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SHORT COMMUNICATION

Quantitative measurement of blood remifentanil concentration: development of a new method and clinical application

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Abstract We have developed a new detection method of blood remifentanil concentration using a gas chromatography-mass spectrometry(GC–MS) with fentanyl as the internal standard(IS). The detection was performed at *m*/*z* 168 and 245 for remifentanil and fentanyl, respectively. In addition, the retention times of remifentanil and fentanyl were 5 min 45 s and 6 min 51 s, respectively. The standard curve of relationship between remifentanil concentration and the ratio of the peak area of remifentanil to fentanyl was satisfactorily fitted as linear regression ($R^2 = 0.998$, $p < 0.01$). Intra- and inter-assay CV was 10.5 and 11.5 %, respectively. In the clinical setting, 21 adult patients undergoing elective surgery under propofol–remifentanil TIVA were enrolled. To determine blood remifentanil concentrations, arterial blood was obtained at 0–30 min after cessation of remifentanil infusion at 0.2 μg/kg/min. Blood samples were given into sample tubes(chilled on ice) containing citric acid 50 % 60 μl which inactivates all esterase, and then stored at −20 °C until assay. Measured blood remifentanil concentration was 3.59 ± 0.74 ng/ml at the end of remifentanil infusion, and the ime for a decrease in blood remifentanil concentration by half was ∼2 min. Remifentanil concentration was below the detection limit 30 min after the cessation. Thus, we have confirmed that this new method is clinically applicable.

Keywords Remifentanil · Fentanyl · Blood concentration · Gas chromatography–mass spectrometry

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Remifentanil is a short-acting opioid in widespread clinical use with a continuous infusion pump as an adjunct to general anesthesia. This agent provides a rapid onset and offset of action by its short blood-effect site equilibration and large clearance. Thus, this agent is ideal for a target-controlled infusion (TCI). However, as a remifentanil TCI pump is not commercially available, an open TCI pump is generally used as a remifentanil TCI pump for research purposes in Japan. To ensure optimal performance of the TCI pump, the most appropriate pharmacokinetic model should be established although the Minto model using blood concentrations [1] is the most popular. In this regards, simultaneous detection of blood remifentanil concentration should be performed. In addition, the actually measured blood concentration of remifentanil must be required to precisely analyze additional/synergistic effects of remifentanil on pharmacokinetics/pharmacodynamics of anesthetic agents. Thus, we have developed a new quantitative method to determine remifentanil concentration using a gas chromatography-mass spectrometry (GC–MS) with fentanyl as the internal standard. In addition, we have also measured blood remifentanil concentrations in the clinical setting.

Remifentanil (Janssen Pharmaceutical KK, Tokyo) was dissolved in saline to make final concentrations of 1.57, 3.13, 6.25, 12.5, 25, 50 and 100 ng/ml; 1 ml of this solution was added into a tube containing 50 ng fentanyl as an internal standard, 4.9 ml of heptane and 0.1 ml of isoamyl alcohol, which was vortexed for 1 min, centrifuged at 2,000×*g* for 10 min. The supernatant 4.5 ml was transferred into a glass tube, and evaporated to dryness in a vacuum oven. The extract was derivatized twice by the addition of 100 % methanol 0.4 and 0.2 ml. The derivatized extract 0.6 ml was evaporated to dryness again under a stream of clean nitrogen on a heatblock at 50 °C, and

then reconstructed with 50 μl of acetonitrile/methanol (1:9) solution. The final sample 3 μl was injected into the GC–MS (AUTOMASS GC–MS system, JEOL, Tokyo, Japan). The GC was equipped with a InerCap17MS capillary column, 0.25 mm × 15 m (GL Sciences Inc, Tokyo, Japan). The oven temperature was held at 200 °C at first, then raised at 18 °C/min up to 270 °C. The GC injection port was set at 235 °C. Helium was used as a carrier gas under a constant pressure of 20 psi. The mass detector operated in electron ionization at 70 eV. Determination of remifentanil and fentanyl were performed on the basis of their abundance and mass-to-change ratio (*m*/*z*). High mass ions were selected for their reproducibility and lack of interference. The *m*/*z* of remifentanil and fentanil were 168 and 245, respectively. In addition, the retention time of remifentanil and fentanil were 5 min 45 s and 6 min 51 s, respectively (Fig. 1a). The relationship between remifentanil concentration and the ratio of the peak area of remifentanil to fentanyl was examined by linear regression analysis

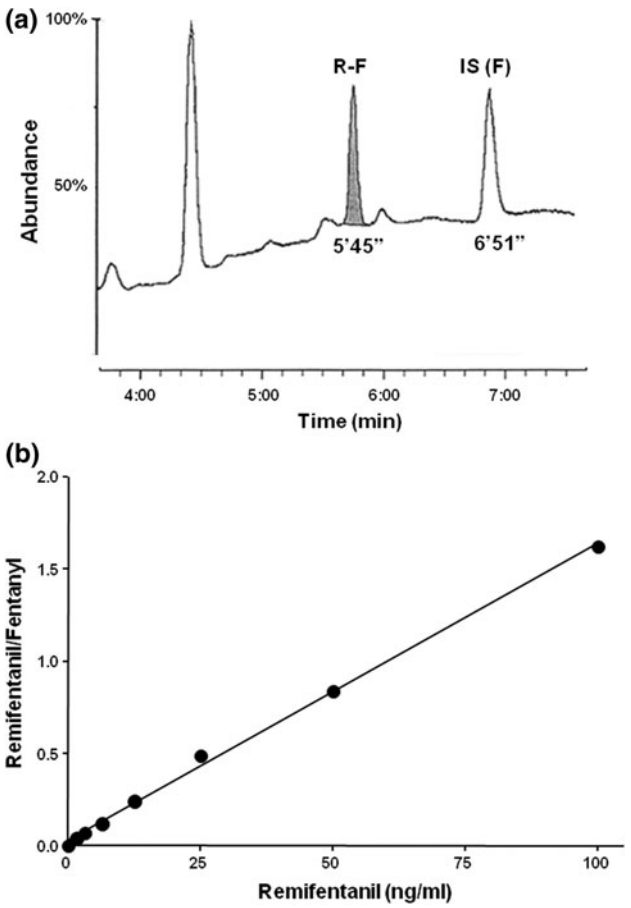

Fig. 1 **a** GC–MS ion chromatography of blood sample containing remifentanil (R–F) and internal standard (IS: Fentanil (F)). **b** A significant linear correlation between measured remifentanil concentration and peak area ratio (remifentanil/fentanyl): $Y = 0.0161X + 0.0268$, $R^2 = 0.998$, $p < 0.01$

(GraphPad Prism 3.0, GraphPad Software Inc, San Diego, USA).

In the clinical setting, the protocol was approved by our University ethics committee on September 28, 2007 (Ref 2007-077), and written informed consent was obtained from all patients. Twenty-four adult patients without either renal dysfunction, anemia or low plasma cholinesterase undergoing elective surgery were enrolled. Patients were also excluded from the study when patients' hematocrit decreased to less than 25 % or required blood transfusion because of blood loss during surgery. Anesthesia was induced with propofol 1–1.5 mg/kg, ketamine 0.5 mg/kg and remifentanil 0.3 μg/kg/min. Following muscle relaxation with iv rocuronium, 0.6 mg/kg endotracheal intubation was performed. Anesthesia was maintained with propofol 5–10 mg/kg/h, remifentanil 0.2 μg/kg/min and morphine 5–10 mg. Muscle relaxation was maintained with intermittent rocuronium. The propofol infusion rate was changed to maintain the bispectral index (BIS) between 40 and 50 during anesthesia. All patients were premedicated orally with diazepam 4–10 mg and roxatidine 75 mg 90 min before induction of anesthesia. To determine blood remifentanil concentrations, arterial blood (3 ml) was drawn from a radial artery catheter at 0 and 1, 3, 5, 7, 10, 15, 20 and 30 min after termination of remifentanil infusion at 0.2 μg/kg/min. Each blood sample was given into a sample tube (chilled on ice) containing citric acid 50 % 60 μl which inactivates all esterase, and then stored at −20 °C until assay. At the assay, the thawed sample 1 ml was mixed with fentanyl 50 ng and 4.9 ml of heptane and 0.1 ml of isoamyl alcohol in a tube. And then GC–MS analysis was performed as described above. Data are presented as mean ± SD. Statistical analysis was performed using the paired *t* test or the Mann–Whitney *U* test, as appropriate.

There was a significant correlation between remifentanil concentration and the ratio of the peak area of remifentanil to fentanyl ($Y = 0.0161X + 0.0268$, $p < 0.01$, $R^2 = 0.998$, Fig. 1b). Intra- and inter-assay CV was 10.5 and 11.5 %, respectively, by 6 experiments. The limit of determination was 0.1 ng/ml.

In the clinical setting, 3 patients were excluded from the study because of blood loss causing less than 25 % hematocrit level. The remaining 21 patients were studied and their demographic characteristics are as follows: male/female = 13/8, age = 60.7 ± 13.1 years, height = 161.3 ± 6.0 cm, body weight = 60.5 ± 12.2 kg, duration of anesthesia = 216 ± 73 min, surgery types = eye surgery ($n = 1$), breast surgery ($n = 1$), thyroid surgery ($n = 1$), otorhinolaryngological surgery ($n = 3$), gynecological surgery ($n = 1$), urological surgery ($n = 7$), gastroenterological surgery ($n = 7$), fluid infusion = 1615 ± 1014 ml, blood loss = 237 ± 342 g, urine output = 346 ± 451 ml. The mean

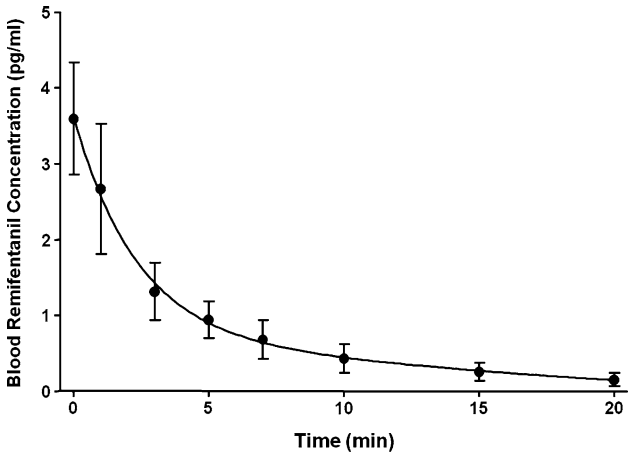

Fig. 2 Changes in blood remifentanil concentrations after cessation of its continuous infusion at 0.2 μg/kg/min. Data are mean ± SD

concentration-time curve for remifentanil after cessation of remifentanil infusion is shown in Fig. 2. Measured blood remifentanil concentration was 3.59 ± 0.74 ng/ml at the end of remifentanil infusion. Concentration of remifentanil was below the detection limit 30 min after cessation of infusion.

In the in vitro study, as the standard curve was satisfactorily fitted with R^2 of 0.998, we have successfully established a new measurement method of remifentanil using fentanyl as an internal standard. In addition, we have also confirmed that this new method could clinically be applied for measurement of blood concentration of remifentanil in the clinical setting. Figure 2 shows that time for a decrease in blood remifentanil concentration by half could be ∼2 min after the cessation of its infusion. Similarly, a previous report shows that the context-sensitive half-time of remifentanil is 2–5 min [2].

In conclusion, our new method for determination of blood remifentanil concentration using GC–MS with fentanyl as an internal standard could clinically be applicable.

References

1. Minto CF, Schnider TW, Egan TD, Youngs E, Lemmens HJ, Gambus PL, Billard V, Hoke JF, Moore KH, Hermann DJ, Muir KT, Mandema JW, Shafer SL. Influence of age and gender on the pharmacokinetics and pharmacodynamics of remifentanil. I. Model development. Anesthesiology. 1997;86:10–23.
2. Michelsen LG, Hug CC Jr. The pharmacokinetics of remifentanil. J Clin Anesth. 1996;8:679–82.