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SHORT COMMUNICATION

Propofol increases the rate of albumin-unbound free midazolam in serum albumin solution

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Abstract Propofol and midazolam have a synergistic anesthetic action. One of the reasons for this is thought to be the inhibitory effect of propofol on midazolam metabolism. However, because both drugs bind strongly to serum protein, their interaction may not only involve the effects of propofol on midazolam metabolism, but may also involve propofol's effects on serum protein-binding. Against this background, we investigated the characteristics of midazolam binding to serum albumin, and evaluated the effects of both propofol and ketamine on this binding. Midazolam was added to a serum albumin solution with propofol or ketamine, and, after incubation for 1 h, albumin-free solution was separated from the sample and the midazolam concentration was measured using a high-performance liquid chromatography system. The albumin-unbound rate of midazolam was evaluated and compared with the rate in the control solution (only midazolam). Propofol significantly raised the rate of albumin-unbound free midazolam, while ketamine had no effect on the binding of midazolam to serum albumin. These findings suggest that the increase in albumin-unbound free midazolam brought about by propofol is involved in the synergistic effect of these two agents.

Keywords Midazolam · Propofol · Pharmacokinetics · Protein-binding

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Some anesthetics are often administered together during general anesthesia and sedation. However, it has been pointed out that unexpected synergistic actions and adverse effects are induced by some of these combinations [1]. Pharmacokinetic and/or pharmacodynamic factors are responsible for the synergistic action when two or more intravenous drugs are administered together. As the pharmacokinetic factor responsible for such synergistic actions, attention has been paid to the effect of each agent exerted on the metabolism of the other agent, such as via the activity of CYP cytochrome P450 (CYP). However, drug interactions not only involve drug metabolism but also affect serum protein-binding.

Propofol and midazolam are commonly employed intravenous anesthetics and are used together in some cases. On investigating interactions in anesthetic effects between midazolam and propofol, a synergistic action has been demonstrated [2–4]. As propofol has been found to decrease the clearance of midazolam by inhibiting cytochrome P450 (CYP) [5], it appears that propofol's inhibition of midazolam metabolism may be the primary reason

for the synergistic action of these two agents. However, both propofol and midazolam bind strongly to serum protein, and the rate of their protein-unbound free fraction was found to be very low [6–8], so even a small change in circulating protein-unbound free midazolam can result in a significant clinical effect [9]. The clinical blood concentration of propofol is usually higher than that of midazolam. If the binding site for propofol in serum protein is the same as that for midazolam, it is possible that propofol inhibits midazolam binding to serum protein, resulting in an elevation of the level of protein-unbound free midazolam in the circulation. Based on in vitro data, protein-binding should be taken into consideration in predicting the effects of drugs [9]. Accordingly, in the present study, we evaluated the effect of propofol on the binding of midazolam to serum albumin, and we also evaluated this effect of ketamine, as another anesthetic.

Human serum albumin was purchased from Wako Pure Chemical Industries (Osaka, Japan) and diluted with 0.9% sodium chloride (Wako Pure Chemical Industries) to a final concentration of 4%. Serum albumin solution was used as the sample for the midazolam binding tests.

The concentration of midazolam in the samples was measured with a high-performance liquid chromatography (HPLC) system (Shimazu, Tokyo, Japan). The mobile phase consisted of 0.01 M potassium dihydrogen phosphate solution and acetonitrile (67:33). The flow rate was 1.0 ml/min, and separation was achieved at 40°C with a reversed-phase column (TSK-GEL ODS-80Ts; Tosoh, Tokyo, Japan). The mobile phase was monitored at 214 nm using an SPD-10Avp UV–VIS detector (Shimazu).

To the sample, diazepam (Wako Pure Chemical Industries) was added as an internal standard (IS). Following liquid–liquid extraction with diethyl ether, the extracted sample was injected into the HPLC column.

The peak-area ratio of midazolam to IS was obtained from the HPLC chromatogram at each concentration, and a calibration curve for midazolam was constructed. The calibration curve of standard midazolam (Cambridge Isotope Laboratories, Andover, MA, USA) at concentrations between 1.25 and 100 ng/ml in serum albumin solution was linear (Fig. 1, correlation coefficient > 0.999). The detection limit for midazolam was 0.50 ng/ml, and 1.25 ng/ml could be quantified with acceptable precision.

The albumin-free solution at each concentration was separated from a sample using centrifugal filter units (Centrifree®; Millipore, Billerica, MA, USA), which can separate free solutes from protein-bound micro-solutes of a biological solution with centrifugation at 2,000*g* and filtration for 1 h. The recovery rate of midazolam through the employed device (Centrifree®) was 99.6 ± 2.6%, and leakage of serum albumin on filtration was 0.012 ± 0.001%.

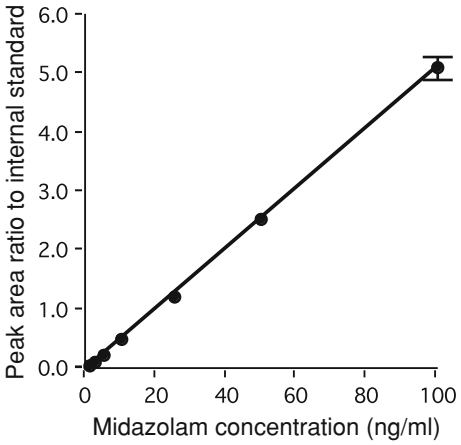

Fig. 1 Calibration curve of midazolam at concentrations between 1.25 and 100 ng/ml in serum albumin solution ($n = 6$). The data are presented as means and standard deviation

Midazolam was added at a final concentration of 100 ng/ml to serum albumin solution and the solution was incubated at 37°C in a glass tube for 1 h with propofol (MP Biomedicals, Eschwege, Germany) at a final concentration of 1 or 5 μg/ml; in the experiments with ketamine (Ketalar; Daiichi Sankyo, Tokyo, Japan) this agent was added to the serum albumin solution at a final concentration of 2 or 10 μg/ml. Using the same method as that described above, we calculated the albumin-unbound rate of midazolam in the serum albumin solution incubated with propofol or ketamine.

Differences among values were analyzed using one-way analysis of variance (ANOVA), followed by the Dunnett test using Prism 4, version 4.0b software (GraphPad Software, San Diego, CA, USA). Significance was defined as $P < 0.05$. The results are presented as means ± standard deviation (range: minimum to maximum).

The rate of albumin-unbound free midazolam was 4.32 ± 0.13% (4.18–4.46%) at a midazolam concentration of 100 ng/ml. The rates of albumin-unbound free midazolam at a midazolam concentration of 100 ng/ml in the serum albumin solution containing propofol at concentrations of 1 and 5 μg/ml were significantly increased, to 4.63 ± 0.23% (4.43–5.01%) ($P < 0.05$) and 4.85 ± 0.16% (4.71–5.10%) ($P < 0.01$), respectively, compared with the control without propofol (Fig. 2). However, ketamine at concentrations of 2 and 10 μg/ml had no effect on the binding of midazolam to serum albumin (Fig. 2).

Protein-bound rates of midazolam have been reported to be more than 95.0% [6, 7]. Therefore, the rate of protein-unbound free midazolam in the circulation is less than 4.0% of the administered dose of midazolam; however, in the present study, the rate of albumin-unbound free midazolam in a serum albumin solution containing midazolam at a concentration of 100 ng/ml was between

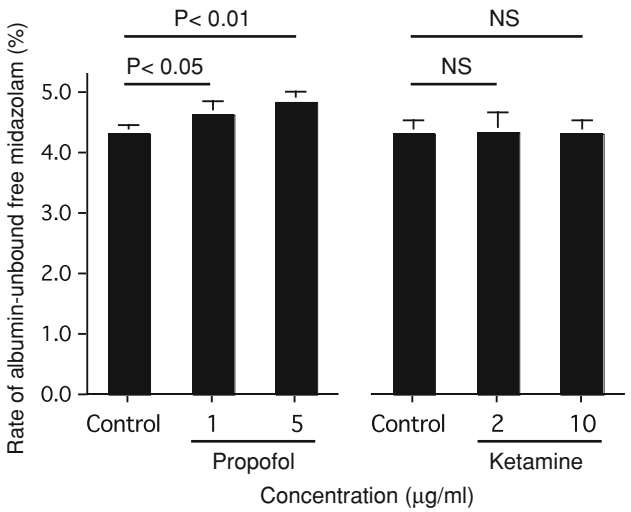

Fig. 2 Effects of propofol or ketamine on the binding of midazolam to serum albumin. The data are presented as means and standard deviation. *NS* not significant ($n = 5$)

4.0 and 5.0%, which is slightly higher. As midazolam can bind not only to albumin but also to other serum components, it is natural that the albumin-unbound rate obtained in our study is slightly higher than that described in previous reports [6, 7].

Because benzodiazepines are thought to bind to a specific site on serum albumin [10], it is possible that albumin-bound midazolam could be displaced by a competitive agent, and the finding in the present study suggests that propofol might be such an agent. On the other hand, another intravenous anesthetic, ketamine, had no effect on the albumin-binding of midazolam in the present study. The albumin-binding rate of ketamine is around 20% [11], which is much lower than that (97–99%) of propofol [8], so even if ketamine binds to the same site as midazolam, ketamine would not compete with midazolam. As the clinical concentration of propofol is much higher than that of midazolam, it seems that propofol can easily displace albumin-bound midazolam in serum. The increase in albumin-unbound free midazolam in combination with the inhibition of midazolam's metabolism by propofol is thought to induce a clinically significant effect. Thus, the order of administration of midazolam and propofol may be clinically important. When midazolam is injected with a continuous infusion of propofol, the administered midazolam may result in an excessive reaction.

The findings in the present study indicate the possibility that not only the inhibition of midazolam metabolism by propofol but also an increase in protein-unbound free midazolam could be involved in the synergistic effect of midazolam and propofol. It has not been clarified how the degree of change in the protein-binding of midazolam can induce a clinically significant effect. However, the change in protein-binding is thought to be responsible for the interaction between these drugs. We need to pay attention to the interactions among drugs when two or more drugs are administered intravenously during general anesthesia or sedation.

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