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Alfentanil-Induced Miosis as a Surrogate Measure of Alfentanil Pharmacokinetics in Patients with Mild and Moderate Liver Cirrhosis

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Abstract

Objectives: (i) To evaluate the pupillary response to alfentanil as a surrogate measure of alfentanil pharmacokinetics in cirrhotic patients and to compare the data observed in cirrhotic patients with those found in healthy volunteers (historical control group); and (ii) to compare this test with other liver function tests in cirrhotic patients.

Methods: Six patients with mild cirrhosis (Child-Pugh grade A) and six patients with moderate cirrhosis (Child-Pugh grade B) were studied after a single 15 μg/kg bolus of alfentanil. Alfentanil plasma concentrations were measured by liquid chromatography-tandem mass spectrometry, and pupillary responses were measured with a Pupilscan II pupillometer. Alfentanil pharmacokinetics (plasma concentration, area under the plasma concentration-time curve from time zero to infinity [$AUC_{\infty(p)}$] and from time zero to 2 hours [$AUC_{2(p)}$], apparent volume of distribution at steady state, clearance and terminal elimination half-life [$t_{½(p)}$]) and miosis pseudo-kinetic parameters [$AUC_{\infty(miosis)}$, $AUC_{2(miosis)}$, $t_{½(miosis)}$] were determined using a noncompartmental analysis method. In six patients (three Child-Pugh grade A and three Child-Pugh grade B), antipyrine (measure of liver intrinsic activity) and D-sorbitol (measure of liver blood flow) tests were performed.

Results: A significant correlation was found between the alfentanil $AUC_{\infty(p)}$ and $AUC_{\infty(miosis)}$ ($r = 0.6$, $p < 0.05$) in cirrhotic patients. This correlation was even more significant if AUC determinations were limited to the first 2 hours after alfentanil administration ($r = 0.9$, $p < 0.01$). Statistically significant differences in pharmacokinetics and miosis pseudo-kinetic parameters were observed between cirrhotic patients and healthy volunteers from our previous experiment (historical control group). The correlations were significant between alfentanil clearance and

antipyrine clearance ($n = 6$, $r = 0.9$, $p < 0.05$), alfentanil clearance and steady-state hepatic blood clearance [$CL_{H(b)}$] measured by the D-sorbitol test ($n = 6$, $r = 0.9$, $p < 0.05$).

Conclusion: Alfentanil pharmacokinetic parameters were correlated with miosis pseudo-kinetic parameters in cirrhotic patients. There was a significant decrease in pharmacokinetics and miosis pseudo-kinetics in cirrhotic patients compared with volunteers from the historical control group. Alfentanil-induced miosis has the advantage of being noninvasive and can be limited to miosis measurements during the first 2 hours after alfentanil administration in cirrhotic patients. We thus propose to substitute the $AUC_{2(miosis)}$ for alfentanil pharmacokinetics in cirrhosis.

Background

Cytochrome P450 (CYP) 3A is clinically the most significant subfamily in human drug metabolism, with CYP3A4 being the most abundant cytochrome in the liver and responsible for the hepatic metabolism of >50% of all drugs.[1,2]

Multiple *in vivo* CYP3A probes have been proposed. However, they all have their limitations and, so far, no substrate has been identified as a probe to quantitatively predict the *in vivo* kinetics of CYP3A drugs.[3-7]

Alfentanil is an opioid analgesic metabolised predominantly by CYP3A4 and partially by CYP3A5, and its pharmacokinetics can be a useful measure of hepatic CYP3A activity.[8-10] The contribution of the intestinal mucosa to the overall metabolism of intravenously administered alfentanil is thought to be limited.[11] Alfentanil induces a dose-related decrease in the pupillary size (miosis) that can be determined easily. Alfentanil may be a useful CYP3A probe since it induces miosis, which could be used as a surrogate measure for plasma concentration and therefore serve as a noninvasive assessment of liver CYP3A activity. The pupillary response after intravenous and oral alfentanil administration has been studied by Kharasch et al.[12-14] in healthy volunteers, showing an excellent correlation between alfentanil pharmacokinetics and miosis pseudo-kinetics. Thereafter, we confirmed the presence of a statistically significant positive correlation between the pharmacokinetics of alfentanil and its induced miosis pseudo-kinetics in healthy volunteers under two different experimental lighting conditions.[15]

To further explore the potential use of alfentanil-induced miosis as an *in vivo* marker of hepatic CYP3A activity, its induced miosis and metabolism should be studied in patients with different pathologies that are likely to alter hepatic CYP3A activity. In hepatic cirrhosis, drug metabolism is impaired, and this is related to alterations in intrinsic hepatic activity (hepatocyte metabolic dysfunction), liver blood flow (portosystemic shunting) and drug protein binding (decreased protein synthesis and binding capacity).[16,17] It has already been demonstrated that the pharmacokinetics of alfentanil are markedly altered in cirrhosis, mainly due to decreased plasma clearance and an increased unbound fraction.[18,19]

To assess the clinical value of alfentanil-induced miosis as a test of liver CYP3A function, we measured miosis after a single intravenous bolus of alfentanil in patients with mild cirrhosis (Child-Pugh grade A) and moderate cirrhosis (Child-Pugh grade B). The primary objectives of this study were to evaluate whether the pupillary response can be used as a surrogate measure of alfentanil pharmacokinetics in cirrhotic patients and to compare the

data observed in cirrhotic patients with those found in healthy volunteers (historical control group). The secondary objective was to compare the results of two other liver function tests, the antipyrine and D-sorbitol tests, with alfentanil pharmacokinetics and pupillary response measurements in cirrhotic patients.

Methods

Patients and Clinical Protocol

Twelve patients (3 women and 9 men; 11 Caucasians and 1 African; age range 41–65 years) with biopsy-proven cirrhosis (6 classified as Child-Pugh grade A and 6 as grade B) were included in the study. The protocol was approved by the Ethics Committee of the Catholic University of Louvain (Brussels, Belgium), and written informed consent was obtained from all patients prior to study inclusion. The patients had no medical history of surgical porto-caval shunting and showed no signs of encephalopathy as assessed by neurological examination and EEG. They had taken no medications known to alter CYP3A4 activity within 1 month preceding as well as during the alfentanil test. The patients had been instructed to avoid beverages containing alcohol (ethanol), caffeine or grapefruit juice for 48 hours prior to and during the study day. Cirrhosis was of alcoholic origin in eight cases (who had abstained from alcohol for at least 3 months), hepatitis C-related in three cases and drug-induced in one case. In nine patients, the presence of oesophageal varices was documented by endoscopy (five patients with grade I varices, three patients with grade II varices and one patient with grade III varices). There was no evidence of renal insufficiency as shown by creatinine clearance (CL_{CR}) calculated by the Cockcroft formula (CL_{CR} values ranged from 82 mL/minute to 151.8 mL/minute). The exclusion criteria included the presence of hepatocellular carcinoma, severe cholestasis (serum total bilirubin >50 mg/dL), primary biliary cirrhosis and known hypersensitivity to opioids.

Experiments

All miosis experiments were carried out in the same room under constant dim lighting conditions. Patients were in the supine position and monitored with a pulse oximeter. As described in our previous paper,[15] an intravenous catheter was inserted into one arm, baseline blood samples were drawn for pharmacokinetic and plasma protein binding determination, and the basal pupil diameter was measured. Subjects received a bolus of alfentanil (15 μg/kg) in the contralateral arm. Blood samples (2.8mL) were drawn 2, 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, 240 and 360 minutes later. Plasma was immediately separated and stored at −20°C until analysis. To evaluate the free drug fraction, one additional sample was taken in 6 of 12 patients 45 minutes after alfentanil administration. At each time of blood sampling, the pupil diameter was assessed using a Pupilscan II Model 12A Pupillometer (Keeler Instruments, Inc., Broomall, PA, USA). The left pupil diameter was measured first at each timepoint to minimise the influence of the measuring procedure on the pupillary response, and subtracted from the diameter taken at baseline ($diameter_0 - diameter_t$). These data were used for subsequent calculations.

Six (three Child-Pugh grade A and three grade B) of 12 patients agreed to participate in two complementary tests. At least 1 week before the alfentanil miosis experiment, antipyrine saliva and D-sorbitol tests were performed to determine intrinsic hepatic activity and blood flow, respectively.[20-23] A washout period of 48 hours was observed between these two tests. Antipyrine powder (600mg) was dissolved in 200mL of water and administered orally. Saliva samples (minimum 2mL) were collected before and 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 36 and 48 hours after intake and frozen at −20°C until analysis.

D-sorbitol (40%) was administered as an intravenous infusion at a rate of 50 mg/minute over 3 hours. The subjects remained in the supine position for 4 hours after the start of the infusion and drank 500mL of water during that period. Blood samples were taken for measurement of plasma D-sorbitol concentrations at 165, 170, 175 and 180 minutes after the start of the infusion, all of which corresponded to the steady state of sorbitol (the mean value of these four concentrations was considered as the sorbitol steady-state concentration). Urine was collected just before and during the 4 hours following the beginning of the sorbitol infusion. Plasma and urine samples were stored at −20°C until analysis.

Analytical Methods and Materials

Fentanyl®[1] (Janssen-Cilag) and alfentanil (Rapifen®, Janssen-Cilag) were provided by Janssen Pharmaceutica (Beerse, Belgium) and used for administration and analysis. Acetonitrile, methanol and ethyl acetate were of high-performance liquid chromatography (HPLC) grade. Antipyrine (Bufa Pharma, Uitgeest, The Netherlands) was weighed and a D-sorbitol sterile solution was prepared by the local St Luc Hospital pharmacy.

Antipyrine concentrations in saliva were measured using a simple HPLC-UV analytical method previously validated by Echizen et al.[24] The HPLC system consisted of a Kontron 420 pump from Beun De Ronde, Serlabo B.V.B.A. (Drogenbos, Belgium) and a Pye Unicam UV detector (Cambridge, UK) set at 254nm. D-sorbitol concentrations in plasma and urine (diluted 1 : 100) were measured by the sorbitol dehydrogenase enzyme-UV method, as previously described by Molino et al.,[25] using a Secomam® Anthelie model spectrophotometer (Domont, France) operating at 366nm.

Plasma alfentanil concentrations were measured by liquid chromatography-tandem mass spectrometry (Waters Micromass Quattro Micro API Mass Spectrometer) and chromatographic analysis was performed using an isocratic HPLC pump and a Lichrocart® 125 × 2mm column packed with Superspher® 100RP-18, using the method previously validated by Baririan et al.[15]

The free fraction of alfentanil in plasma was determined using centrifugal filter devices (Centricon® YM-30, Millipore, Billerica, MA, USA) for ultrafiltration. One millilitre of patient plasma was added to the sample reservoir of devices and centrifuged at 3500 rpm for 30 minutes at room temperature. The filtrate was extracted and analysed in a way similar to that described above for plasma samples. The alfentanil free fraction was calculated as follows: $C_{filtrate}/C_{total} \cdot 100\%$, where $C_{filtrate}$ is the concentration of filtrate and C_{total} is the measured plasma concentration of alfentanil.

Albumin levels, bilirubin levels and the prothrombin time were determined in the St Luc Hospital laboratory using routine clinical laboratory methods.

Data Analysis

Data analysis was performed using the same methods as those used in volunteers, as previously described by Baririan et al.[15] Alfentanil plasma and antipyrine saliva concentrations were analysed using noncompartmental pharmacokinetic analysis with WinNonlin® 3 Professional Version software (Pharsight Corporation, Mountain View, CA, USA). Antipyrine pharmacokinetic parameters were determined by analysis of salivary concentrations.[26,27]

The pupillary response at each timepoint was defined as the baseline diameter minus the diameter at each timepoint. The elimination rate constant (k_e) was estimated by log-linear regression analysis of miosis versus time semi-logarithmic plots, and the elimination half-life [$t_{½(miosis)}$] was calculated as

1 The use of trade names is for product identification purposes only and does not imply endorsement.

$\ln 2/k_e$. The area under the curve from time zero to infinity [$AUC_{\infty(miosis)}$] value was calculated by the trapezoidal rule.

Correlations between pupillary effect kinetics, alfentanil pharmacokinetics and the free fraction were assessed by Spearman's correlation coefficient. Logarithmic transformations of all pharmacokinetic and pseudo-kinetic parameters were done to normalise their distributions (confirmed by the Shapiro-Wilk W test). The Student's t-test was used to assess the significance of differences in alfentanil pharmacokinetics and its induced miosis pseudo-kinetic parameters between the different groups. The choice of the Student's t-test was justified by the analysis of variances (one-way ANOVA), which proved the equality of variances in the compared groups. The sample size of 12 cirrhotic patients was justified by the power of the applied t-test (JMP® 5.1, SAS Institute, Cary, NC, USA) which was >90%. For most clinical trials, a statistical test power of 80% or 90% is frequently chosen as adequate.[28] The data are presented as mean ± standard deviation.

Pharmacokinetic/pharmacodynamic analysis was performed using WinNonlin® 3 Professional Version software, using the sigmoid maximum effect (E_{max}) model. According to the model, the following individual pharmacodynamic parameters were estimated: E_{max} (alfentanil-induced maximum miosis), EC_{50} (concentration of alfentanil producing 50% of E_{max}) and γ (sigmoidicity parameter).

The steady-state hepatic blood clearance of sorbitol [$CL_{H(b)}$] was calculated by commonly used formulae, as described in detail by Molino et al.[25] In patients with liver disease, $CL_{H(b)}$ is considered an estimate of the functionally effective liver parenchymal perfusion.[22,25,29]

Results

The patient's clinical characteristics are presented in table I.

The alfentanil concentration-time and miosis-time curves are depicted in figure 1 and figure 2, respectively. The noncompartmental method of curves analysis was applied for both concentration- and miosis-time curves. Thereafter, pharmacokinetics and miosis pseudo-kinetic parameters were derived, as presented in table II.

A statistically significant linear correlation was found between the alfentanil $AUC_{\infty(p)}$ and the $AUC_{\infty(miosis)}$ ($r = 0.6$, $p < 0.05$) in cirrhotic patients (figure 3). This correlation was even more significant when AUC determinations were limited to the first 2 hours after alfentanil administration ($r = 0.9$, $p < 0.01$). The variability in the $AUC_{2(miosis)}$ (CV = 52%) was about 2-fold lower than that of the $AUC_{\infty(miosis)}$ (CV = 77%) in cirrhotic patients. Beyond 2 hours, the mean plasma alfentanil concentration remained at ≤50 ng/mL (≈20% of the mean maximal concentration) and the mean pupillary response was ≤0.5mm (≈25% of the mean maximal response).

Statistically significant differences were observed for alfentanil clearance (CL) [$p < 0.01$], $AUC_{(miosis)}$ ($p < 0.05$) and alfentanil AUC ($p < 0.05$) when data obtained in cirrhotic patients were compared with those from our previous experiment performed in healthy volunteers (historical control group, study performed with the same design, protocol and data analysis methods). As observed with cirrhotic patients, the correlation between miosis and plasma alfentanil in healthy volunteers was more significant when AUC determinations were limited to the first 2 hours after alfentanil administration [$r = 0.9$, $p < 0.05$ for the $AUC_{2(p)}$ versus $r = 0.8$, $p < 0.01$ for the $AUC_{\infty(p)}$]. In addition, the variability in the $AUC_{2(miosis)}$ among volunteers (CV = 73%) was also lower than that determined for the $AUC_{\infty(miosis)}$ (CV = 105%).

The sigmoid E_{max} model was applied for pharmacokinetic/pharmacodynamic analysis in all cirrhotic patients. Three patients (2 Child-Pugh

Table I. Clinical characteristics and hepatic tests of patients with cirrhosis

Patient no.	Age (y)	Sex	BMI[a]	Child-Pugh score	Plasma albumin (g/dL)	Plasma bilirubin (mg/dL)	INR[b]	Ascites	Antipyrine CL (mL/min/kg) [n = 6]	$CL_{H(b)}$ (mL/min/kg) [n = 6]	Alfentanil free fraction (%) [n = 6]
1	58	Female	31.3	5	3.9	0.9	1.0	No			
2	52	Female	29.2	5	3.8	0.9	1.2	No			
3	65	Male	26.2	6	3.8	1.4	1.1	Yes	0.7	19.8	10.6
4	47	Male	27.7	6	3.8	0.8	1.1	Yes	5.6	30.8	4.8
5	38	Male	25	6	3.9	1.1	1.1	Yes	2	15.5	3.9
6	62	Male	23	6	3.9	1.6	1.4	Yes			
7	56	Female	28	7	3.0	1.5	1.2	Yes	0.4	9	16.2
8	53	Male	25	7	3.7	2.1	1.1	Yes	0.4	11.8	15.7
9	57	Male	25.7	8	2.1	0.7	1.2	Yes			
10	59	Male	31.2	8	3.1	2.0	1.2	Yes			
11	64	Male	29.2	8	3.1	2.4	1.1	Yes			
12	41	Male	23.2	8	3.1	1.1	1.4	Yes	0.9	16.8	18.1
Mean (± SD)	54.3 ± 8.7		27 ± 2.8		3.4 ± 0.6	1.4 ± 0.6	1.2 ± 0.1		1.7 ± 2.2	17.2 ± 7.7	11.6 ± 6.1

a BMI = weight in kg/height in m^2.

b INR = (quick time of patient/quick time of pool blood)^{ISI}.

BMI = body mass index; **CL** = clearance; $\mathbf{CL_{H(b)}}$ = steady-state hepatic blood clearance measured by D-sorbitol; **INR** = international normalised ratio; **ISI** = International Sensitivity Index.

grade A, 1 Child-Pugh grade B) were excluded from pharmacokinetic/pharmacodynamic analysis because of very large CVs in the estimated pharmacodynamic parameters. The following values were observed: $E_{max} = 2.4 \pm 1.3$mm, $EC_{50} = 110.5 \pm 108.9$ ng/mL, $\gamma = 1.8 \pm 1.5$. The differences in pharmacodynamic parameters between cirrhotic patients and the historical control group (with the applied sigmoid E_{max} model) were not found to be statistically significant, suggesting that the pharmacodynamic properties of alfentanil (e.g. miosis) are similar in cirrhotic patients and in healthy subjects (altered opioid receptor sensitivity or density would be expected to alter E_{max} or EC_{50} values).

Alfentanil clearance did not correlate with the alfentanil free fraction. The alfentanil free fraction showed a higher variability in cirrhotic patients (average free fraction = $11.6 \pm 6.1\%$) and was significantly different from that observed in the historical control group (average free fraction = $8.9 \pm 2.8\%$) [$p < 0.05$].

In cirrhotic patients, a highly significant correlation was observed between the alfentanil CL and antipyrine CL ($r = 0.9$, $p < 0.01$) and between the alfentanil CL and $CL_{H(b)}$ ($r = 0.9$, $p < 0.05$) but not between antipyrine CL and $CL_{H(b)}$.

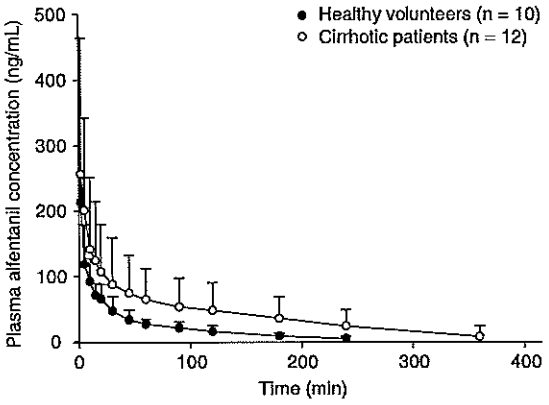

Fig. 1. Alfentanil plasma concentration-time curves in healthy volunteers (historical control group) and cirrhotic patients. The data points represent the mean plasma alfentanil concentration values of subjects with positive error bars (SD).

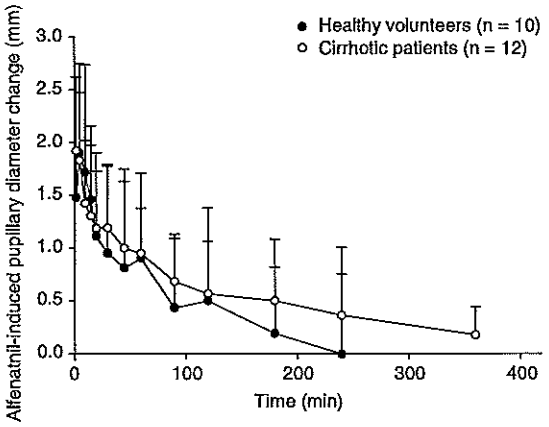

Fig. 2. Alfentanil-induced pupillary size change-time curves in healthy volunteers (historical control group) and cirrhotic patients. The data points represent the alfentanil-induced mean miosis values of subjects with positive error bars (SD).

With regard to side effects, nausea and vomiting occurred in two patients 35 and 60 minutes after alfentanil administration. Three other patients displayed brief blood desaturation at room air (90%, 88% and 87% of oxygen saturation) after the alfentanil bolus, which responded to supplemental oxygen administration. Some patients were slightly sedated during the first hour following alfentanil administration.

Discussion

Our work was designed to study alfentanil-induced miosis as a surrogate noninvasive marker of altered alfentanil pharmacokinetics (CYP3A activity) in cirrhotic patients compared with healthy volunteers.

First, statistically significant correlations were found between miosis and the plasma AUCs in cirrhotic patients. This observation sustains the hypothesis that alfentanil-induced miosis can be used as a surrogate measure of alfentanil pharmacokinetics not only in healthy volunteers but also in patients with mildly or moderately impaired hepatic function. Moreover, the plasma alfentanil and miosis AUC_2 were also highly correlated, and AUC_2 varia-

Table II. Alfentanil pharmacokinetic parameters and pseudo-kinetic parameters of alfentanil-induced miosis in patients with cirrhosis and healthy volunteers (historical control group)

Subject groups	Pharmacokinetic parameters[a]					Miosis pseudo-kinetic parameters[a]		
	$AUC_{\infty(p)}$ (ng • min/mL)	$AUC_{2(p)}$ (ng • min/mL)	CL (mL/min/kg)	$t_{1/2(p)}$ (min)	$V_{ss(p)}$ (mL/kg)	$AUC_{\infty(miosis)}$ (mm • min)	$AUC_{2(miosis)}$ (mm • min)	$t_{1/2(miosis)}$ (min)
Cirrhosis								
Child-Pugh grade A (n = 6)	15 635 ± 9 231	10 092 ± 6 763	1.1 ± 0.8	99.9 ± 40.8	132.1 ± 70.7	372 ± 174	141 ± 53	234.7 ± 198.2
Child-Pugh grade B (n = 6)	19 698 ± 19 323	8 361 ± 6 840	1.6 ± 1.6	182.9 ± 159.5	222.7 ± 114.7	290 ± 218	136 ± 96	245.7 ± 158.0
total (n = 12)	17 667 ± 14 593*	9 227 ± 7 059*	1.4 ± 1.3*	142.2 ± 120.9	177.4 ± 102.5*	340 ± 261*	139 ± 73*	240.2 ± 171.0*
Volunteers (n = 10)	5 106 ± 1 010	3 864 ± 925	2.9 ± 0.6	50.5 ± 9.9	217.8 ± 65.5	153 ± 161	83 ± 61	67.8 ± 34.3

a All values are presented as mean ± SD.

$AUC_{\infty(miosis)}$ = area under the miosis-time curve from time zero to infinity; **$AUC_{\infty(p)}$** = area under the plasma concentration-time curve from time zero to infinity; **$AUC_{2(miosis)}$** = miosis AUC from 0 to 2 hours; **$AUC_{2(p)}$** = plasma AUC from 0 to 2 hours; **CL** = total plasma alfentanil clearance; **$t_{1/2(miosis)}$** = elimination half-life of miosis; **$t_{1/2(p)}$** = elimination half-life of plasma alfentanil; **$V_{ss(p)}$** = apparent volume of alfentanil distribution; * $p < 0.05$ vs volunteers.

bility was lower than AUC_∞ variability in both cirrhotic patients and healthy volunteers. Thus, in practice, the miosis test can be limited to 2 hours after alfentanil administration in patients with mild and moderate cirrhosis and in healthy volunteers. Consequently, hepatic CYP3A activity can be assessed on the basis of the $AUC_{2(miosis)}$.

It has already been demonstrated that alfentanil pharmacokinetics are markedly altered in cirrhosis due, almost exclusively, to its hepatic metabolism.[19,30,31] The alfentanil pharmacokinetics observed in our group of cirrhotic patients were very similar to those published previously.[30,31] Furthermore, significant differences in various pharmacokinetic and miosis pseudo-kinetic parameters were found when data obtained in cirrhotic patients were compared with those in healthy volunteers (historical control group).[15] Thus, and in contrast to what has been reported for several other CYP3A probes (e.g. midazolam, lidocaine [lignocaine]),[32-34] alfentanil pharmacokinetics and miosis pseudo-kinetics can be used to distinguish cirrhotic patients from healthy volunteers.

Second, we compared the alfentanil-induced miosis test with two other quantitative tests of liver function: the antipyrine test, which is influenced by intrinsic liver activity, and the D-sorbitol test, which depends on liver blood flow.[20-23] Based on its hepatic extraction ratio, alfentanil has been classified by several authors as belonging to the group of drugs with an intermediate hepatic extraction coefficient ($E = 0.3–0.6$).[18,19,35,36] In that case, alfentanil plasma (and miosis) clearance should be influenced by both intrinsic liver activity and blood flow, and should translate into statistically significant correlations between alfentanil plasma clearance and antipyrine and sorbitol clearance. Our results do not favour the hypothesis that alfentanil is a low-extraction coefficient drug ($E < 0.3$), as suggested by others.[9,37] The impairment of drug metabolism in liver disease is the result of a combination of func-

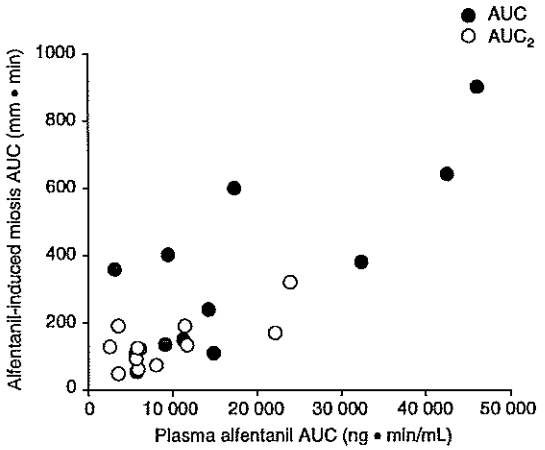

Fig. 3. Relationship between the area under the concentration-time curve (AUC) from time zero to infinity (AUC_∞) and from time zero to 2 hours (AUC_2) of alfentanil-induced miosis and the alfentanil plasma concentration in cirrhotic patients. Each data point represents a single subject ($n = 12$); $r = 0.6$, $p < 0.05$ for the AUC_∞ and $r = 0.9$, $p < 0.01$ for the AUC_2.

tional intrahepatic shunts, which effectively reduce blood flow to the lobules, and a reduced number of normally functioning hepatocytes.[38,39] Many shunts between various branches of the portal vein, hepatic veins and hepatic artery are responsible for reduction in the blood flow through sinusoids and reduced oxygen supply to hepatocytes. Moreover, the loss of endothelial fenestrae, together with the formation of basal lamina beneath the endothelial cells, impair the diffusion of protein-bound substrates. Thus it is difficult to distinguish between the contribution of hepatocyte dysfunction and anatomical disturbances of portal flow to impaired drug metabolism in cirrhotic livers.[38,40]

Since limited adverse effects (slight sedation, nausea, limited blood oxygen desaturation) were observed during the first minutes after alfentanil administration, we suggest that this miosis test must be done under medical control and pulse oximeter monitoring during the first hour after alfentanil administration.

Conclusion

This study demonstrated that miosis kinetics can be used as a surrogate measure of alfentanil pharmacokinetics in cirrhotic patients and thus may reflect CYP3A activity not only in healthy volunteers but also in patients with mild and moderate liver dysfunction. Alfentanil-induced miosis has the advantage of being noninvasive and can be limited to miosis measurements during the first 2 hours after alfentanil administration. We propose to use $AUC_{2(miosis)}$ as an *in vivo* marker of hepatic CYP3A activity.

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