Propofol inhibits phagocytosis and killing of *Staphylococcus aureus* and *Escherichia coli* by polymorphonuclear leukocytes *in vitro*

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Polymorphonuclear leukocytes (PMNL) are important components of the immunological defence system which protects the human organism from invading bacteria. Using a fluorescence microscopic method, we examined the influence of propofol and its solvent intralipid on phagocytosis and killing of *Staphylococcus aureus* and *Escherichia coli* by PMNL *in vitro*. Propofol inhibited ($P \leq 0.001$) phagocytosis of *Staphylococcus aureus* as well as *Escherichia coli*. Killing of *Staphylococcus aureus* ($P \leq 0.001$) and of *Escherichia coli* ($P \leq 0.01$) was suppressed. Intralipid, by itself, impaired phagocytosis of *Escherichia coli* ($P \leq 0.05$). Apart from that, intralipid produced no relevant effects. Additional clinical studies regarding the influence of propofol on PMNL function are recommended.

Les polynucléaires sont des éléments importants du système immunologique de défense qui protège l'organisme humain de l'envahisseur bactérien. Nous avons étudié *in vitro*, à l'aide d'une méthode de fluorescence microscopique, l'influence du propofol et de son solvant, l'intralipide, sur la phagocytose et la bactéricidie du *Staphylococcus aureus* et de l'*Escherichia coli* par les polynucléaires. Le propofol inhibe ($P \leq 0,001$) la phagocytose du *Staphylococcus aureus* et de l'*Escherichia coli*. La bactéricidie du *Staphylococcus aureus* ($P \leq 0,001$) et de l'*Escherichia coli* ($P \leq 0,01$) est abolie. L'intralipide entrave par lui-même la phagocytose de l'*Escherichia coli* ($P \leq 0,05$). L'in-

tralipide ne produit pas d'effets pertinents. Des études cliniques supplémentaires concernant l'influence du propofol sur l'activité des polynucléaires sont recommandées.

About a century ago Platania¹ observed an intensification of bacterial infections in laboratory animals caused by anaesthetic drugs. He regarded a disturbance of the nervous system as the reason for his discovery. This opinion, however, was not shared by all scientists, some of whom had the idea that anaesthetics might inhibit the bactericidal effect of the blood. A few years later Graham² discovered a suppression of phagocytosis of bacteria by polymorphonuclear leukocytes (PMNL) caused by ether. Since then, several studies have been conducted concerning the influence of anaesthetic agents on PMNL function. A current survey was published by Kress and Eberlein.³ However, there are few findings regarding the effect of propofol. In particular, its influence on the confrontation between PMNL and human pathogenic bacteria has not been examined up to now. This induced us to investigate the effects of propofol and its solvent intralipid on phagocytosis and killing of *Staphylococcus aureus* and *Escherichia coli*, *in vitro*.

Methods

Permission to conduct the study was obtained from the Ethics Committee of the Medical Faculty of Justus-Liebig-University Giessen.

Blood samples

Venous blood samples (1.5 mg ethylenediaminetetraacetic-dipotassium ml⁻¹; Sigma, Deisenhofen) were obtained from ten healthy male volunteers (age: 29 ± 4 yr; body weight: 75 ± 8 kg; height: 1.80 ± 0.05 m (mean and SD)).

Isolation of PMNL

The PMNL were isolated using a method described by

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Eggleton *et al.*⁴ In order to lyse erythrocytes, the blood samples were mixed with distilled water in a proportion of one to five. After 20 sec, isotonia was restored by application of 2.7% saline (Merck, Darmstadt). Subsequent to centrifugation at $160 \times g$ for ten minutes at 20°C , the supernatant fluid was decanted. The cell pellet was resuspended with 10 ml Hanks' balanced salt solution (HBSS; Sigma, Deisenhofen) and centrifuged at $55 \times g$ for 10 min at 20°C . After decanting and adding 5 ml HBSS, a further centrifugation was conducted at $55 \times g$ for five minutes at 20°C . The purity of PMNL yield was evaluated microscopically, and the viability was confirmed as $\geq 95\%$ by the trypan blue exclusion test.⁵ The PMNL concentration was adjusted to 5×10^6 cells ml^{-1} by adding HBSS.

Phagocytosis and bactericidal activity

A modification of the method described by Bellinati-Pires *et al.*⁶ was used to examine the influence of propofol on phagocytosis and killing of bacteria by PMNL. After having been cultured on blood agar plates at 37°C for 24 hr, *Staphylococcus aureus* (serovar K:807) and *Escherichia coli* (serovar O:6) were inoculated to Todd Hewitt nutrient broth (Biotest, Dreieich) and incubated at 37°C for 24 hr. Subsequent to centrifugation at $1000 \times g$ for ten minutes at 5°C , the concentration of bacteria was adjusted to 1×10^9 ml^{-1} using phosphate buffered saline (PBS; Gibco, Karlsruhe). After additional diluting with HBSS in a proportion of one to ten, 1 ml pool serum of the blood group AB was added per 10 ml suspension. Following vibromixer incubation at 37°C for 30 min, centrifugation was conducted at $1000 \times g$ for three minutes. The supernatant was decanted, and the concentration of bacteria was adjusted to 100×10^6 ml^{-1} using HBSS. Then 195 μl PMNL suspension, 195 μl bacteria, and 5 μl correspondingly diluted anaesthetic were poured into a test tube. After incubation for 30 min at 37°C , phagocytosis was brought to an end by adding 1 ml ice-cold HBSS. Subsequent to centrifugation at $160 \times g$ for seven minutes at 4°C , the supernatant was carefully removed, 200 μl acridine orange ($20 \mu\text{g} \cdot \text{ml}^{-1}$ HBSS; Merck, Darmstadt), and 800 μl ice-cold HBSS were added. After centrifugation at $160 \times g$ for seven minutes at 4°C , the supernatant was decanted. Then 200 μl crystal violet ($50 \mu\text{g} \cdot \text{ml}^{-1}$ isotonic saline; Sigma, Deisenhofen) and 800 μl ice-cold HBSS were added. Following centrifugation at $160 \times g$ for seven minutes at 4°C , the supernatant was removed. After a time of rest of 15 min at 4°C in darkness, 30 μl of the suspension were applied on a glass slide. By means of a fluorescence microscope (Laborlux K; Leitz, Wetzlar) 100 PMNL were in each case examined. Intracellular killed (orange fluorescence) and living bacteria (green fluores-

cence) were counted. Extracellular fluorescence was quenched by crystal violet. A quotient of phagocytosis (number of phagocytised bacteria per 100 PMNL), a quotient of killing (number of killed bacteria per 100 PMNL), and the percentage of killed related to phagocytised bacteria were calculated.

Anaesthetic and solvent

The following concentrations were tested:

- Propofol (Diprivan; ICI Pharma, Plankstadt): 5 and $50 \mu\text{g} \cdot \text{ml}^{-1}$.
- Intralipid 10% (Kabi/Pfrimmer, Munich): 0.975 and $9.75 \mu\text{l} \cdot \text{ml}^{-1}$.

The lower propofol concentration is similar to plasma concentrations after intravenous injection of an induction dose.⁷

Statistics

All tests were performed in duplicate, and the results were mean values of two estimations. The Pearson Stephens test was used to check normal distribution. The Bartlett test examined homogeneity of variance ($P \leq 0.1$). If the requirements were met, analysis of variance for repeated measures and Scheffé test were conducted. If the requirements were not fulfilled, Friedman analysis of variance and Miller test were performed. A probability of $P \leq 0.05$ was regarded as significant.

Results

Means and standard deviations are specified in Tables I–III.

Both concentrations of propofol tested caused an inhibition of phagocytosis of *Staphylococcus aureus* as well as *Escherichia coli* ($P \leq 0.001$) (Table I).

There was no effect of intralipid on phagocytosis of *Staphylococcus aureus*. Phagocytosis of *Escherichia coli*, however, was reduced ($P < 0.05$) by both solvent concentrations (Table I). While killing of *Staphylococcus aureus* was inhibited by both concentrations of propofol ($P < 0.001$), killing of *Escherichia coli* was reduced by the low concentration only ($P < 0.01$) (Table II). There was no influence of intralipid on the killing of *Staphylococcus aureus* and *Escherichia coli* (Table II).

Neither propofol nor intralipid changed the percentage of killed to phagocytised bacteria (Table III).

Discussion

Despite all medical efforts, perioperative infection is still an important clinical problem. Polymorphonuclear leukocytes (PMNL) play a crucial part in the defence against invading bacteria.^{8,9} Passing through several intermediate stages, PMNL are created in bone marrow within about

TABLE I The influence of various concentrations of propofol and its solvent intralipid on phagocytosis of *Staphylococcus aureus* and *Escherichia coli* by PMNL *in vitro* (phagocytised bacteria per 100 PMNL; $n = 10$; means $\pm$ SD)

	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
No additive	391 $\pm$ 64	203 $\pm$ 37
5 μ g propofol ml ⁻¹	317 $\pm$ 83*	134 $\pm$ 34*
50 μ g propofol ml ⁻¹	300 $\pm$ 70*	145 $\pm$ 36*
0.495 μ l intralipid ml ⁻¹	358 $\pm$ 69	171 $\pm$ 47†
4.95 μ l intralipid ml ⁻¹	396 $\pm$ 76	172 $\pm$ 47†

* $P \leq 0.001$.

† $P \leq 0.05$.

TABLE II The influence of various concentrations of propofol and its solvent intralipid on killing of *Staphylococcus aureus* and *Escherichia coli* by PMNL *in vitro* (killed bacteria per 100 PMNL; $n = 10$; means $\pm$ SD)

	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
No additive	234 $\pm$ 35	110 $\pm$ 21
5 μ g propofol ml ⁻¹	187 $\pm$ 49*	75 $\pm$ 20†
50 μ g propofol ml ⁻¹	157 $\pm$ 26*	80 $\pm$ 21
0.495 μ l intralipid ml ⁻¹	215 $\pm$ 40	92 $\pm$ 25
4.95 μ l intralipid ml ⁻¹	249 $\pm$ 43	91 $\pm$ 24

* $P \leq 0.001$.

† $P \leq 0.01$.

TABLE III The influence of various concentrations of propofol and its solvent intralipid on the percentage of killed related to phagocytised bacteria (%; $n = 10$; means $\pm$ SD)

	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
No additive	60.3 $\pm$ 4.2	54.5 $\pm$ 6.7
5 μ g propofol ml ⁻¹	59.5 $\pm$ 8.3	56.7 $\pm$ 8.2
50 μ g propofol ml ⁻¹	53.4 $\pm$ 4.0	56.4 $\pm$ 8.0
0.495 μ l intralipid ml ⁻¹	60.8 $\pm$ 5.2	54.6 $\pm$ 7.3
4.95 μ l intralipid ml ⁻¹	62.4 $\pm$ 4.2	53.5 $\pm$ 6.6

14 days and are subsequently delivered to the blood. In infected regions of the body they increasingly adhere to vascular endothelium. After permeating the vessel wall by diapedesis, PMNL migrate amoeboidly in the direction of bacteria. In this process, chemotactic factors (e.g., leukotriene B₄, complement protein C5a) are of great importance. While PMNL are approaching, bacteria are normally coated with antibodies (primarily IgG) and complement factors (first of all C3b), an action called opsonisation. After the establishing of contact between PMNL and opsonic molecules by specific receptors, bac-

teria are phagocytised, usually killed, and degraded. A distinction can be made between oxygen-dependent and oxygen-independent bactericidal mechanisms. Oxygen-dependent killing is performed by superoxide anion, hydrogen peroxide, singlet oxygen, and hydroxyl radicals. Oxygen-independent bactericidal agents are, for example, defensins and cationic proteins.

Propofol¹⁰ is a rapidly acting intravenous induction agent, and it can be used for maintenance of anaesthesia and sedation of patients of an intensive care unit.

Until now, only a few investigators have examined the influence of propofol on PMNL functions. In 1990, Tufano *et al.*¹¹ reported a dose-dependent increase of chemiluminescence, chemotaxis, phagocytosis of *Saccharomyces cerevisiae*, which is a non-pathogenic fungus, and phagosome-lysosome fusion. Since anaesthetics, in particular the induction agent thiopentone, tend to depress PMNL functions, these results were surprising and were not confirmed by others. For example, Jensen *et al.*¹² found suppression of PMNL locomotion, and Kellermann *et al.*¹³ observed inhibition of the generation of bactericidal hydrogen peroxide by propofol. A disturbance of PMNL change of form under the influence of a chemotactic stimulus was discovered by O'Donnell *et al.*¹⁴ Our present study, however, is the first to examine the effect of propofol on the struggle between PMNL and vital human pathogenic bacteria.

Phagocytosis and bactericidal activity are among the most important polymorphonuclear leukocyte functions. Concerning *Staphylococcus aureus*, a gram-positive bacterium, we found significant inhibition of phagocytosis as well as killing caused by a clinically relevant concentration of propofol. The solvent intralipid, which consists of soya bean oil, egg phosphatide, and glycerol, was tested separately. However, it did not have any effect regarding *Staphylococcus aureus*. Concerning gram-negative *Escherichia coli*, again propofol suppressed phagocytosis, but in this case intralipid alone caused a moderate inhibition. Clinically relevant concentrations of propofol impaired the killing of *Escherichia coli*. Again there was no influence of intralipid on bactericidal activity. In conclusion, propofol is able to suppress phagocytosis and killing of *Staphylococcus aureus* and *Escherichia coli*, *in vitro*. However, the underlying mechanisms are not clear. Since *in vitro* observations are not in accordance with *in vivo* conditions necessarily, additional clinical studies should be conducted.

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