Plasma concentration and protein binding of alfentanil during high-dose infusion for cardiac surgery

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SUMMARY

We have studied plasma protein binding of alfentanil in 10 patients given a mean total dose of 543 μg kg⁻¹ as the principal anaesthetic agent for coronary artery bypass grafting. The mean unbound fraction of plasma alfentanil increased from 0.08 to 0.16 after administration of heparin and to 0.26 after beginning cardiopulmonary bypass (CPB). After CPB until the end of surgery, the unbound fraction decreased to 0.12. These changes in the unbound fraction were associated with significant changes in plasma total and unbound concentrations of alfentanil also. Within the first 1 min of CPB, total alfentanil concentration had decreased by more than the unbound concentration and the decrease observed in the latter disappeared rapidly. From induction of anaesthesia until awakening of the patient, plasma protein binding of alfentanil was related significantly (P = 0.0166) to the serum concentration of orosomucoid (α₁-acid glycoprotein). (Br. J. Anaesth. 1994; 72: 571-576)

KEY WORDS


High-dose alfentanil anaesthesia has been reported for cardiac surgery in several studies [1-7]. It has been suggested [2] that, in patients undergoing coronary artery bypass grafting (CABG) after premedication with lorazepam 80 μg kg⁻¹, a plasma concentration of alfentanil of 1000 μg litre⁻¹ or greater is needed to block the haemodynamic responses to surgical stimuli before bypass. Similarly, Hug and colleagues [5] reported that a mean plasma concentration of alfentanil of 1178 μg litre⁻¹, again with lorazepam 80 μg kg⁻¹ as premedication, was associated with satisfactory anaesthesia and haemodynamic conditions during the pre-bypass phase of CABG. During and after cardiopulmonary bypass (CPB), smaller plasma concentrations of alfentanil might be sufficient for adequate anaesthesia and haemodynamic stability [2].

Drugs exist in plasma in two fractions: a protein-bound and unbound fraction, the latter being the pharmacologically active moiety of the drug. Therefore, in addition to the more generally reported total drug concentration in plasma, it is important to consider the unbound concentration also [8]. This is important particularly in cardiac surgical patients, as several factors may affect plasma protein binding of drugs [9,10]. Furthermore, determination of unbound alfentanil concentrations at a higher dose range may be of interest as binding sites for alfentanil on plasma proteins may become saturated [11]. Therefore, we have measured plasma protein binding of alfentanil in patients undergoing CABG under such anaesthesia. Because plasma protein binding of alfentanil has been determined previously only immediately before, during and after CPB [10], we have extended the study from induction of anaesthesia until awakening.

PATIENTS AND METHODS

We studied 10 patients (nine males) undergoing elective CABG. The study was approved by the Ethics Committee of the Department of Anaesthesia and informed consent was obtained from each patient.

On the morning of surgery, the patients received their regular doses of β-adrenergic blockers, calcium channel blockers and long-acting nitrates. The patients were premedicated with lorazepam 80 μg kg⁻¹ i.m. at least 1 h before arrival in the operating theatre. Before induction of anaesthesia, a peripheral venous catheter and a radial arterial catheter were inserted under local anaesthesia.

Anaesthesia

Anaesthesia was induced with alfentanil (Rapifen, Orion Pharmaceutica, Espoo, Finland) 120 μg kg⁻¹ given within 10 min with pancuronium (Pavulon, NV Organon, Oss, Holland) 0.1 mg kg⁻¹. Thereafter, the maintenance infusion of alfentanil 1.2 μg kg⁻¹ min⁻¹ was started and continued at this rate until the start of surgery. After tracheal intubation, the lungs were ventilated mechanically with a mixture of oxygen in air (F₁O₂ = 0.5). Normocapnia was maintained and assessed continuously by measurement of end-tidal carbon dioxide concentration and intermittently by arterial blood-gas analyses.
Before the start of surgery, alfentanil 240 μg kg⁻¹ was given and the rate of the alfentanil infusion was increased to 3.6 μg kg⁻¹ min⁻¹. Alfentanil was infused at this rate until the hypothermic phase of CPB (nasopharyngeal temperature 28 °C), when the infusion was discontinued. The infusion was continued again, at a rate of 1.8 μg g⁻¹ min⁻¹, from the start of the rewarming phase of CPB until the end of surgery. Before and after CPB, anaesthesia was supplemented with enflurane or halothane when required and during CPB with diazepam 5–15 mg. Throughout anaesthesia and surgery, pancuronium 1–2 mg was given for neuromuscular block, as needed.

**Cardiopulmonary bypass**

The aorta and the superior and inferior venae cavae (nine patients) or the right atrium (one patient) were cannulated for CPB. A left ventricular decompression tube was introduced through a pulmonary vein. CPB was conducted with a semi-occlusive roller pump (Stöckert, Munich, Germany), to produce non-pulsatile flow and a bubble oxygenator (S 100 A, Shiley, Irvine, CA, U.S.A.). The CPB circuit was primed with Ringer's acetate solution 35 ml kg⁻¹ containing heparin 500 u. For systemic anticoagulation, the dose of heparin was adjusted according to the heparin dose-activated coagulation time (ACT) response curve [12]. Thus, the first dose of heparin was 200 u. kg⁻¹ and subsequent doses were given to maintain the ACT value longer than 480 s during CPB. During the cooling and rewarming phases of CPB, the pump flow was 2.4 litre min⁻¹ m⁻² and during the hypothermic phase 1.8 litre min⁻¹ m⁻². Topical myocardial cooling and cold crystalloid potassium cardioplegia solution (Plegisol, Abbott, North Chicago, IL, U.S.A.) were used for myocardial protection during aortic cross-clamping. The cardioplegia solution was given at 20-min intervals. After CPB, heparin was neutralized by protamine 1.3 mg for each calculated 100 u. of heparin circulating in the blood [12]. Protamine was mixed in 100 ml of isotonic saline and infused within 15 min. Blood flow concentration of haemoglobin was maintained greater than 80 g litre⁻¹ by giving packed red cells as needed and mannitol 15 g was given to enhance urine output. Mean arterial pressure was maintained between 40 and 80 mm Hg; phenylephrine (increments of 200 μg) was given to increase arterial pressure when needed, while phenotolamine (increments of 1–2 mg) or sodium nitroprusside (50 μg min⁻¹ or more) was administered to decrease arterial pressure. Before weaning from CPB, patients were rewarmed to a nasopharyngeal temperature of at least 36.5 °C. Residual blood remaining in the extracorporeal circuit was collected and transfused to the patient after CPB.

After surgery, the degree of neuromuscular block was assessed with the aid of a peripheral nerve stimulator and pancuronium was antagonized with neostigmine when required. The patients were transported to the recovery room for postoperative observation. The time to return of appropriate response to the command: "Move your legs and arms!" and to the question: "Do you feel any pain?" was defined as the awakening time. For evaluation of intraoperative awareness and recall of intraoperative events, patients were interviewed on the first and seventh days after operation.

Blood samples were collected from the radial artery at the following times: before induction of anaesthesia; 45 min after induction of anaesthesia, but before the start of surgery; 45 min after the start of surgery, but before administration of heparin (in five patients only); immediately before CPB, but 15 min after administration of heparin; 1 min after commencement of CPB, after achievement of the hypothermic phase of CPB, but before discontinuation of the alfentanil infusion; during the hypothermic phase, but immediately before the start of rewarming and re-institution of the alfentanil infusion; at termination of CPB; at the end of surgery (after skin closure, when administration of protamine had been concluded); and at awakening of the patient.

Blood samples were obtained for measurement of packed cell volume (PCV) and concentrations of serum total protein, albumin and orosomucoid (α₁-acid glycoprotein) and plasma alfentanil. Samples for measurement of total protein, albumin and orosomucoid were taken into glass tubes. Blood for measurement of plasma concentration of alfentanil was collected into pre-chilled heparinized tubes, placed on ice and centrifuged within 20 min. Plasma was stored at −70 °C until assayed.

**Protein binding**

**Ultrafiltration.** The unbound fraction of plasma alfentanil in blood samples was separated by centrifugation (10 min at 1500 × g) using ultrafiltration tubes (a micropartition system MPS-1 with YMT ultrafiltration membrane, Centrifree, Amicon Division, WR Grace & Co, Danvers, CO, U.S.A.) according to the manufacturer's directions (manual for *in vitro* diagnostic use, Amicon). The procedure was performed at room temperature (25 °C) and with no pH adjustment, with the possible influences of temperature and pH on the unbound fraction of alfentanil being explored in a separate evaluation with plasma obtained from healthy volunteers, as described in detail below. In addition, the effectiveness of the ultrafiltration method to separate the unbound fraction was compared with that of equilibrium dialysis in plasma from healthy volunteers.

**Effect of pH and temperature.** Plasma samples from healthy volunteers were mixed with [H]alfentanil and dissolved in ethanol to obtain a final concentration of alfentanil of 200 μg litre⁻¹ in plasma. pH adjustment was made by adding 100 μl of sodium phosphate buffer 1 mol litre⁻¹ (pH 7.03), per milli- litre of plasma, resulting in a final pH 7.4. In the control samples (no pH adjustment) 100 μl of distilled water was used instead of buffer. After pH adjustment or addition of water, 1.1 ml of the fortified plasma samples were pipetted into the ultrafiltration tubes, which were incubated at room temperature or at 37 °C in a water bath for 30 min. After incubation, the ultrafiltration units were centrifuged for 10 min at 1500 × g, producing approximately 200 μl of ultrafiltrate. Centrifugation was performed at room temperature using a fixed angle.
rotor. The pH of plasma samples was measured immediately before incubation.

**Dialysis technique.** Plasma samples mixed with [3H]alfentanil were subjected to equilibrium dialysis also against a Sörensens phosphate buffer 0.067 mol litre⁻¹, pH 7.17, at 36 °C, using a Dianorm system with 20 identical macro-1 Teflon cells (Diachema). Spectrapor-2 membranes (cut-off 12000–14000 Da) were boiled in distilled water and tightened between two compartments which were filled with 1.0-ml aliquots of plasma and buffer, using glass pipettes. Cells were rotated at 20 rpm in a Heto thermostat bath at 37 °C. The pH of all dialysed plasma samples was measured immediately after dialysis.

Radioactivity measurements were made in a liquid scintillation spectrometer (Packard 200 CA or 4530) with automatic calculation from cpm to dpm, using Ultima Gold as scintillation cocktail.

**Alfentanil and protein concentrations**

Plasma and ultrafiltrate concentrations of alfentanil in the patient samples were measured by capillary gas chromatography with a nitrogen-sensitive detector [4]. During the preliminary phase of the study, it appeared that diltiazem and its main metabolite, desacetyl diltiazem, eluted at the same retention time as alfentanil. Therefore, patients receiving regular medication with diltiazem were not included in the study. Other drugs used regularly by the patients or given as premedication did not interfere with the analysis. The detection limit of the assay was 1 µg litre⁻¹.

Plasma total protein concentration was determined by a biuret reaction method (coefficient of variation 0.9 %) and plasma albumin concentration by a biuret reaction method (coefficient of variation 0.9 %) using a Hitachi 737 analyser. An immunoturbidimetric method with N-protein-standard serum (Behringwerke AG, Marburg, Germany) was used for measurement of plasma concentration of orosomucoid using a Monarch 2000 Chemistry System analyser.

**Statistical analysis**

Analysis of variance for repeated measures [13] was used to detect significant change over time in the humoral variables. The Bonferroni correction was applied for subsequent multiple comparisons. Analysis of covariance for repeated measures [13] was used to define the relationships between the ratio of bound to unbound alfentanil concentration in plasma and blood PCV and serum protein concentrations (total protein, albumin and orosomucoid). Linear regression analysis was used to test the relationship between the time to awakening and plasma concentration of total or unbound alfentanil at the end of the alfentanil infusion and also the total dose of alfentanil administered. P < 0.05 was considered statistically significant. Data are expressed as mean (range or SD).

**RESULTS**

**Comparison of ultrafiltration and equilibrium dialysis**

The results obtained using ultrafiltration and equilibrium dialysis were similar (table I). Binding amounted to 92.2 %, determined by ultrafiltration, and to 90.8 % determined by equilibrium dialysis. These data are comparable with those reported previously [14]; pH (range 7–8) had only a minor influence on the binding of alfentanil and it was not affected by the temperature at which ultrafiltration was performed.

**Patient study**

Patient characteristics before operation and intraoperative data are shown in table II. The total dose of alfentanil administered was 949 (767–1076) µg kg⁻¹. Plasma concentrations of total and unbound alfentanil and the unbound fraction of alfentanil, PCV and serum protein concentration data are summarized in table III.

With the increase in alfentanil dose from the sample before surgery to the sample before bypass, there was also an increase in the concentration of both total and unbound alfentanil (P < 0.001). The fraction of unbound alfentanil increased also (P < 0.01). In the five patients in whom the additional sample was obtained before administration of heparin, the concentrations of total and unbound alfentanil were 1121 (983–1230) µg litre⁻¹ and 93 (63–130) µg litre⁻¹ before heparin and 1007 (907–1119) µg litre⁻¹ and 155 (133–196) µg litre⁻¹ after heparin, respectively (P < 0.05). In these five patients, the unbound fraction of alfentanil increased from 0.08 (0.05–0.11) to 0.16 (0.12–0.22) (P < 0.05) with administration of heparin.

Initiation of CPB decreased total alfentanil concentration abruptly (P < 0.001), but the concentra-

**Table I. Effects of separation method, temperature and pH on plasma protein binding of alfentanil at a concentration of 200 µg litre⁻¹. Mean (SD) of five experiments**

<table>
<thead>
<tr>
<th>Method</th>
<th>Temperature (°C)</th>
<th>pH</th>
<th>Alfentanil bound (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrafiltration</td>
<td>25</td>
<td>8.09 (0.04)</td>
<td>92.5 (1.0)</td>
</tr>
<tr>
<td>Equilibrium dialysis</td>
<td>25</td>
<td>7.41 (0.02)</td>
<td>92.8 (1.1)</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>7.41 (0.01)</td>
<td>92.2 (1.2)</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>7.42 (0.01)</td>
<td>90.8 (1.3)</td>
</tr>
</tbody>
</table>

**Table II. Patient characteristics before operation and operative data for 10 study patients (mean (range) or No. of patients). *From induction of anaesthesia to the end of surgery**

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>Ejecction fraction of left ventricle</th>
<th>Regular drug therapy</th>
<th>β adrenergic blocker</th>
<th>Calcium channel blocker</th>
<th>Long-acting nitrate</th>
<th>Duration of anaesthesia* (min)</th>
<th>Duration of CPB (min)</th>
<th>Aortic cross-clamp time (min)</th>
<th>Hypothermic interval of CPB (min)</th>
<th>Number of distal grafts</th>
<th>Inhalation anaesthetic given before CPB</th>
<th>Inhalation anaesthetic given after CPB</th>
<th>Vasoconstricor given during CPB</th>
<th>Vasodilator given during CPB</th>
</tr>
</thead>
<tbody>
<tr>
<td>58 (52–68)</td>
<td>83 (59–104)</td>
<td>176 (165–187)</td>
<td>0.63 (0.35–0.75)</td>
<td>8</td>
<td>6</td>
<td>8</td>
<td>33 (1–5)</td>
<td>297 (218–375)</td>
<td>97 (48–163)</td>
<td>47 (20–79)</td>
<td>37 (17–65)</td>
<td>3.3 (1–5)</td>
<td>9</td>
<td>7</td>
<td>3</td>
<td>8</td>
</tr>
</tbody>
</table>
tration of unbound alfentanil did not change as markedly ($P < 0.05$), because the onset of CPB increased the unbound fraction of alfentanil ($P < 0.01$). Both total and unbound concentration of alfentanil increased ($P < 0.05$) compared with the sample obtained immediately after commencement of CPB until the hypothermic phase (10 (5–14) min after onset of CPB). Discontinuation of the infusion of alfentanil after achievement of hypothermia decreased the plasma concentration of both total ($P < 0.001$) and unbound ($P < 0.001$) alfentanil. During the rewarming period (i.e. from the start of rewarming until the end of CPB), when the infusion of alfentanil had been reinstituted, concentrations of alfentanil remained stable. Compared with the sample obtained 1 min after the start of CPB, the unbound fraction of alfentanil remained unchanged during the whole duration of CPB. Up to the end of surgery, total concentration of alfentanil increased significantly ($P < 0.001$) from that measured at the end of CPB. Simultaneously, unbound alfentanil concentration decreased, but this decline was not significant. Concomitantly, the unbound fraction of alfentanil decreased by 50% ($P < 0.01$). At the time of awakening, the total concentration of alfentanil decreased ($P < 0.01$) to less than that measured during anaesthesia before surgery, but the unbound concentration of alfentanil was only slightly less than that measured before surgery. At awakening, the unbound fraction of alfentanil was not changed from that calculated at the end of surgery.

PCV and serum protein concentrations decreased during the period before bypass. After commencement of CPB, these values decreased significantly ($P < 0.001$), but then remained unchanged during the whole CPB period. After discontinuation of CPB, PCV and plasma protein concentrations increased ($P < 0.01$ or $P < 0.001$) from those measured during CPB, but remained at a lower concentration ($P < 0.001$) than that observed before CPB.

The ratio of bound to unbound alfentanil concentration was related significantly to serum orosomucoid concentration ($P = 0.0166$), but not to PCV ($P = 0.55$), serum total protein ($P = 0.23$) or albumin ($P = 0.17$) concentration.

Total and unbound alfentanil concentration in plasma at discontinuation of the alfentanil infusion and the total dose of alfentanil administered did not correlate significantly with the period from the end of alfentanil infusion to awakening. All patients were adequately awake 2.8 (0.5–4.4) h after discontinuation of the alfentanil infusion. No patient reported awareness during operation or any recall of intraoperative events.

**DISCUSSION**

Variable rate infusion of alfentanil was chosen for the present study because different plasma concentrations of alfentanil appear to be required for adequate anaesthesia and haemodynamic stability during various phases of CABG [2]. After induction of anaesthesia but before surgery, the mean total plasma concentration of alfentanil was 380 $\mu$g litre$^{-1}$. During surgery, before CPB, the mean concentration was approximately 1100 $\mu$g litre$^{-1}$ before administration of heparin and 950–1000 $\mu$g litre$^{-1}$ after heparin.

Because cardiac surgery is associated with marked changes in plasma protein binding of drugs [9], the unbound concentration of alfentanil, in parallel with its total concentration, might be of interest during such a procedure. In *vitro*, the binding sites for alfentanil to plasma proteins become saturated at alfentanil concentrations greater than 1000 $\mu$g litre$^{-1}$ [11]. In patients undergoing CABG surgery, Kumar and colleagues [10] reported plasma unbound concentration of alfentanil during a fixed rate infusion of alfentanil in the low to moderate dose range. Immediately before, during and immediately after CPB, they reported mean unbound alfentanil concentrations of 30–35 $\mu$g litre$^{-1}$. In contrast, in the present study, we have found greater concentrations (90–150 $\mu$g litre$^{-1}$) which have not been reported previously in surgical patients.

The unbound fraction of alfentanil, calculated in our patients during anaesthesia but before surgery, was similar to that reported previously by others in healthy volunteers [11, 14] and in non-cardiac surgical patients [15, 16]. As assessed in five of our patients, about 45 min after plasma total alfentanil concentration had been increased from 380 to approximately 1000 $\mu$g litre$^{-1}$ during surgery, the unbound fraction of alfentanil remained unchanged. This appears to indicate that, in our patients, the capacity of plasma proteins to bind alfentanil was not limited. It remains to be determined if the binding
sites for alfentanil to plasma proteins become saturated at very large plasma concentrations of total alfentanil, such as 3000 μg litre⁻¹ (reported in some studies [5, 7]).

The unbound fraction of alfentanil in our patients after administration of heparin was 0.16, which is identical to the mean value reported by Kumar and colleagues [10] immediately before the start of CPB. In five of our patients, the unbound fraction of alfentanil could be calculated both immediately before and after administration of heparin, when alfentanil had been infused at an unchanged rate for more than 1 h. In these patients the unbound fraction of alfentanil increased after heparin. Administration of heparin has been reported to increase the unbound fraction of various other drugs [17, 18], a phenomenon attributed to activation of lipoprotein lipase by heparin and a subsequent increase in the concentration of free fatty acids with the displacement of the drug from its binding sites [19]. There is no definite consensus as to whether or not this finding is an in vivo phenomenon or, for the major part, an in vitro artefact, in which case the cascade is initiated in vivo and continues in vitro in the collecting tube after collection of the blood sample [19, 20]. As assessed in five of our 10 patients, concomitantly with the increase in the unbound fraction of alfentanil after administration of heparin, there was a decrease in the total concentration of alfentanil and an increase in its unbound concentration. If the increase in the unbound fraction was an in vitro artefact only, a decrease in the total concentration of alfentanil in plasma would not be expected, particularly as the distribution and binding of alfentanil to red cells is low [11, 14]. Thus, our observation appears to suggest that the increase in the unbound fraction of alfentanil after heparin is, at least to a considerable extent, a true in vivo process. After CPB until the end of surgery, when protamine had been administered to neutralize heparin, the unbound fraction of alfentanil decreased. Again, the concomitant increase in the total concentration of alfentanil does not fit an explanation based on an in vitro phenomenon only. A shift of alfentanil from tissues to plasma must have occurred, although the increase observed in serum protein concentrations after CPB is a confounding factor in determining the contribution of protamine to the changes observed.

The commencement of CPB increased further the unbound fraction of alfentanil from its value of 0.16 before bypass and after heparin to 0.26. This was caused by the dilution of the binding protein concentration in plasma by the protein-free priming solution of the CPB circuit. Kumar and colleagues [10] noticed also doubling of the unbound fraction of alfentanil with the start of CPB. The increase in the unbound fraction at initiation of CPB has been demonstrated also for various other drugs used in patients undergoing cardiac surgery [21, 22]. In the present study, the unbound fraction of alfentanil remained stable for the whole duration of CPB.

The initiation of CPB decreased plasma total alfentanil concentration. However, after the first 1 min of CPB, as long as the infusion rate of alfentanil was maintained unchanged, the unbound concentration was not smaller than that measured before the start of CPB. A similar lack of effect of CPB on unbound drug concentration has been demonstrated previously for alfentanil [10], thiopentone [21, 23] and methohexitone [21]. These studies suggest that, in spite of the decrease in total drug concentration in plasma that accompanies connecting the patient to the extracorporeal circuit, the concentration of the active moiety of the drug remains unchanged. Therefore, the level of anaesthesia may not become lighter.

Orosomucoid (α₁-acid glycoprotein) is the principal binding protein for alfentanil in plasma; albumin is responsible for binding to a lesser degree [14]. Accordingly, throughout our study period, we did observe a significant relationship between the binding of alfentanil and serum orosomucoid concentration, whereas serum albumin and total protein concentrations were not related significantly to alfentanil binding. The time to awakening after discontinuation of the alfentanil infusion in the present study was 2.8 (0.5–4.4) h. This is comparable with values reported with the high-dose alfentanil technique for CABG surgery [2, 4, 6, 7].

In conclusion, there exists great variability in plasma protein binding of alfentanil during the various phases of coronary bypass operation. During cardiopulmonary bypass, the unbound fraction of alfentanil almost tripled from its value before surgery, associated with administration of heparin and haemodilution. However, by the end of operation, the unbound fraction of alfentanil had returned to near its value before surgery.

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REFERENCES