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# Alfentanil-Induced Miosis Clearance as a Liver CYP3A4 and 3A5 Activity Measure in Healthy Volunteers: Improvement of Experimental Conditions

Narine Baririan, Yves Horsmans, MD, PhD, Jean-Pierre Desager, DSc, Roger Verbeeck, PhD, Roger Vanbinst, DSc, Pierre Wallemacq, PhD, and Luc Van Obbergh, MD, PhD

The aims of this study were to demonstrate the correlation between alfentanil-induced miosis evaluation and alfentanil pharmacokinetics (PK) as a CYP3A4 and 3A5 activity probe in volunteers and to explain the variability in pupilar response and in alfentanil PK. In ambient light, the miosis kinetic parameters were significantly correlated with PK (CLs:  $r = 0.9$ ,  $P = .00$ ; AUCs:  $r = 0.8$ ,  $P = .01$ ). In dark, a similar correlation was observed between miosis and alfentanil clearances ( $r = 0.85$ ,  $P = .03$ ). In 6 volunteers, the sigmoid  $E_{max}$  model was applicable (average  $E_{max} = 2.5 \pm 0.7$  mm,  $\gamma = 2.5 \pm 1.6$  and  $EC_{50} = 76.8 \pm 22.3$  ng/mL), and in 3, the simple  $E_{max}$  model was applicable (average  $E_{max} = 2.8 \pm 0.3$  mm and  $EC_{50} = 19.9 \pm 8.5$  ng/mL).

There was a large interindividual variability in PK parameters (coefficient of variation = 19.7%-31.2%). Free drug fraction concentrations were negatively correlated with plasma  $\alpha_1$ -AGP ( $r = -0.9$ ,  $P = .04$ ) and albumin levels ( $r = -0.94$ ,  $P = .02$ ). Alfentanil-induced miosis clearance as a noninvasive CYP3A4 and 3A5 activity measure can be done in both ambient and dark conditions. Drug free fraction may be responsible for large intersubject variability in alfentanil PK.

**Keywords:** Alfentanil; pharmacokinetics; CYP3A4; miosis  
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Several probe-based tests have been proposed as a measure of CYP3A activity in vivo: erythromycin, midazolam, lidocaine, cortisol, dapsone, nifedipine, and dexamethasone.<sup>1-8</sup> However, there are many potential problems in the development and validation of these probe tests. For example, beside CYP3A4, the additional involvement has been demonstrated of CYP2E1 and CYP3A5 in dapsone metabolism<sup>9</sup> and CYP1A2 in lidocaine N-deethylation<sup>10</sup>. Moreover, as most of these

substrates are characterized by high or intermediate extraction coefficient, their metabolism is influenced by liver blood flow. There is no correlation between erythromycin and midazolam, dextromethorphan, dapsone, and cortisol in vivo probes.<sup>11-13</sup> Finally, the common disadvantages of these CYP3A4 in vivo tests are the need to repeat blood sampling or urine collection, the use of expensive analytical methods, and extensive analytical effort.

Alfentanil is an opioid analgesic with some pharmacokinetic advantages to be evaluated as a CYP3A4 substrate test. First, CYP3A4 is the major P450 isoform responsible for human liver microsomal alfentanil metabolism.<sup>14,15</sup> In addition, the involvement of CYP3A5 has been recently demonstrated.<sup>16,17</sup> Its intestinal metabolism remains poorly known. However, after intravenous administration, its impact should remain limited as it should be delivered to the site of intestinal metabolism from the systemic circulation, and plasma protein binding can act as a limiting factor of diffusion.<sup>18</sup> Thus, the contribution of the intestinal me-

From the Clinical Pharmacology Unit, St Luc University Hospital, Brussels, Belgium (Ms Baririan, Dr Horsmans, Dr Desager); Pharmacokinetics, Metabolism, Nutrition and Toxicology Unit, Université Catholique de Louvain, Brussels, Belgium (Dr Verbeeck); Therapeutic Monitoring and Toxicology Laboratories, St Luc University Hospital Brussels, Belgium (Dr Vanbinst, Dr Wallemacq); and the Anesthesiology Unit, Université Catholique de Louvain, Brussels, Belgium (Dr Van Obbergh). Submitted for publication June 11, 2005; revised version accepted September 16, 2005. Address for reprints: Yves Horsmans MD, PhD, Department of Gastroenterology, Cliniques Universitaires Saint-Luc, Avenue Hippocrate, 10, Brussels 1200, Belgium.  
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tabolism for high protein-binding drugs, such as alfentanil, can be considered as relatively limited after its intravenous administration. Second, alfentanil has a low hepatic extraction coefficient, and the pharmacokinetics of alfentanil is not dependent on liver blood flow.<sup>19</sup> These data suggest that alfentanil metabolism may be an excellent *in vivo* probe for hepatic CYP3A4 and 3A5 activity.<sup>14,20</sup>

Alfentanil administration also induces a decrease in pupilar size (miosis), which is known to be dose related.<sup>21,22</sup> This effect is easy to measure and can be used for pseudo-kinetic analysis. A pilot investigation by Phimassone et al compared alfentanil plasma clearance ( $CL_{pl}$ ) and miosis clearance ( $CL_{miosis}$ ) after induction and inhibition of CYP3A4 by rifampicin and troleandomycin in 6 healthy volunteers.<sup>23</sup> These preliminary results seem to suggest that the alfentanil effect (miosis) can be a surrogate measure for plasma alfentanil concentration and hepatic CYP3A4 and 3A5 activity.<sup>23,24</sup>

The purpose of our investigation was to validate alfentanil-induced miosis as a CYP3A4 and 3A5 probe by assessment of its metabolism and pupilar response correlation in 10 healthy volunteers, to develop well-defined experimental conditions (light intensity) and to study some of the parameters playing a role in the interpatient pharmacokinetic results variability.

## SUBJECTS AND METHODS

### Subjects and Clinical Protocol

Ten volunteers (4 women and 6 men; 8 whites and 2 North Africans; age range, 24-34 years) participated in the study, which was approved by the Ethical Committee of the Catholic University of Louvain. Written informed consent was obtained from all subjects. Volunteers were in good health, with a mean body mass index of  $22.8 \pm 3.6$ , and were taking no medications known to alter CYP3A4 and 3A5 activity. Subjects were instructed to avoid beverages containing alcohol or caffeine and not to consume grapefruit for 48 hours before and during the study day.

All experiments were done in the same room with constant ambient (or dark) light conditions; subjects were supine and monitored with pulse oximeter. An intravenous catheter was inserted in one arm and after baseline blood samples for pharmacokinetic and plasma protein determination; basal pupil diameter measure was obtained. The subjects then received a bolus of alfentanil (15 g/kg) in the contralateral arm. Blood (2.8 mL) was sampled 2, 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, and 240 minutes later. Plasma was imme-

diately separated and stored at  $-20^{\circ}\text{C}$  until analysis. During the experiment, 1 additional sample was taken 45 minutes after alfentanil administration, to evaluate the free drug fraction. At each time of blood sampling, pupil diameter was assessed with the use of a Pupil scan II Model 12A Pupillometer (Keeler Instruments, Inc, Broomall, Pa). Left pupil diameter was measured first at each time point in all experiments, to minimize the influence of measure procedure on the pupilar response, and subtracted from the ambient (or dark) adapted diameter taken at baseline ( $diameter_0 - diameter_t$ ). These data were therefore used for subsequent calculations.

Five of 10 subjects agreed to participate in a second experiment under the same design but in dark conditions.

### Analytical Methods and Materials

Fentanyl (Janssen-Cilag) and alfentanil (Rapifen; Janssen-Cilag) were provided by Janssen Pharmaceutica (Beerse, Belgium) and used for administration and analysis. Acetonitrile (SDS, Peypin, France), methanol (CHROMASOLV; Sigma-Aldrich Laborchemikalien GmbH) and ethyl acetate (Merck Kga, Darmstadt, Germany) were of high-performance liquid chromatography (HPLC) grade.

Plasma alfentanil concentrations were measured by liquid chromatography dual mass spectrometry (Waters Micromass Quattro micro API Mass Spectrometer) using a modification of previous methods.<sup>25,26</sup> Calibrators (0, 1, 10, 50, 150, and 300 ng/mL) were prepared by adding standards to blank serum. A total of 200  $\mu\text{L}$  of calibration standards or patient plasma samples, 200  $\mu\text{L}$  carbonate buffer pH 9.2, and 750  $\mu\text{L}$  of the internal standard, solution in the ethyl acetate (25 ng/mL fentanyl), were added to a screw-top tube. After vortexing for 10 seconds, the tubes were placed on the stirrer for 10 minutes and then centrifuged for 5 minutes. A total of 650  $\mu\text{L}$  of top organic phase was transferred to a clean tube and evaporated to dryness under vacuum (SC210A Speed Vac Plus; Thermo Savant). The drug remainder was dissolved in 100  $\mu\text{L}$  pure methanol and transferred to a glass autosampler vial for analysis.

The chromatographic analysis was performed using an isocratic HPLC pump and a Lichrocart 125  $\times$  2 mm column packed with Superspher 100RP-18. Separation of the compounds was obtained at a flow rate of 0.3 mL/min with 20% aqueous acetonitrile containing 2 mM ammonium acetate and 0.1% (v/v) formic acid within 5 minutes. A volume of 20  $\mu\text{L}$  was injected.

A Quattro micro triple quadrupole mass spectrometer fitted with Z SPRAY ion interface was used for detection and analyzed by the multiple reaction monitoring method. Collision gas (argon) pressure was maintained at  $2.5 \times 10^{-3}$  mbar. Ionization was achieved using electrospray in the positive ionization mode. The instrument was run in multiple response monitor mode and used the ratio of parent/daughter ion pairs of 417/197 and 336.92/104.98 atomic mass units to identify and quantify alfentanil and fentanyl, respectively. The following conditions were used: capillary voltage, 3.5 V; block temperature, 140°C; and desolvation gas (nitrogen) heated to 300°C. All aspects of system operation and data acquisition were controlled using Mass Lynx 3.4 software with automated data processing using the Mass Lynx Quantify program.

Before study, the bioanalytical method of alfentanil determination was validated. No significant interference was observed in extracted blank air samples or wipes at the retention time of the compounds (specificity). Linear regression analysis of the calibration plots (3 replicate samples at each calibration level) resulted in a good linearity ( $r^2 > 0.95$ ). The limit of detection and the limit of quantification were calculated by linear regression analysis of 10 times injected calibrator responses (Area/IS Area) and were found to be 0.1 ng/mL and 0.03 ng/mL, respectively (sensitivity). Extraction recovery was about  $97\% \pm 13\%$  for alfentanil and  $92\% \pm 7\%$  for fentanyl (evaluated with 6 calibrators). The intraassay precision of the sampling method was defined as the intraday coefficient of variation and was found to be 1% for highest calibration concentration of alfentanil and 8% for lowest calibration concentration. The interassay precision of the sampling method was defined as the interday coefficient of variation and was found to be 5% for highest calibration concentration of alfentanil and 14% for lowest calibration concentration. Long-term stability of alfentanil in plasma during the samples conservation (at  $-20^\circ\text{C}$ ), short-term stability of alfentanil in plasma during the extraction period (at  $23^\circ\text{C}$ ), and autosampler stability in chromatograph during the analysis period (at  $4^\circ\text{C}$ ) were defined to be  $>95\%$ .

The free fraction of alfentanil in plasma obtained from the same healthy subjects was determined using Centrifugal Filter Devices for ultrafiltration (Centricon YM-30, Millipore). The devices were prerinsed prior to analysis with 2 mL of deionized water. After removing the remaining rinse, 1 mL of patient plasma was added to the sample reservoir of the devices and centrifuged at 3500 rpm for 30 minutes. The filtrate was extracted and analyzed analogously to plasma samples.

$\alpha_1$ -AGP and albumin were analyzed in St Luc Hospital's local laboratory of clinical biology. Serum  $\alpha_1$ -AGP was quantified by an in vitro immunochemical reaction with specific antibodies (N antiserum to human  $\alpha_1$ -acid glycoprotein) with the BN\* systems (Behring Nephelometer Analyser II, Dade Behring Marburg GmbH, Germany). Serum albumin was measured by Beckman LX20 (USA) colorimeter using Bromocresol purple as the reagent.

### Data Analysis

Alfentanil plasma concentrations were analyzed, and pharmacokinetic parameters were calculated using the noncompartmental pharmacokinetic analysis method with WinNonlin 3 Professional version software (Pharsight Corp, Mountain View, Calif). The pupilar response at each time point was defined as the baseline diameter minus the diameter at each time point. The elimination constant ( $K_{el}$ ) was estimated by log-linear regression analysis of miosis versus time semilogarithmic plots, the elimination half-life ( $t_{1/2 \text{ miosis}}$ ) was calculated as  $\ln 2/K_{el}$ . The area under the curve to the last time point ( $\text{AUC}_{0-t \text{ miosis}}$ ) value was calculated by trapezoidal rule. The pupilar effect clearance ( $\text{CL}_{\text{miosis}}$ ) was calculated as  $\text{DOSE}/\text{AUC}_{0-\infty \text{ miosis}}$ .

The relationship between alfentanil plasma concentration and its pharmacological effect (miosis; pharmacokinetic/pharmacodynamic analysis) was performed by a nonlinear regression analysis by WinNonlin 3 Professional version software using sigmoid  $E_{\text{max}}$  and/or ordinary  $E_{\text{max}}$  models. For an ordinary or simple  $E_{\text{max}}$  model, the relationship between concentration and pharmacological effect can be described by the Hill equation:

$$E = E_{\text{max}} \cdot C/EC_{50} + C.$$

For a sigmoid  $E_{\text{max}}$  model, this relationship is described by a sigmoid version of the Hill equation, which should be viewed as an extension of the ordinary  $E_{\text{max}}$  model to account for the curvature:

$$E = E_{\text{max}} \cdot C^\gamma/EC_{50}^\gamma + C^\gamma.$$

Individual pharmacodynamic parameters were estimated:  $E_{\text{max}}$  (alfentanil-induced maximum miosis),  $EC_{50}$  (concentration of alfentanil that produces 50% of  $E_{\text{max}}$ ), and  $\gamma$  (sigmoidicity parameter).

Correlations between pupilar effect kinetics and alfentanil pharmacokinetics and between alfentanil free fraction and plasma protein concentrations were assessed by least squares regression analysis (SPSS Science, Chicago, Ill). Spearman's correlation coefficient

was calculated. A Student's paired *t* test was used to assess the significance of pupilar response differences among 2 results in different conditions of luminosity (SPSS Science, Chicago, Ill). Results were considered statistically significant when  $P < .05$  (95% confidence interval [CI]).

## RESULTS

No side effects were observed among the volunteers after the alfentanil injection. Some subjects were briefly and mildly sedated for 10 to 15 minutes; however, no one required administration of supplemental oxygen.

The first experiment (A) was done in 10 healthy volunteers in whom alfentanil pupilar responses in ambient lighting conditions and alfentanil plasma concentrations were studied. The alfentanil concentration versus time curve showed the classic pharmacokinetics of alfentanil. The same noncompartmental method of curves analysis was used for both pharmacokinetics and miosis; conventional pharmacokinetic parameters and the effect pseudo-kinetic parameters were derived (Table I; for 1 subject the  $CL_{\text{miosis}}$  was not calculated because of measurement inaccuracy). The pseudo-kinetic parameters for alfentanil miosis were correlated with the plasma pharmacokinetic parameters. There was a large linear correlation between  $CL_{\text{pl}}$  and  $CL_{\text{miosis}}$  ( $r = 0.9$ ,  $P = .00$ ,  $n = 9$ ; Figure 1) and between the  $AUC_{\text{all pl}}$  and  $AUC_{\text{all miosis}}$  ( $r = 0.8$ ,  $P = .01$ ,  $n = 9$ ; data not shown), with both curves being extrapolated to infinity. No correlation was found between the elimination half-lives ( $t_{1/2 \text{ pl}}$  and  $t_{1/2 \text{ miosis}}$ ).

The pharmacokinetic/pharmacodynamic analysis results are shown in Table II. The sigmoid  $E_{\text{max}}$  model was applied for only 6 volunteers, while the ordinary  $E_{\text{max}}$  model was compatible for the other 3 volunteers (the same subject with inaccuracy of miosis measurement was excluded from pharmacokinetic/pharmacodynamic analysis).

Table I shows that the greatest interindividual variability in derived pharmacokinetic parameters was observed for the apparent volume of distribution. Since plasma protein binding could be a factor susceptible to affect alfentanil pharmacokinetics, plasma albumin,  $\alpha_1$ -AGP, and free drug fraction were measured.  $\alpha_1$ -AGP and free drug fraction showed a large interindividual variability (coefficient of variation = 26.5% and 23.2%, respectively). All values of plasma  $\alpha_1$ -AGP and albumin remained in normal physiological ranges (50-120 mg/dL), except for 1 subject with  $\alpha_1$ -AGP of 40 mg/dL. Free drug fractions varied from 5.2% to 9.8% and were negatively correlated with plasma  $\alpha_1$ -AGP and albu-

min contents ( $r = -0.9$ ,  $P = .04$  and  $r = -0.94$ ,  $P = .02$ , respectively; data not shown).

Studying the alfentanil miosis response in experiment A, a considerable interindividual variability in baseline pupil diameters (varying from 4.2 mm to 7.2 mm) and in maxima pupil responses (varying from 1.8 mm to 4.7 mm) was observed (Table I). There was a linear correlation between the initial and minima pupil diameters ( $r = 0.8$ ,  $P = .01$ ). To establish the importance of initial pupil diameter in alfentanil-induced miosis evaluation, the same test was performed in 5 of the 10 volunteers a few months (7-10 months) after the first experiment in dark lighting (experiment B<sub>dark</sub>). Miosis versus time 2 plots are shown in Figure 2. Concentration versus time plots were similar in both experiments. Alfentanil caused immediate and profound miosis in both ambient and dark experiments, inducing a maximum left pupil diameter change of  $2.3 \pm 0.8$  mm and  $3.6 \pm 0.3$  mm, respectively. The initial pupil diameter of 5 volunteers in dark lighting varied from 6.3 to 7.0 mm (vs 5.5-6.6 mm in ambient conditions). The difference between the 2 maximum miosis values is due to the larger pupil diameter at baseline in dark conditions, which offered the greatest dynamic range of alfentanil-induced miosis compared to the ambient lighting response ( $P = .00$ , 95% CI). The differences were statistically significant between  $AUC_{0 \rightarrow \infty \text{ miosis}}$  ( $P = .01$ , 95% CI) and  $CL_{\text{miosis}}$  ( $P = .03$ , 95% CI) and not significant between the  $t_{1/2 \text{ miosis}}$  in 2 different luminosity experiments. As observed in experiment A, there were significant correlations between the alfentanil pharmacokinetic parameters and its induced miosis kinetic parameters in dark lighting conditions (data not shown).

## DISCUSSION

In the first part of our investigation, the pupil diameter changes after an alfentanil intravenous bolus was studied in 10 volunteers in ambient lighting conditions (experiment A). Miosis was treated similarly to alfentanil plasma concentration, and its disposition was analyzed using the same noncompartmental method. There were excellent correlations between the derived  $AUC_{0 \rightarrow \infty \text{ miosis}}$  and  $AUC_{0 \rightarrow \infty \text{ pl}}$ ,  $CL_{\text{miosis}}$ , and  $CL_{\text{pl}}$ . The elimination half-lives were not correlated, probably because they concerned the terminal phases of alfentanil concentration and miosis versus time plots, when the concentration is still dropping, but miosis already remains stable. This indicates that alfentanil plasma and miosis half-lives are not useful parameters to compare and/or correlate when developing an alfentanil-induced miosis test as a measure of CYP3A and 3A5 activity.

**Table I** Alfentanil Pharmacokinetic and Alfentanil-Induced Miosis Pseudo-kinetic Parameters in Volunteers After 15 g/kg Intravenous Bolus, in Ambient and Dark Lighting Condition Experiments

Patient Group	Miosis Pseudo-kinetics				Alfentanil Pharmacokinetics				
	AUC <sub>0-∞</sub> <sup>a</sup> mm·min	Elimination t <sub>1/2</sub> , min	CL, μg·min <sup>-1</sup> ·mm <sup>-1</sup>	Initial Pupil Diameter, mm	Maximal Pupilar Response, mm	AUC <sub>0-∞</sub> <sup>b</sup> μg·min·mL <sup>-1</sup>	Elimination t <sub>1/2</sub> , min	CL, mL·min <sup>-1</sup> ·kg <sup>-1</sup>	V <sub>d area</sub> <sup>c</sup> L/kg
A	153 ± 161	67.8 ± 34.3	157.8 ± 132.3	5.8 ± 1.0	2.1 ± 0.7	5106 ± 1023	50.5 ± 9.9	2.9 ± 0.6	0.2 ± 0.0
B <sub>dark</sub>	437 ± 284	108.8 ± 4.8	54.1 ± 43.3	6.6 ± 0.4	3.6 ± 0.3	8802 ± 1708	67.4 ± 8.5	1.7 ± 0.3	0.1 ± 0.0
B <sub>amb</sub>	196 ± 225	110.5 ± 3.7	165.4 ± 127.7	5.8 ± 0.9	2.3 ± 0.8	5407 ± 1213	51.8 ± 5.8	2.3 ± 0.5	0.2 ± 0.0

Experiment A: 10 volunteers in ambient lighting conditions; experiment B<sub>dark</sub>: 5 volunteers (of 10 volunteers from experiment A) in dark lighting conditions; B<sub>amb</sub>: 5 volunteers (same volunteers from B<sub>dark</sub> group) in ambient lighting conditions. Results are mean ± SD (n = 10 for experiment A and n = 5 for experiment B). AUC<sub>0-∞</sub><sup>a</sup> = area under the miosis/concentration-time curve extrapolated to the infinity; E<sub>max</sub> = maximum pupil diameter change; elimination t<sub>1/2</sub> = elimination half-life of miosis/plasma alfentanil; CL = total clearance of miosis/plasma alfentanil; V<sub>d area</sub><sup>c</sup> = apparent volume of alfentanil distribution.

## ALFENTANIL-INDUCED MIOSIS CLEARANCE

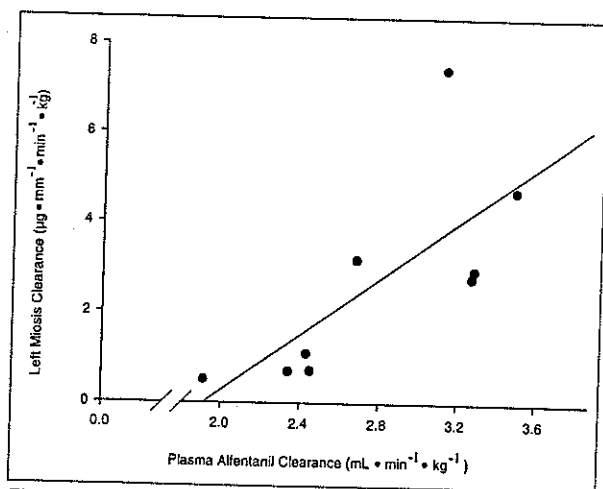


Figure 1. Relationship between the clearances for alfentanil-induced miosis and for plasma concentration. Each data point represents a single subject ( $n = 9$ ). For 1 volunteer, the miosis CL was impossible to calculate because of the inaccuracy of the miosis measurement.  $r = 0.9$ . The correlation is significant at the .01 level (2-tailed).

Table II Pharmacodynamic Parameters in Volunteers (Ambient Lighting Experiment) Estimated by Pharmacokinetic/Pharmacodynamic Analysis

Pharmacokinetic/ Pharmacodynamic Model Applied	n	$E_{max}$ , mm	$EC_{50}$ , ng/mL	$\gamma$
Sigmoid $E_{max}$	6	$2.5 \pm 0.7$	$76.8 \pm 22.3$	$2.5 \pm 1.6$
Ordinary $E_{max}$	3	$2.8 \pm 0.3$	$19.9 \pm 8.5$	1

$E_{max}$  = alfentanil-induced maximum miosis;  $EC_{50}$  = concentration of alfentanil that produces 50% of  $E_{max}$ ;  $\gamma$  = sigmoidicity parameter. One volunteer was excluded from pharmacokinetic/pharmacodynamic analysis. Results are mean  $\pm$  SD.

To further study the relationship between alfentanil-induced miosis and plasma concentration, a pharmacokinetic/pharmacodynamic analysis was also performed. In our volunteers, sigmoid  $E_{max}$  could be applied in 6 cases, and for the other 3 cases, the ordinary  $E_{max}$  model was applied. These choices were based on visual observation of the pharmacokinetic/pharmacodynamic curves and justified by minimal deviation between predicted and observed values. In Phimmason and Kharasch's pilot investigation, a sigmoid  $E_{max}$  model was described in 5 of 6 volunteers. However, the estimated pharmacodynamic parameters were not provided by these authors.<sup>23</sup> The sigmoid  $E_{max}$  model represents the same ordinary  $E_{max}$  model with the addition of a single exponent parameter  $\gamma$  in the Hill equation, which modifies curvature of the response-

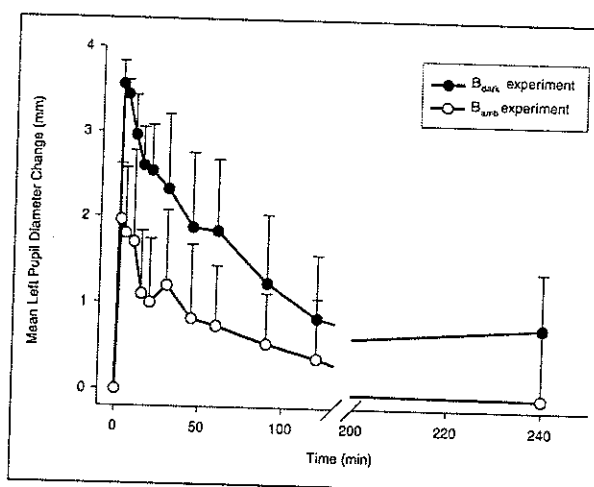


Figure 2. Effect of alfentanil on pupil diameter in ambient ( $B_{amb}$ ) and dark ( $B_{dark}$ ) lighting. Results are represented as mean values  $\pm$  SD ( $n = 5$ ).

concentration curve. For the simple  $E_{max}$  model, the exponent  $\gamma$  is equal to 1. The larger the value of  $\gamma$ , the more curvature there is in the line around  $EC_{50}$  (sigmoid  $E_{max}$  model).<sup>27</sup> In our volunteers in whom the sigmoid  $E_{max}$  model was applied, the  $\gamma$  values were small. Thus, the differences between the 2 groups were not very marked, and the choice of 2 models was essentially driven by the willingness to obtain the best visual fitting. Although it seems to be a difference between the  $EC_{50}$  values, there was no clear cut between the 2 groups; furthermore, the variability of this value was large. To determine whether the 2 patterns of response really exist, more data should be obtained in a larger group of volunteers.

The second purpose of our investigation was to find factors linked to the variability of pharmacokinetics and pupilar response in volunteers to improve the test conditions. In the pharmacokinetic results of volunteers, interindividual variability in  $AUC_{0-\infty}$  (20%), CL (17.6%), and  $V_{d,area}$  (20.6%) was observed. This variability could be explained by hepatic CYP3A4 and/or CYP3A5 activity or by plasma protein content because alfentanil has a low hepatic extraction coefficient.<sup>28,29</sup> The present data of plasma alfentanil free fraction in 10 volunteers ( $f_u = 8.2 \pm 1.9$ ) were consistent with the data of Piasfski.<sup>30</sup> The individual  $f_u$  varied from 5.2% to 9.8%; thus, in some subjects,  $f_u$  increased 2-fold. Free drug fraction variability may therefore be involved in the interindividual variability of alfentanil pharmacokinetics.

The other factor susceptible to modifying the results of pupilar response to alfentanil may be lighting conditions during the experiment. A second study was performed in dark conditions with 5 of 10 volunteers (ex-

periment  $B_{\text{dark}}$ ). The aim was to obtain a maximal mydriasis at baseline, compared to experiment A. No changes in pharmacokinetics were observed in subjects during the second experiment. The time courses of miosis in ambient and dark experiments were similar. However, in dark conditions, the initial pupil diameters of all subjects were lesser, and the maxima responses were greater. Consequently,  $AUC_{0 \rightarrow \infty \text{ miosis}}$  and  $CL_{\text{miosis}}$  were significantly different from the ambient experiment ( $P < .05$ , 95% CI), with similar coefficients of variation. Significant correlations were also observed between the  $AUC_{0 \rightarrow \infty \text{ miosis}}$  and  $AUC_{0 \rightarrow \text{pl}}$ ,  $CL_{\text{miosis}}$  and  $CL_{\text{pl}}$  in dark conditions experiment. Thus, both ambient and dark lighting conditions can be used in alfentanil-induced miosis probe.

Plasma alfentanil pharmacokinetics can be replaced by the determination of alfentanil-induced miosis, at least in healthy volunteers. More important, this miosis test can be performed in dark or ambient light conditions. Clearances of miosis and plasma alfentanil are the suitable parameters to be used in this test. Furthermore, the results of this investigation might suggest a contribution of the free drug fraction, susceptible to modify the pharmacokinetics of alfentanil.

To further validate the test in clinical practice, alfentanil miosis and metabolism should be studied in patients with different pathologies susceptible to alter the CYP3A4 and 3A5 activity and alfentanil metabolism.

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