration.

## RESULTS

*Thiopentone.* Thiopentone affected neither granulocyte recruitment nor phagocytosis activity at low concentrations, which correspond to concentrations found in patients and the ten-fold of these values (data not shown).

With increasing concentrations beginning at 100-fold of the clinically found concentration ($7.6 \times 10^{-3}$ M), thiopentone significantly reduced granulocyte recruitment in a dose-dependent manner. At $7.6 \times 10^{-3}$ M, a significant retardation of granulocyte recruitment occurred at the early time points of 2.5 mins (70.9%, $p < .001$) and 5 mins (81.7%, $p < .002$). However, at later time points, granulocyte recruitment did not differ from controls. At $9.5 \times 10^{-3}$ M thiopentone, granulocyte recruitment was significantly reduced over the whole investigated time-range; the maximal effect (<20% granulocyte recruitment compared with controls) was observed at the highest concentration ($1.9 \times 10^{-2}$ M, $p < .008$ at all time points).

Phagocytosis activity was also found to be suppressed from concentrations starting at $7.6 \times 10^{-3}$ M. At $7.6 \times 10^{-3}$ M, significant retardation was observed at 5, 10, and 20 mins ($p < .04$), but the values found at 40 mins did not differ from controls. At concentrations of $9.5 \times 10^{-3}$ M and $1.9 \times {}^{-2}$ M, phagocytosis activity was found to be significantly reduced at 5, 10, 20, and 40 mins ($p < .035$), with a maximal effect of <5% of the phagocytosis activity of controls at the highest concentration (Fig. 1).

*Methohexitone.* Methohexitone significantly attenuated granulocyte recruitment and phagocytosis activity beginning at $1.1 \times 10^{-3}$ M, which corresponded approximately to ten times the plasma levels found in patients. Granulocyte recruitment was significantly inhibited at 10, 20, and 40 mins ($p < .045$ all). In contrast, for thiopentone in the lowest inhibiting concentration, only a retardation of granulocyte recruitment was observed at 2.5 and 5 mins. At $1.1 \times 10^{-2}$ M methohexitone, granulocyte recruitment was significantly suppressed to <20% of that of controls at 5, 10, 20, and 40 mins ($p < .0002$ for all time points).

Phagocytosis activity was also significantly attenuated beginning at a concentration of $1.1 \times 10^{-3}$ M. This was observed at 10, 20, and 40 mins ($p < .0001$ for all time points). Again, no retardation was seen as with thiopentone in the lowest inhibitory concentration. The maximal effect, <5% phagocytosis activity of controls, was observed at the highest concentration of $1.1 \times 10^{-2}$ M methohexitone at 10, 20, and 40 mins ($p < .0001$ in each case), as shown in Figure 2.

*Propofol.* Propofol affected neither granulocyte recruitment nor phagocytosis activity at any of the investigated concentrations (Fig. 3).

*Dose-Dependent Effects.* Since both barbiturates showed a strong inhibition of granulocyte recruitment and phagocytosis activity, dose-response curves were fitted to compare the observed effects.

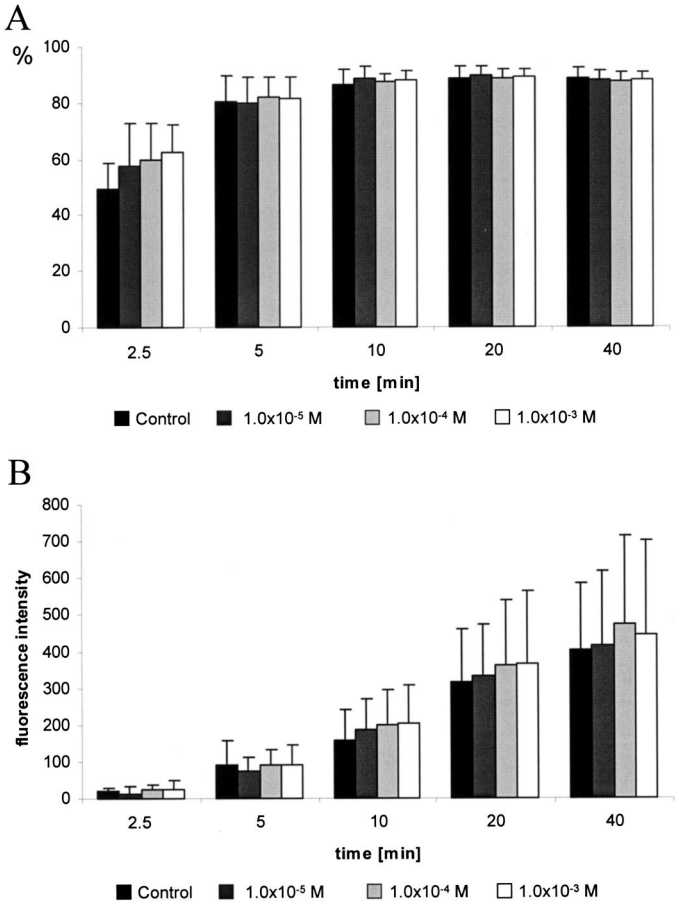


Figure 3. Effects of different concentrations of propofol on (*A*) granulocyte recruitment and (*B*) phagocytosis activity. Results are presented as mean ± SD.

Whereas methohexitone showed a gradually increasing inhibition over the investigated dose range of $1.1 \times 10^{-4}$ M to $1.1 \times 10^{-2}$ M, thiopentone exerted no effects over a comparable range of concentrations but was followed, however, by an abrupt and maximal inhibitory effect beyond the 2.5-fold concentration (Fig. 4). This observation was confirmed by lower Hill coefficients for thiopentone effects on granulocyte recruitment (−4.8) and phagocytosis activity (−8.8) than for methohexitone on granulocyte recruitment (−1.3) and phagocytosis activity (−2.2).

Comparing the effect strength on the investigated variables, both barbiturates inhibited phagocytosis activity at lower concentrations than granulocyte recruitment. For thiopentone, the calculated concentration at which granulocyte recruitment was reduced to 50% (IC50) was $1.5 \times 10^{-2}$ M; the IC50 for phagocytosis activity was $1.0 \times 10^{-2}$ M. The corresponding values for methohexitone were $3.6 \times 10^{-3}$ M for granulocyte recruitment and $1.1 \times 10^{-3}$ M for phagocytosis activity, respectively.

## DISCUSSION

Our data confirm a significant inhibition of granulocyte phagocytosis of viable *S. aureus* in the presence of barbiturates. Both granulocyte recruitment and phagocytosis activity were significantly attenuated at minimal concentrations of $7.6 \times 10^{-3}$ M for thiopentone and $1.1 \times 10^{-3}$ M for methohexitone. At higher concentrations, the two barbiturates caused nearly complete inhibition of the investigated variables. For thiopentone, the IC50 for granulocyte recruitment was $1.5 \times 10^{-2}$ M, and the IC50 for phagocytosis activity was $1.0 \times 10^{-2}$ M. The corresponding values for methohexitone were $3.6 \times 10^{-3}$ M for granulocyte recruitment and $1.1 \times 10^{-3}$ M for phagocytosis activity. In contrast, propofol was not observed to inhibit granulocyte recruitment or phagocytosis activity.

This study corroborates previous data on the inhibitory effect of thiopentone on phagocytosis of human leukocytes. Furthermore, our study expands these results to phagocytosis of live *S. aureus*, which is a predominant pathogen, especially in patients with brain trauma (9, 10). More important, the results indicate substantial differences between the two barbiturates thiopentone and methohexitone with respect to their inhibitory potency and a differential pattern of inhibition.

However, our whole blood model with viable *S. aureus* is an *in vitro* assay, and the limitations of such a model should be considered. Mechanical manipulation, contact with foreign surfaces, and the lack of interaction with other cell types in tissues may alter phagocytosis. Besides this, phagocytosis is only one relevant part in the complex system of host defense, in which other components may compensate a depression of another component. Additionally, the lack of metabolism of the anesthetics by the liver and a potential interaction of metabolites with the host defense cannot be excluded. Furthermore, whole blood samples can only be incubated with barbiturates for a limited time. Compared with the clinical situation, these incubation times are rather short, considering that barbiturate sedation is applied over several days to control raised intracranial pressure. Additionally, blood was drawn from healthy volunteers and not from critically ill patients, so the results cannot be extrapolated to a clinical setting.

Thiopentone was previously shown to inhibit phagocytosis of heat-inactivated *S. aureus* and *E. coli* (6, 7, 18). Davidson and coworkers (6) observed decreased phagocytosis in granulocytes at $7.6 \times 10^{-3}$ M. The inhibition of phagocytosis at this concentration was confirmed by our data and expanded to viable *S. aureus*. In addition, our data show a strong dose-dependent inhibition of granulocyte recruitment and phagocytosis activity beginning at concentrations of $7.6 \times 10^{-3}$ M and leading to an almost complete inhibition of these two variables at $1.9 \times 10^{-2}$ M. In contrast, Heller and coworkers (7) found inhibition of phagocytosis of fluorescence labeled *E. coli* at concentrations of only $7.6 \times 10^{-5}$ M thiopentone. This discrepancy might be explained by the use of different strains of bacteria and different bacterial concentrations. Bacterial ingestion depends on the bacterial species, as identical amounts of *S. aureus* are ingested to a greater extent than *E. coli* (18) Additionally, granulocyte re-

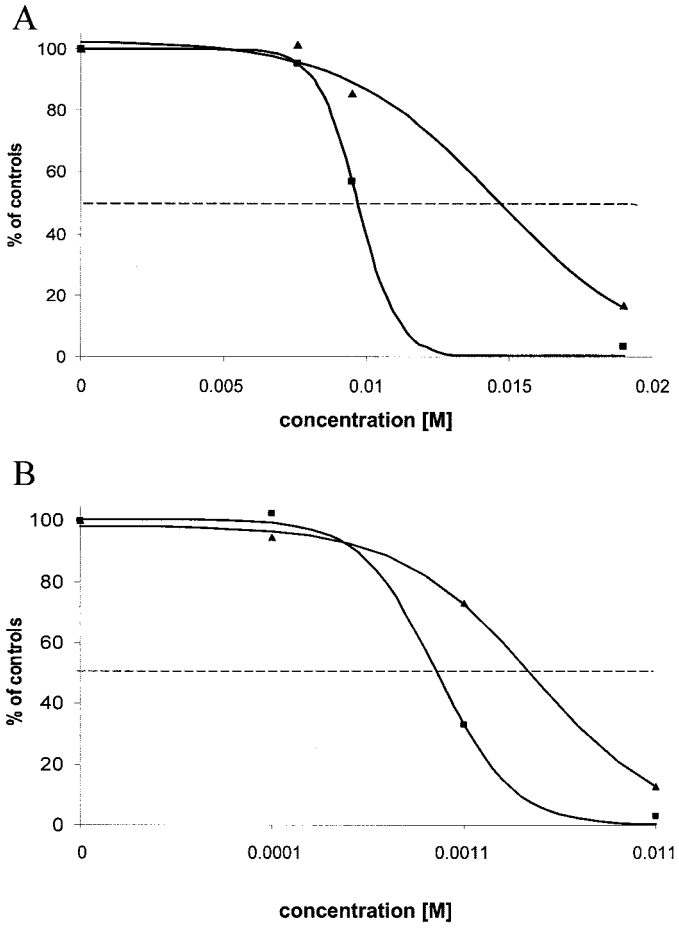


**Figure 4.** Concentration-dependent inhibition of granulocyte recruitment (*triangles*) and phagocytosis activity (*squares*) after exposure to (*A*) thiopentone and (*B*) methohexitone. Curve fitting using the Hill equation revealed an inhibitory concentration (IC 50) of $1.5 \times 10^{-2}$M for granulocyte recruitment and $1.0 \times 10^{-2}$M for phagocytosis activity for thiopentone and an inhibitory concentration of $3.5 \times 10^{-3}$M for granulocyte recruitment and $1.1 \times 10^{-3}$M for phagocytosis activity for methohexitone.

cruitment and phagocytosis activity of *S. aureus* depend directly on the bacterial concentration. Previously, we investigated the effect of local anesthetics on phagocytosis and applied a 5:1 ratio of bacteria to leukocytes (11). In our present study, we used a ratio of 25:1 bacteria to leukocytes, which resulted in a marked increase in both granulocyte recruitment and phagocytosis activity and allowed a better calculation of dose-response curves.

For methohexitone, our results demonstrate for the first time inhibitory effects on the phagocytosis of *S. aureus* at concentrations corresponding to only ten times those described in the clinical setting. Methohexitone showed a dose-dependent inhibition over a broad dose range from $1.1 \times 10^{-4}$ M to $1.1 \times 10^{-2}$ M, whereas comparable dose ranges of thiopentone showed no inhibition, followed abruptly by maximal inhibition in a narrow concentration range just above these levels. Since intracellular pathways were not investigated in this study, this obvious difference cannot be explained by our results. It might be speculated, however, that thiopentone and methohexitone affect different pathways due to structural differences, possibly mediated by the sulfur- and the oxy-substituents (19). Furthermore, barbiturates have been shown to inhibit protein kinase C activation, which plays a crucial role in cell membrane signal transduction and the regulation of inflammatory systems (20), and for thiopenone an inhibition of nuclear factor-κB, a transcription factor that plays a central role in the regulation of inflammatory gene expression, has been described (21, 22).

Conflicting results have been published for propofol (5). Propofol *in vitro* did not affect lymphocyte proliferation (5) but has been shown to inhibit adhesion of platelets to leukocytes (23), oxidative burst (24), cytokine release (25), T-cell functions (5), and phagocytosis (5–7). Our data corroborate the observation of Davidson and coworkers (6), who found no inhibition of phagocytosis after 30 mins up to a concentration of $2.5 \times 10^{-3}$ M. In contrast, inhibition of phagocytosis was described for *E. coli* in whole blood ($2.75 \times 10^{-5}$ M propofol) (24) as well as for *S. aureus* and *E. coli* in a model with isolated leukocytes ($3.3 \times 10^{-4}$ M propofol) (26). In a rabbit model, bacterial clearance during propofol anesthesia was significantly attenuated (27). However, a comparable effect was observed with the lipid vehicle, which induced enhanced organ colonization with *E. coli*. Since in our study no inhibitory effects could be observed even in the 100-fold clinical concentration, the effects of the lipid carrier were not investigated. It seemed unlikely that potential inhibitory effects of propofol in our study might be masked by the lipid carrier itself, since in the majority of studies *in vitro*, no or only minor inhibitory effects were observed for the lipid carrier (23, 24, 26, 28). Again, the choice of different bacterial species and methodological differences between the studies seem to account for the observed differences.

In patients with increased intracranial pressure, barbiturates are usually applied until a burst suppression pattern is seen in the electroencephalographic monitoring. Here, the plasma levels are up to 40 times higher than after the induction of anesthesia, and the patients may receive such treatment for several days (12–15). These concentrations, however, are ten times lower than the minimal inhibitory concentrations that we observed for methohexitone and more than 100 times lower than the minimal inhibitory concentration of thiopentone. On the other hand, it has to be kept in mind that in the clinical setting the exposure to high barbiturate concentrations may be maintained over several days and various drug interactions can be expected, especially when additional immunosuppressive treatment such as corticosteroids is given.

Despite the fact that in our study inhibitory effects occurred in supraphysiological concentrations, an impact on the susceptibility to infectious complications in critically ill patients cannot be excluded. Our results might suggest that thiopentone—due to its lower inhibitory effects—should be preferred to metho-

*Our* in vitro *model points at substantially different effects of barbiturates and propofol on phagocytosis of* S. aureus*, which is one of the most important pathogens in patients who need neuroprotective therapy.*

hexitone for sedation of patients with critically elevated intracranial pressure. Since our study investigated a small aspect of the complex system of host defense, the fact that barbiturates may inhibit granulocyte functions deserves further attention and warrants clinical studies addressing this issue.

## CONCLUSIONS

Our *in vitro* study using human leukocytes in a whole blood model shows different inhibitory effects of barbiturates on the phagocytosis of viable *S. aureus*, which is clinically one of the most relevant pathogens. In addition, our data allow a direct comparison of the inhibitory potency of thiopentone and methohexitone with concentration effect curves and suggest a more pronounced impact of methohexitone on granulocyte recruitment. This difference is even more dramatic with respect to phagocytosis activity.

However, the inhibitory effects occurred at dose ranges beyond the clinically observed plasma barbiturate concentrations and were observed in blood samples from healthy volunteers. Thus, direct extrapolation of these *in vitro* results to the clinical setting is not possible but should be the focus of future clinical trials, especially in critically ill or immunocompromised patients. Moreover, the differences between the two barbiturates deserve further attention both in investigations of molecular interactions and in clinical studies.


## ACKNOWLEDGMENTS

We thank Christoph Zanke, Michaela Hoch-Gutbrodt, and Alice Mager for their scientific support and technical assistance. We thank Diane Blaurock for copyediting the manuscript.


## REFERENCES


1. Stover JF, Stocker R: Barbiturate coma may promote reversible bone marrow suppression in patients with severe isolated traumatic brain injury. *Eur J Clin Pharmacol* 1998; 54:529–534
2. Eberhardt KEW, Thimm BM, Spring A, et al: Dose-dependent rate of nosocomial pulmonary infection in mechanically ventilated patients with brain oedema receiving barbiturates: A prospective case study. *Infection* 1992; 20:12–21
3. Krueger WA, Lenhart F-P, Neeser G, et al: Influence of combined intravenous and topical antibiotic prophylaxis on the incidence of infections, organ dysfunctions, and mortality in critically ill surgical patients. *Am J Respir Crit Care Med* 2002; 166:1029–1037
4. Nadal P, Nicolas JM, Font C, et al: Pneumonia in ventilated head trauma patients: The role of thiopental therapy. *Eur J Emerg Med* 1995; 2:14–16
5. Galley HF, DiMatteo MA, Webster NR: Immunomodulation by anesthetic, sedative and analgesic agents: Does it matter? *Intensive Care Med* 2000; 26:267–274
6. Davidson JAH, Boom SJ, Pearsall FJ, et al: Comparison of the effects of four i.v. anaesthetic agents on polymorphonuclear leukocyte function. *Br J Anaesth* 1995; 74:315–331
7. Heller A, Heller S, Blecken S, et al: Effect of intravenous anaesthetics on bacterial elimination in human blood *in vitro*. *Acta Anaesthesiol Scand* 1998; 42:518–526
8. Rello J, Quintana E, Ausina V, et al: Risk factors for *Staphylococcus aureus* nosocomial pneumonia in critically ill patients. *Am Rev Respir Dis* 1990, 142:1320–1324
9. Bronchard R, Albaladejo P, Brezac G, et al: Early onset pneumonia. *Anesthesiology* 2004; 100:234–239
10. Campbell W, Hendrix E, Schwalbe R, et al: Head-injured patients who are nasal carriers of *Staphylococcus aureus* are at high risk for *Staphylococcus aureus* pneumonia. *Crit Care Med* 1999; 27:798–801
11. Kiefer RT, Ploppa A, Krueger WA, et al: Local anesthetics impair human granulocyte phagocytosis activity, oxidative burst, and CD11b expression in response to *Staphylococcus aureus*. *Anesthesiology* 2003; 98: 842–848
12. Russo H, Dubboin MP, Bressole F, et al: Time-dependent pharmacokinetics of high dose thiopental infusion in intensive care patients. *Pharmaceutical Research* 1997; 14: 1583–1588
13. Russo H, Urien S, Duboin MP, et al: Pharmacokinetics of high-dosage thiopental sodium in patients with cerebral injuries. *Clin Drug Invest* 1997; 13:255–269
14. Turcant A, Delhumeau A, Premel-Cabic A, et al: Thiopental pharmacokinetics under conditions of long-term infusion. *Anesthesiology* 1985; 63:50–54
15. Schickendanz J, Funk W, Ittner KP, et al: Elimination of methohexitone after long term, high dose infusion in patients with critically elevated intracranial pressure. *Crit Care Med* 1999; 27:1570–1576
16. Barr J, Egan TD, Sandoval NF, et al: Propofol dosing regimens for ICU sedation based upon an integrated pharmacokinetic-pharmacodynamic model. *Anesthesiology* 2001; 95: 324–333
17. Steiner LA, Johnston AJ, Chatfield DA, et al: The effects of large dose propofol on cerebrovascular pressure autoregulation in head-injured patients. *Anesth Analg* 2003; 97: 572–576
18. 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
19. Kress HG, Eberlein T, Hörber B, et al: Suppression of neutrophil migration and chemiluminescence is due to the sulphur atom in the thiobarbiturate molecule. *Acta Anaesthesiol Scand* 1989; 33:122–128
20. Mikawa K, Maekawa N, Hoshina H, et al: Inhibitory effect of barbiturates and local anaesthetics on protein kinase C activation. *J Internatl Med Res* 1990; 18:153–160
21. Loop T, Liu Z, Humar M, et al: Thiopental inhibits the activation of nuclear factor kB. *Anesthesiology* 2002; 96:1202–1213
22. Haeberle HA, Kiefer RT: Thiopental and nuclear factor kB: Some questions. *Anesthesiology* 2003; 98:1020
23. Rossi L, Wessiepe M, Buhre W, et al: Effects of propofol on adhesion of activated platelets to leukocytes in human whole blood. *Intensive Care Med* 2003; 29:1157–1163
24. Heine J, Jaeger K, Weingaertner N, et al: Effects of different preparations of propofol, diazepam, and etomidate on human neutrophils in vitro. *Acta Anaesthesiol Scand* 2001; 45:213–220
25. Galley HF, Dubbels AM, Webster NR: The effect of midazolam and propofol on interleukin-8 from human polymorphonuclear leukocytes. *Anesth Analg* 1998; 86: 1289–1293
26. Krumholz W, Endrass J, Hempelmann G: Propofol inhibits phagocytosis and killing of *Staphylococcus aureus* and *Escherichia coli* by polymorphonuclear leucocytes *in vitro*. *Can J Anaesth* 1994; 41:446–449
27. Kelbel I, Koch T, Weber A, et al: Alterations of bacterial clearance induced by propofol. *Acta Anaesthesiol Scand* 1999; 443:71–76
28. Skoutelis A, Lianou P, Papageorgiou E, et al: Effects of propofol and thiopentone on polymorphonuclear lukocyte functions *in vitro*. *Acta Anaesthesiol Scand* 1994; 38:858–862