Inhibition of neutrophil respiratory burst and chemotaxis in vitro by thiopentone but not methohexitone

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Administration of high-dose barbiturates may be used as an appropriate adjunctive treatment for control of intracranial pressure. The thiobarbiturate, thiopentone, has been reported to increase the rate of nosocomial pulmonary infection. This may be a substance-related effect of thiobarbiturates and it may be clinically important in barbiturate-sedated patients with severe head injury. Thus, the effects of the dose-response relationship of two commonly used barbiturates (thiopentone and methohexitone) on two vital aspects of neutrophil function were tested. We studied the production of superoxide anion during the respiratory burst by means of a flow cytometric method, and we assessed *N*-formyl-methionyleucylphenylalanine-induced neutrophil chemotaxis using the results produced by specific migration. The concentrations of thiopentone and methohexitone tested in vitro were adjusted to conform to the plasma concentrations reported for anesthesia and also to 10-fold higher concentrations. Only thiopentone dose dependently decreased respiratory burst and *N*-formyl-methionyleucylphenylalanine-induced chemotaxis. Methohexitone produced minimal effects in both concentrations. It was demonstrated that thiopentone had a direct effect on the intracellular respiratory burst oxidase enzyme system. The postulated free radical scavenging capacity of thiopentone was ruled out.

Key Words * barbiturates * respiratory burst * chemotaxis * neutrophils

Anesthetic agents inhibit some aspects of neutrophil function.[12] This fact may be clinically important in treating intensive care patients in whom long-term sedation is necessary. In addition to other brain protection drug therapies the early and aggressive use of barbiturates is effective in controlling generalized posttraumatic increased intracranial pressure (ICP) in patients with severe head injury.[7,10] A report based on a five-center study has shown that administration of high-dose pentobarbital is useful in controlling elevations in ICP. The authors concluded that high-dose barbiturates can be considered an appropriate adjunctive treatment for control of ICP.[4] On the other hand the thiobarbiturate, thiopentone, has been reported to increase the rate of nosocomial pulmonary infection in a dose-dependent manner in mechanically ventilated patients with brain edema.[3] This may be a substance-related effect of thiobarbiturates. We investigated the effects of thiopentone and methohexitone on two vital aspects of neutrophil function. We studied superoxide anion production during the respiratory burst by means of flow cytometry, and the *N*-formyl-methionyleucylphenylalanine

(FMLP)-induced chemotaxis by means of specific migration. The concentrations of thiopentone and methohexitone tested in vitro were adjusted to conform to the plasma concentrations reported for anesthetized patients and to 10-fold higher concentrations.[2]

MATERIALS AND METHODS

This study was approved by the university's Committee on Ethics.

Heparinized peripheral blood samples were obtained from 20 healthy donors just before the initiation of these experiments. Thiopentone and methohexitone were obtained in the form of dry material and were dissolved with isotone saline solution (0.9% NaCl). The desired concentrations of the test drugs were standardized by using phosphate-buffered saline (PBS) and Dulbecco's medium without Ca^{++} and MgCl_2 .

Respiratory Burst

For the flow cytometric evaluation of the respiratory burst, leukocytes were obtained as a supernatant after sedimentation. The cell suspensions (containing 5×10^5 cells/ml) were incubated with the drug and the concentration was tested at 37°C for 10 minutes. The effects of the given anesthetic and 10-fold anesthetic plasma concentration on respiratory burst were measured by the intracellular oxidation of 10^4 mol dihydrorhodamine-123 to fluorescent rhodamine-123.[9] Neutrophils from the samples were stimulated by 10^{-6} mol phorbol 12-myristate 13-acetate (PMA) at 37°C for 20 minutes. The stimulation was then terminated by transferring the samples onto ice. Viability discrimination was performed by adding 3×10^{-3} mol propidium-iodide (PI) just before measurement. All results were expressed as the percentage of inhibition compared with the positive control response which was defined as respiratory burst after full stimulation by PMA in the absence of any anesthetic agents. Prestimulation of neutrophils was excluded by means of negative controls that were not stimulated with PMA.

Induced Chemotaxis

The test kits used in these experiments were prepared as described elsewhere.[8] Briefly, 10 ml of heparinized blood was mixed (2:1) with 6% hydroxyethyl starch to precipitate sedimentation of erythrocytes. After 30 minutes of sedimentation the leukocyte-enriched supernatant was harvested by centrifugation at 250 G and washed twice for 10 minutes with cold PBS. The remaining erythrocytes were lysed by using equal volumes of 0.83% ammonium chloride. After an additional washing, cells were counted and adjusted to 10^8 ml^{-1} . For each test drug, 1 ml of the remaining cell suspension was incubated with the respective anesthetic or 10-fold anesthetic plasma concentration for 3 hours at 37°C in 5% CO_2 in air. We investigated the random and chemotactic locomotion of the neutrophils toward a 2×10^{-7} mol gradient of FMLP in an agarose assay. The neutrophil samples were then fixed with 3.7% formalin and stained according to Papenheim's technique. The chemotactic locomotion toward the center containing FMLP and the migration in the opposite direction was measured under a microscope. The difference between these distances was considered to be the specific migration.

Statistical Analysis

Data are presented as means $\pm$ standard deviations (SDs). The data showed a Gaussian distribution and thus the paired two-tailed Student's t-test was applied to evaluate the differences ($p < 0.05$). In the case of nonparametric data, the Mann-Whitney U-test was used.

Sources of Supplies

The thiopentone (Trapanal) was purchased from Byk Gulden, Konstanz, Germany, and the methohexitone (Brevimytal) was purchased from Eli Lilly, Bad Homburg, Germany. The dihydrorhodamine-123 was obtained from MoBitec, Berlin, Germany. The PMA, gelatin, and RPMI 1640 were supplied by Sigma, Deisenhofen, Germany. The hydroxyethyl starch (Steril) was obtained from Fresenius, Bad Homburg, Germany. The agarose assay (Agarose A 73) was purchased from Reactifs IBF, Villeneuve, France.

RESULTS

Respiratory Burst

The percentages of PI-positive neutrophils (necrotic cells) were similarly low for both drugs in both concentrations tested ($< 5\%$). At concentrations required for anesthesia, $40\text{ }\mu\text{g/ml}$ of thiopentone ($4.5 \pm 5.3\%$) produced a significantly higher suppression of the neutrophil respiratory burst than $10\text{ }\mu\text{g/ml}$ of methohexitone ($0.9 \pm 1.6\%$). At the 10-fold anesthetic concentration inhibition by $400\text{ }\mu\text{g/ml}$ of thiopentone ($29.2 \pm 15.1\%$) was nearly seven times higher. Thiopentone produced significantly more suppression than $100\text{ }\mu\text{g/ml}$ of methohexitone, which produced only minimal effects even at the corresponding 10-fold anesthetic concentration ($1.8 \pm 1.7\%$)(Fig. 1).

Fig. 1. Bar graph showing the percentages of inhibition of the neutrophil respiratory burst produced by $40\text{ }\mu\text{g/ml}$ and $400\text{ }\mu\text{g/ml}$ thiopentone (shaded bars) and $10\text{ }\mu\text{g/ml}$ and $100\text{ }\mu\text{g/ml}$ of methohexitone (solid bars). These are the concentrations likely to be required for anesthesia and 10-times those concentrations. Twenty measurements were obtained per group (* $p < 0.05$).

We also examined whether addition of the test drugs after induction of respiratory burst by PMA influenced the superoxide anion production. The percentage of rhodamine-positive cells remained unchanged compared with the positive controls. No significant difference between thiopentone and methohexitone could be found (data not shown). Because respiratory burst was not suppressed when thiopentone was added after stimulation, the presumed radical scavenging could not have been responsible for the inhibition of superoxide anion production.

Induced Chemotaxis

Specific migration of the neutrophils was significantly decreased by thiopentone in anesthetic (40 µg/ml, 1.21 ± 0.34 mm) and 10-fold anesthetic concentrations (400 µg/ml, 0.13 ± 0.31 mm) in comparison to the control samples (1.40 ± 0.35 mm). No significant effects on chemotaxis were caused by methohexitone in either concentrations (10 µg/ml, 1.34 ± 0.4 mm or 100 µg/ml, 1.37 ± 0.38 mm) (Fig. 2).

Fig. 2. Bar graph illustrating the specific migration of neutrophils in soft agar in the presence of thiopentone (40 µg/ml and 400 µg/ml) and methohexitone (10 µg/ml and 100 µg/ml) at concentrations likely to be required for anesthesia (solid bars) and 10-times those concentrations (open bars) compared with the control samples (shaded bars). Twenty measurements were obtained per group (*p < 0.05).

DISCUSSION

We investigated the inhibitory effects of various concentrations of the thiobarbiturate thiopentone and the oxibarbiturate methohexitone on two important aspects of the elimination of bacteria and fungi: neutrophil respiratory burst and chemotaxis. The respiratory burst enzyme in the plasma membrane of neutrophils catalyzes the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH), which leads to the production of superoxide anion. The oxygen radicals are responsible for killing phagocytized microorganisms.[1,5] Migration of neutrophils induced by chemotactic stimuli is one of the earliest events during the nonspecific cellular defense against bacterial and fungal infection. In the present study thiopentone, in contrast to methohexitone, caused significant and dose-related suppression of neutrophil respiratory burst and chemotaxis.

The multiparameter flow cytometric technique allows evaluation of several parameters of each cell at the same time. Using this technique, it is possible to investigate the intracellular oxygen radical content of a selected cell population. Thus, it was possible to ascertain that thiopentone, even within the therapeutic plasma level range, has a direct effect on the intracellular respiratory burst oxidase enzyme system. We were able to clarify that depression of free radical production is caused by the interaction of the barbiturates with neutrophil function and not by free radical scavenging. Therefore, we do not agree with other authors who claim that thiopentone is a good free radical scavenger.[11,13]

The ascribed enhanced inhibitory potency of thiopentone on neutrophil function could be a substance-related effect of thiobarbiturates, attributable to the sulfur atom in the thiobarbiturate molecule.[6]

Induced depression of neutrophil function by long-term sedation with thiopentone in neurotraumatized patients may lead to an increased incidence of infection. It has been reported that after 7 days of ventilation patients with brain edema who received thiopentone as a sedative had a significantly higher rate of nosocomial pneumonia (43.8%) than the control patients who did not receive thiopentone (7.7%).[3] Infection was even more common when patients received a higher dose of thiopentone. Because neutrophils are important for defense against microbial infection in the lung, the higher rate of pneumonia is probably due to the suppression of neutrophil functions by the thiobarbiturate.

CONCLUSIONS

These results should be taken into consideration when choosing an intravenous barbiturate for long-term sedation in neurotraumatized patients. The reported beneficial effects of high-dose barbiturates as an appropriate adjunctive treatment for control of ICP[4] should be balanced against the inhibitory effect of thiopentone on cellular immunity. The oxibarbiturate, methohexitone, which did not alter neutrophil respiratory burst and chemotaxis, may therefore reduce the rate of nosocomial infections in patients receiving barbiturates for brain protection.

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