

## LABORATORY REPORTS

# *Etomidate Reversibly Depresses Human Neutrophil Chemiluminescence*

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The effect of etomidate on human neutrophil chemiluminescence was evaluated. The latter is an index of oxygen free-radical generation, which is an important bactericidal mechanism. Five drug concentrations representing the free plasma concentrations ranging from deep anesthesia (0.5 µg/ml) to the plasma level that may be found approximately 1 h after an etomidate anesthetic (0.0156 µg/ml) were studied. The importance of the duration of drug incubation was assessed by incubating the neutrophils with the drug concentrations for 10, 20, 30, 40, or 50 min before stimulation. The reversibility of any depression in chemiluminescence was assessed by gently washing the cells twice after incubation and then stimulating them. The two higher etomidate concentrations (0.5 µg/ml and 0.125 µg/ml) produced reductions in chemiluminescence of 43% and 35%, respectively ( $P < 0.05$ ). The reductions in activity only occurred when the cells had been incubated with etomidate for 50 min and were completely reversed by washing. These results show that clinically relevant concentrations of etomidate will reversibly depress neutrophil chemiluminescence *in vitro* when the cells are exposed to the drug for more than 50 min. (Key words: Anesthetics, intravenous: etomidate. Blood: neutrophils. Immune response.)

ETOMIDATE HAS qualities that would make it a desirable intravenous anesthetic for use in the operating room and for continuous infusion in the intensive care unit (ICU). However, Ledingham and Watt observed a more than three-fold increase in the mortality of ICU patients sedated with etomidate.<sup>1,2</sup> They noted that "most patients acquired some form of infection and outcome appeared to be related to the subsequent course of the infective process."<sup>1</sup> Although this may be adequately explained by etomidate-induced adrenocortical suppression, other factors, including an alteration in the patients' immune status, may also have contributed.<sup>3</sup> We have accordingly examined the effects of etomidate on human neutrophil chemiluminescence. This is an index of excited oxygen radical generation, a major mechanism of bacterial killing.<sup>4,5</sup> We addressed three questions: 1) does etomidate alter leukocyte chemiluminescence? 2) is there a critical duration of exposure to the drug necessary to produce an effect? and 3) is the effect reversible?

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## Methods

Ten milliliters of heparinized blood and 20 ml of clotted blood were obtained from each of 14 healthy human volunteers. The neutrophils were obtained by gentle centrifugation of the heparinized blood with dextran (150 mol wt) at  $15 \times g$  for 10 min. The cells were washed twice with phosphate-buffered saline (PBS) after lysis of the erythrocytes. The cell concentration was adjusted to 500,000 cells/ml, viability was confirmed with trypan blue exclusion, and a differential count was done to confirm the presence of greater than 80% neutrophils.

Zymosan<sup>®</sup>, 10 mg/ml (Sigma Chemical Co., St. Louis, MO), was opsonized by incubation with the subject's serum at 37° C for 60 min, then centrifuged and resuspended in PBS in a concentration of 10 mg/ml.

Five concentrations of etomidate were studied. These were 0.5 µg/ml, 0.125 µg/ml, 0.0625 µg/ml, 0.0313 µg/ml, and 0.0156 µg/ml. These concentrations are the free plasma concentrations ranging from deep anesthesia (0.5 µg/ml) to the plasma levels found approximately 1 h after an etomidate anesthetic (0.0156 µg/ml).<sup>6,7</sup> As it is generally believed that the protein-bound drug does not actively participate in pharmacologic effects, all our studies were done in an aqueous medium (PBS) using drug concentrations equivalent to the free plasma levels found clinically.

One milliliter of etomidate diluted in PBS was incubated with 1 ml of cell suspension (500,000 cells) at 37° C. The final drug concentration was as described earlier. One milliliter of cell suspension with 1 ml of PBS was used as a control solution. All assays were done in triplicate.

Following incubation, 100 µl of Luminol<sup>®</sup> (Sigma Chemical Co.) was added to the test vials, and chemiluminescence was measured after neutrophil activation with 200 µl of the previously opsonized Zymosan<sup>®</sup> (10 mg/ml). The light emission was measured using a Beckman<sup>®</sup> Liquid Scintillation Counter (Model 230) in the in-coincidence mode, and the counts were measured for 0.1 min every 10 min for 1 h. The use of the in-coincidence mode ensured a low background activity before stimulation and avoided the need for dark adaptation of the counting vials. Basal background activity was also measured.

The following studies were performed: 1) to assess the effects of etomidate, cells were incubated with one of the

five etomidate drug concentrations for 1 h prior to stimulation; 2) in order to assess the importance of duration of incubation, the cells were incubated with 0.125  $\mu\text{g}/\text{ml}$  or 0.5  $\mu\text{g}/\text{ml}$  for 10, 20, 30, 40, or 50 min prior to stimulation; and 3) in the third study, cells were incubated for 1 h with 0.125  $\mu\text{g}/\text{ml}$  or 0.5  $\mu\text{g}/\text{ml}$  of etomidate and then gently washed twice with PBS before stimulation to assess the reversibility of the depression. A control group of cells were incubated with PBS and similarly washed twice before Zymosan<sup>®</sup> stimulation.

The data were recorded in counts per min (CPM) and analyzed by analysis of variance of basal activity, peak activity, and the area under the time/activity curve. The area under the curve was calculated by numerical integration using Simpson's Formula. When a critical F ratio was achieved, further statistical analysis was by unpaired *t* test with Bonferonni's modification.

### Results

The normal control curve showed a peak of activity at 20 min following stimulation with Zymosan<sup>®</sup> (fig. 1). Basal background activity of the unstimulated cells was always less than 100 CPM and was unaffected by the presence of the drug.

There was a progressive dose-dependent reduction in chemiluminescence with etomidate 0.125  $\mu\text{g}/\text{ml}$  and 0.5  $\mu\text{g}/\text{ml}$ , producing statistically significant reductions (table 1 and fig. 1). The area under the chemiluminescence curve was reduced by 35% and 43%, respectively. The general shape of the chemiluminescence curve was unchanged.

The reductions in activity only occurred when the cells had been incubated with etomidate for 50 min (fig. 2). Gentle washing of the cells completely reversed the effects of etomidate and did not result in counts greater than control.

### Discussion

The major findings of this study were that clinically relevant concentrations of etomidate depress neutrophil chemiluminescence. Fifty minutes of cell incubation with the drug is required to achieve this depression, and gentle washing of the cells after incubation removes the drug-induced depression.

Evidence for the importance of leukocyte oxidative metabolism in bacterial killing comes from the study of patients with chronic granulomatous disease.<sup>8,9</sup> This is an inherited disorder characterized by severe, recurrent, and often fatal pyogenic infections. Their leukocytes exhibit normal phagocytic activity and granule content but no chemiluminescence whatsoever. Furthermore, *in vitro* studies using suitably opsonized bacteria have shown a good correlation between the extent of bacterial killing

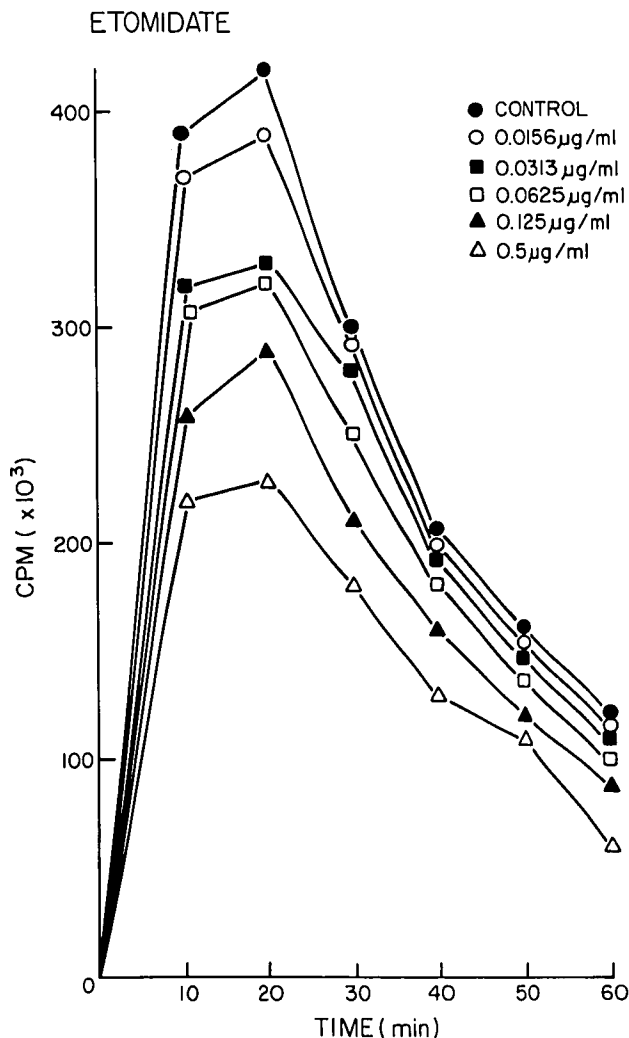


FIG. 1. The effect of increasing concentrations of etomidate on human neutrophil chemiluminescence. All cells were incubated with the drug for 60 min. Chemiluminescence is shown in counts per minute (CPM).

and chemiluminescence, and human leukocytes exposed to chemical and enzyme inhibitors of oxygen radical reactions have a reduced ability to kill bacteria.<sup>4,5,10</sup> Thus, there is evidence in support of a relationship between

TABLE 1. The Effect of Etomidate on Chemiluminescence

| Drug Concentration ( $\mu\text{g}/\text{ml}$ ) | Per Cent Reduction of Chemiluminescence* (mean $\pm$ SEM) |
|------------------------------------------------|-----------------------------------------------------------|
| 0.5                                            | 43 $\pm$ 5†                                               |
| 0.125                                          | 35 $\pm$ 5‡                                               |
| 0.0625                                         | 14 $\pm$ 13                                               |
| 0.0313                                         | 13 $\pm$ 5                                                |
| 0.0156                                         | 9 $\pm$ 21                                                |

\* Area under the curves compared with no added drug.

† *P* < 0.01.

‡ *P* < 0.05.

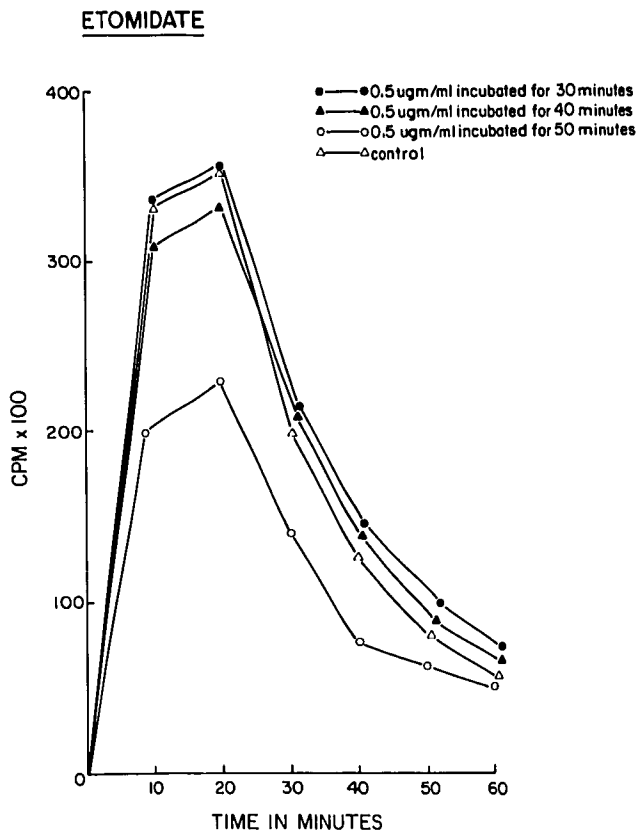


FIG. 2. The effect of duration of incubation on chemiluminescence. Depression only occurs after 50 min of incubation.

chemiluminescence and bacterial killing. We believe, however, that our demonstration of a reduction in chemiluminescence with etomidate should be viewed as qualitative evidence of a reduction in neutrophil competence rather than as quantitative data as it is possible that, *in vivo*, other defense mechanisms may partially or totally compensate for this reduction in neutrophil oxygen radical generation. A 40% reduction in chemiluminescence may therefore not be clinically equivalent to a 40% reduction in leukocyte competence.

The effects of other anesthetic agents on chemiluminescence have been studied previously.<sup>11-14</sup> While high concentrations of diazepam inhibit neutrophil oxidative metabolism, more clinically relevant concentrations do not alter chemiluminescence.<sup>12,13</sup> Similarly, clinical concentrations of narcotics do not alter chemiluminescence.<sup>12</sup> Clinical concentrations of both thiopental and alfathesin reduce chemiluminescence to a similar extent as etomidate.<sup>12</sup> The relative slowness of etomidate is in stark contrast to the effects of thiopental and alfathesin. Thiopental inhibits chemiluminescence even if given up to 10 min after triggering the reaction, while alfathesin requires only 10 min of incubation (Gelb, unpublished data). The effects of all three drugs are easily reversed by washing. This

needs to be borne in mind in any clinical study of leukocyte function during anesthesia. Cells are often washed during the separation process, and this may alter the effects of the anesthetic, leading to the possibly erroneous conclusion that anesthetics do not alter leukocyte function *in vivo*.

The mechanisms producing depression in chemiluminescence were not directly investigated in the study. The depression was dose dependent, and the kinetics of the depression suggest that there was a reduction in all excited oxygen radical production rather than a reduction in a specific radical, as this leads to a change in the shape of the chemiluminescence curve.<sup>15</sup> The drug may: 1) interfere with the interaction of Zymosan® with surface receptors; 2) inhibit the membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase; 3) alter intracellular enzyme processes; 4) alter the fusion of the phagocytic vacuole with the enzyme containing cytoplasmic granules; or 5) act as a free-radical scavenger. The fact that 50 min of incubation was required before an effect was seen makes it unlikely that etomidate was blocking or altering the surface receptor. We are unaware of any data showing etomidate to be a free-radical scavenger. The fungicidal imidazole compounds (ketoconazole, miconazole) have structural and functional similarities with etomidate, and many of these compounds are peroxidase and catalase inhibitors, suggesting that etomidate may behave similarly.<sup>3,16</sup> As the absorbance spectrum of etomidate is in the 220–240 nm range and the light generated during chemiluminescence in the 450–600 nm range, it is unlikely that etomidate was merely absorbing the photons without altering the leukocyte.<sup>15</sup> It is possible that the alcohol in which etomidate is solubilized influenced the chemiluminescence and not the anesthetic compound itself. The alcohol, however, would also have been diluted to the concentrations that would be found clinically if this drug preparation were used.

In conclusion, these results show that clinically relevant concentrations of etomidate reversibly depress human neutrophil chemiluminescence. Fifty minutes of incubation with the drug was required before a reduction in free-radical production became apparent.

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