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Involvement of α_1-acid glycoprotein in inter-individual variation of disposition kinetics of ropivacaine following epidural infusion in off-pump coronary artery bypass grafting

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Abstract

The influence of drug interaction and protein variants on the binding disposition of ropivacaine to α_1-acid glycoprotein (AGP) was examined. The subjects were five patients who received epidural infusion of ropivacaine for 24–54 h in off-pump coronary artery bypass grafting followed by drug combination therapy, and 10 healthy volunteers.

The post-operation plasma albumin concentration showed little overall change, while the AGP concentration in the five patients decreased for 6 h, then increased gradually to about 3-times the initial value by 54 h. The unbound fraction in plasma (f_u) of ropivacaine gradually decreased as the AGP concentration increased, but there was large inter-individual variation among the five patients. In contrast, there was a good correlation between the f_u value and AGP concentration when ropivacaine was added to blood samples from the 10 healthy volunteers. Among the volunteers, eight showed F_1S variants and two showed F_1 variant without S variant of AGP. The f_u value of ropivacaine did not differ between these two groups. However, when ropivacaine was added in combination with dipyridamole, the f_u values of ropivacaine in blood from volunteers with F_1S variants were greater than those in blood from volunteers without S variant. In the case of co-administration of disopyramide or lidocaine, there was no such difference. Among the patients, one showed F_1S variants and four showed F_1 variant without S variant. The results indicate that variability in the side-effects of therapy with ropivacaine alone is caused by the change of the unbound concentration upon changes in the AGP concentration. However, in combination therapy, it is also important to consider the AGP variant-dependence of the inhibitory effect of concomitantly administered drugs.

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Introduction

Ropivacaine is a basic drug with a high protein binding ratio of 94% and is widely used as an amide-type local anaesthetic (Svensson et al 1986; Lee et al 1989). In our hospital, it has been administered as an epidural infusion to patients receiving off-pump coronary artery bypass grafting. However, there is marked inter-individual variation in the effectiveness or side-effects of ropivacaine, so that there is a great need for drug concentration monitoring in clinical practice.

It is well known that α_1-acid glycoprotein (AGP) is an acute-phase reactant that is particularly important in protein binding of lipophilic basic drugs. It has been reported that the AGP concentration rises gradually to about twice the pre-operative concentration by Day 4 after cardiac surgery, resulting in a decrease of the unbound fraction in plasma (f_u) of lidocaine (Holley et al 1984; Booker et al 1996). Further, the f_u value of ropivacaine in combined drug regimens may be influenced by drug–drug interactions associated with AGP binding. The nature of the binding sites should also be taken into consideration. For example, albumin has at least three types of drug-binding sites, typically binding warfarin, diazepam and digitoxin. The binding site(s) of AGP have not yet been characterized.

Recently, genomic DNA cloning studies have established that there are three adjacent AGP coding genes, AGP-A, AGP-B and AGP-B′, which are identical in exon–intron organization (Dente et al 1987; Nakamura et al 2000; Yuasa et al 2001). Yuasa et al (2001) reported that human AGP exists as a heterogeneous population of three genetic variants of ORM1 F1 and ORM1 S derived from the AGP-A gene, and ORM2 A derived from the AGP-B/B′ genes in the plasma of most individuals (Yuasa et al 1987, 1988, 1990). The expression ratio of the two genes changes in various disease states (Mittermuller & Weidinger 1992; Duche et al 2000) and there is marked inter-individual variation (Duche et al 1998). Moreover, it has been clarified that various drugs bind specifically to certain variants (Eap et al 1990; Herve et al 1996; Taheri et al 2003).

In this study, we examined the influence of AGP concentration and AGP variants on the disposition kinetics of ropivacaine.

Materials and Methods

Materials

Anapeine injection (ropivacaine hydrochloride) and Xylocaine injection (lidocaine hydrochloride) were purchased from AstraZeneca Co., Ltd (Osaka, Japan). Persantin injection (dipyridamole) was purchased from Boehringer Ingelheim Co., Ltd (Tokyo, Japan). Vasolan injection (verapamil hydrochloride) was purchased from Eisai Co., Ltd (Tokyo, Japan). Rythmodan P injection (disopyramide phosphate) was purchased from Chugai Pharmaceutical Co., Ltd (Tokyo, Japan). Mepivacaine and human AGP were purchased from Sigma-Aldrich Co., Ltd (MO, USA). Other chemicals were of reagent grade.

Anaesthetic protocol for patients

The five patients were given an intravenous injection of ropivacaine (50 mg) before off-pump coronary artery bypass grafting and simultaneously an epidural infusion of ropivacaine (25 mg h^{-1}) was started. After completion of the procedure, epidural infusion of ropivacaine (10 mg h^{-1}) was continued for 24–54 h for the control of pain.

Sampling of blood

Arterial blood samples from the five patients were withdrawn though a cannula at designated time intervals after drug administration. Blood samples (100 mL) from the 10 healthy volunteers were withdrawn in two portions from a vein. The plasma was separated by centrifugation and stored at −80°C until assay. This clinical study was approved by the Institutional Review Board of Kanazawa University Hospital, Kanazawa, Japan, and informed consent was obtained from each patient and volunteer.

Determination of laboratory data

Measurements of the concentration of albumin and AGP in plasma were conducted by SRL Co. Ltd (Tokyo, Japan).

Protein binding studies

The f_u values of ropivacaine in the plasma samples from the five patients were measured by ultrafiltration (Centrifree MPS-3; Amicon Co. Ltd, CA, USA). Aliquots (10 μL) of various concentrations of ropivacaine were added to 1 mL of plasma from the 10 healthy volunteers. For the inhibitor studies, aliquots (10 μL) of various concentrations of dipyridamole, disopyramide or lidocaine were added to 1 mL of ropivacaine (final concentration 10 μm)-containing plasma from the 10 healthy volunteers. The samples were incubated at 37°C for 30 min and f_u was measured using Centrifree MPS-3.

Assay for ropivacaine

Concentrations of ropivacaine were determined by gas chromatography–mass spectrometry (GC-MS) (Model GC-17 system Class 5000; Shimadzu, Kyoto, Japan). The assay for ropivacaine was carried out according to Engman et al (1998).

Aliquots (100 μL) of each sample were mixed with 900 μL of saline, 500 μL of 50 ng mL^{-1} mepivacaine in saline, as an internal standard, 500 μL of 10% Na_2CO_3 buffer and 4 mL of *n*-heptane:dichloromethane (4:1 v/v). The mixture was shaken for 20 min and centrifuged for 5 min at 3000 *g*. The supernatant organic phase was transferred to another glass tube and pre-concentrated under a stream of nitrogen gas at room temperature. Then, 100 μL of *n*-heptane:ethanol (9:1 v/v) was added to the residue and an aliquot (1 μL) of the mixture was injected into the GC-MS system. Analyses were carried out in the selected-ion monitoring mode, with monitoring at m/Z 126 and m/Z 98 for ropivacaine and mepivacaine, respectively. Chromatographic separation of ropivacaine was achieved with a 5% phenyl-methylpolysiloxane-crosslinked capillary column (DB-5; 30 m × 0.315 mm I.D.; J&W Scientific Inc., Folsom, CA, USA) in a gas chromatograph equipped with a splitless injector. The oven temperature was set at 60°C for 1 min and then programmed to 280°C at 10°C min^{-1}. The final temperature was maintained for 22 min.

Method for isoelectric focusing of AGP

Isoelectric focusing was carried out according to Eap & Baumann (1988). A mixture of 5 μL of plasma and 5 μL of 4 M urea was incubated at 37°C for 1 h. Then, 20 μL of 1.5 U of neuraminidase (Wako Pure Chemicals Co., Ltd, Osaka, Japan) was added and incubation was continued at 37°C for 24 h. The gel used was Immobiline Dryplate pH 4.5–5.5 (Amersham Pharmacia Biotech Co., Ltd, UK). The gel buffer was prepared by mixing 8 M urea (final concentration), 60 mg of dithiothreitol (Wako Pure Chemicals Co., Ltd), 0.5 mL of Pharmalyte narrow range 4.5–5.5 (Amersham Pharmacia Biotech Co., Ltd) and 0.1 mL of Triton-X with 12 mL of distilled water. The gel was used after distension with the buffer at room temperature for 18 h. Isoelectric focusing was performed using a NA-1410-R apparatus (Eido Co., Ltd, Tokyo, Japan), with the cooling plate set at 10°C. A 20-μL aliquot of sample was applied and run at 500 V for 1 h, followed by 2000 V for 10 h. For immunoblotting, the gel was blotted

onto a single sheet of polyvinylidene difluoride (Millipore, USA) in Tris-glycine buffer (pH 8.3) for 1 h. Then, the sheet was blocked with Tris-buffered saline (pH 7.6) with 5% skim milk at 4°C for 1 h, washed with washing buffer (0.1% Tween-20 in phosphate-buffered saline) and incubated overnight at 4°C with primary antibody, rabbit anti-human orosomucoid (DAKO Co., Ltd, Kyoto, Japan) and then for 4 h with secondary antibody, biotinylated anti-rabbit IgG (Cell Signaling Technology, MA, USA). The sheet was extensively washed with phosphate-buffered saline and immunopositive bands were measured by means of the ECL Plus Western Blotting Detection System (Amersham Pharmacia Biotech). The pI values for the A, S and F_1 variants of AGP were 5.06, 5.03 and 4.93, respectively.

Data analysis

Comparisons of numerical data among groups in the protein binding studies were made by the Jonckheere test, with $P < 0.05$ indicating a significant difference, using SPSS 10 (SPSS Inc. Chicago, IL, USA).

Results

Profiles of plasma albumin and AGP concentrations and binding disposition of ropivacaine in five patients

Figure 1 shows the profiles of plasma albumin and AGP concentrations after the operation in the five patients. The albumin concentrations decreased for 2–6h after the operation, except with patient E, and were below the usual range (3.8–5.3 g dL^{-1}). On the other hand, the AGP concentrations clearly decreased for the first 2–6 h, then increased substantially up to 54 h.

Figure 2 shows the profiles of plasma total and unbound concentrations of ropivacaine in the patients following epidural infusion of ropivacaine. The total concentrations gradually rose until the medication was discontinued, and did not reach a steady state. In contrast, the unbound concentrations did not increase after 6 h, but showed marked inter-individual variations.

Figure 3 shows the time-courses of the f_u values of ropivacaine in the patients following epidural infusion of ropivacaine. The f_u values tended to increase transiently until 6 h and subsequently decreased. Figure 3 also shows the correlation between the f_u value of ropivacaine and plasma AGP concentration in the patients. The f_u values tended to decrease with an increase of AGP concentration. There were large inter-individual variations in f_u for a given AGP concentration. The correlation between f_u and AGP concentration was poor ($r = 0.663$).

Isoelectric focusing analysis after desialylation of AGP in plasma of healthy volunteers

Figure 4 shows the immunoblots of desialysed ORM protein after isoelectric focusing of commercial human AGP and plasma from the 10 healthy volunteers. From the anode side, the F_1 and S bands derived from ORM1 and the A band derived from ORM2 were observed, respectively. A mixture of F_1, S and A variants was seen in eight subjects, but the S variant was absent in two volunteers (nos 2 and 8).

Binding disposition of ropivacaine in blood sampled from healthy volunteers

In the 10 healthy volunteers, the plasma concentrations of albumin were over the range of 4.32 to 5.07 g dL^{-1} (4.57 ± 0.23, mean ± s.d.), and those of AGP were over the range of 41 to 68 mg dL^{-1} (55.0 ± 8.6).

Figure 5 shows the correlation between the plasma AGP concentrations and the f_u values of ropivacaine (10 μM). The f_u values of the 10 healthy volunteers were over the range of

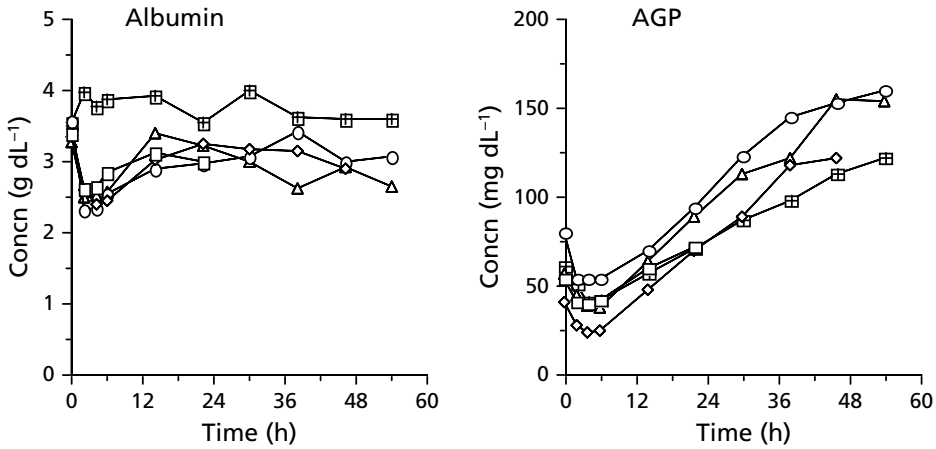

Figure 1 Plasma albumin and α_1-acid glycoprotein (AGP) concentration–time courses of five patients after epidural infusion during off-pump coronary artery bypass grafting. Five patients were administered an intravenous injection of ropivacaine (50 mg) initially, with simultaneous initiation of epidural infusion of ropivacaine (25 mg h^{-1}) for 6 h, followed by epidural infusion of ropivacaine (10 mg h^{-1}) for 48 h after the operation. □, Patient A; ◊, patient B; ○, patient C; △, patient D; ⊞, patient E.

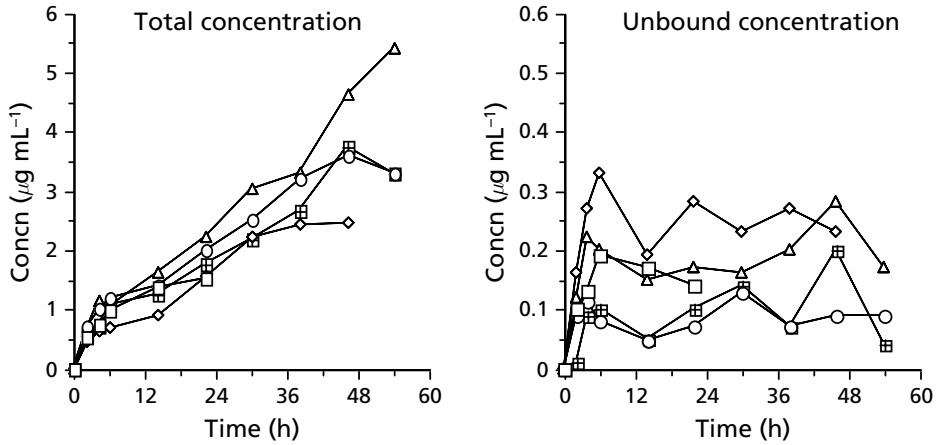

Figure 2 Time courses of plasma total and unbound concentrations of ropivacaine in five patients following epidural infusion of ropivacaine for off-pump coronary artery bypass grafting. Ropivacaine administration was as described in Figure 1. □, Patient A; ◊, patient B; ○, patient C; δ, patient D; ⊞, patient E.

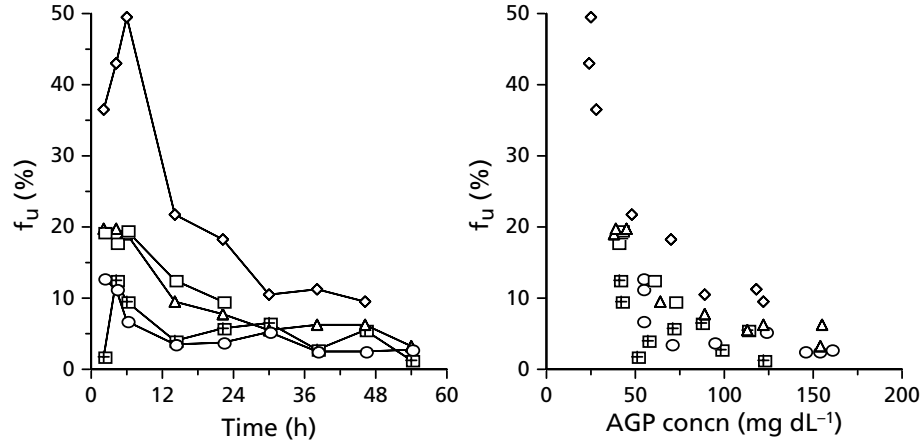

Figure 3 Time-courses of unbound fraction (f_u) of ropivacaine and correlation between the f_u and plasma α_1-acid glycoprotein (AGP) concentration in five patients following epidural infusion of ropivacaine for off-pump coronary artery bypass grafting. Ropivacaine administration was as described in Figure 1. □, Patient A; ◊, patient B; ○, patient C; △, patient D; ⊞, patient E.

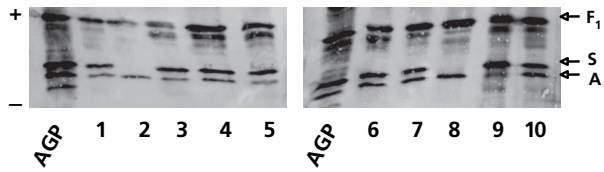

Figure 4 ORM phenotypes in plasma of 10 healthy volunteers analysed by isoelectric focusing after desialylation of α_1-acid glycoprotein (AGP).

approximately 10–25%, but they correlated well with AGP concentration regardless of the variant (r = 0.861).

Figure 6 shows the profile of increase ratio of f_u of ropivacaine in plasma in relation to the AGP variants present. The increase ratio of f_u is defined as the ratio of f_u at various concentrations of ropivacaine to the f_u at 10 μM ropivacaine. The mean increase ratio of f_u for eight volunteers with the F_1S variants coincided well with those for the two volunteers without S variant.

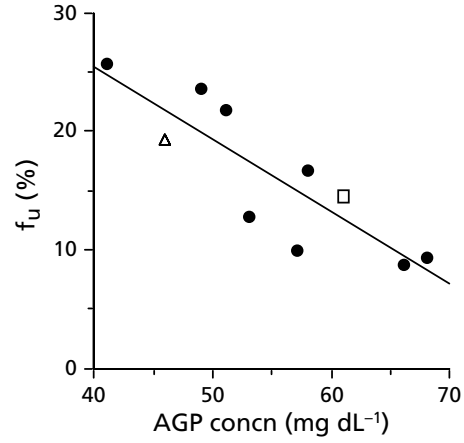

Figure 5 Correlation between the unbound fraction (f_u) of ropivacaine (10 μM) and plasma α_1-acid glycoprotein (AGP) concentration in 10 healthy volunteers: •, eight volunteers with F_1S variant; □, △, two volunteers without S variant.

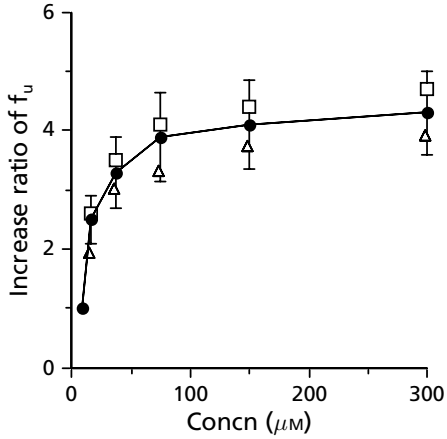

Figure 6 Profiles of the unbound fraction (f_u) of ropivacaine in the plasma of 10 healthy volunteers: •, eight volunteers with F_1S variant (mean ± s.d.); □, △, two volunteers without S variant.

Figure 7 shows the inhibitory effect of dipyridamole, lidocaine and disopyramide on the binding of ropivacine (10 μM) in plasma. The increase ratios of f_u of disopyramide and lidocaine were essentially the same in the groups with the F_1S variants and without the S variant, but the increase ratio of dipyridamole in the group without the S variant was significantly less than that in the group with the F_1S variants.

Isoelectric focusing analysis after desialylation of AGP in plasma of five patients

Figure 8 shows the immunoblots of desialysed ORM protein after isoelectric focusing of commercial human AGP and plasma of the five patients. A mixture of F_1, S and A variants was seen only in patient D, whereas the S variant was absent in the other four patients.

Discussion

We confirmed that ropivacaine binds mainly with AGP, the binding to albumin being negligible, using commercial human AGP and albumin. In agreement with previous findings (Holley et al 1984), the plasma concentrations of AGP in five patients decreased for about 6 h after the operation and subsequently gradually increased to about 3-times the initial value by 54 h (Figure 1). We found that the plasma total concentration of ropivacaine following epidural infusion continued to increase up to 48 h and did not reach a steady-state level, whereas the unbound concentration reached a steady-state level within 6 h (Figure 2). The reason for this is thought to be a decrease of hepatic clearance owing to the decrease in the f_u value with time as the concentration of AGP increased (Figure 3).

However, there was a wide inter-individual variation among f_u values of the patients at the same concentration of AGP (Figure 3). It is well known that toxicity of local anaesthetics is closely related to the unbound concentration, rather than the total concentration. It has been reported that toxicity

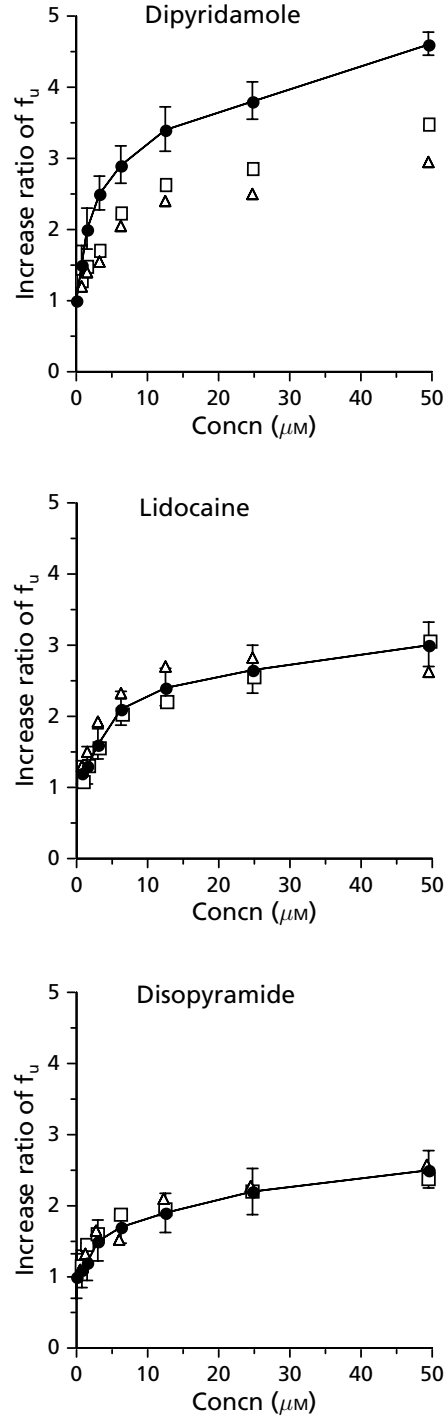

Figure 7 Effect of dipyridamole, lidocaine or disopyramide on the unbound fraction (f_u) of ropivacaine (10 μM) in the plasma of 10 healthy volunteers: •, eight volunteers with F_1S variant (mean ± s.d.); □, △, two volunteers without S variant.

of ropivacaine to the central nervous system and heart appears in the unbound concentration range of 0.34–0.85 μg mL^{-1} (Wiedemann et al 2000). The f_u value of patient B was greater than that of the other patients, even though the AGP concentration tended to be lower immediately after the operation.

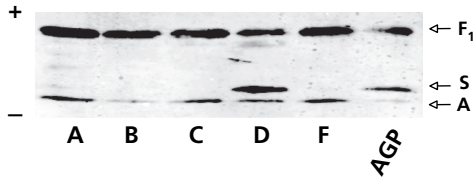

Figure 8 ORM phenotypes in the plasma of five patients were analysed by isoelectric focusing after desialylation of α_1-acid glycoprotein (AGP) before epidural infusion of ropivacaine during off-pump coronary artery bypass grafting.

Therefore, the infusion of ropivacaine to patient B was discontinued after 40 h because of possible toxicity.

Recently, it has been reported that three main AGP phenotypes of F_1S/A, F_1/A and S/A are distributed in 48, 35 and 16% of the general population, respectively (Yuasa et al 1988). Further, it has been clarified that some drugs bind selectively to different AGP variants (Eap et al 1990; Herve et al 1996; Taheri et al 2003). Ropivacaine and dipyridamole bind selectively to the F_1S variants, disopyramide to the A variant, and lidocaine to the F_1S and A variants. In this study, we found that the 10 healthy volunteers could be classified into two groups: eight with F_1S/A and two with F_1/A without S (Figure 4).

Although the AGP concentration and the f_u value of ropivacaine (10 μM) in blood from 10 healthy volunteers show a wide inter-individual variation, there was a good correlation between them (Figure 5). There was little difference in the binding disposition of ropivacaine between the group with F_1S variants and the group without S variant (Figure 6). Although it is reported that ropivacaine combines selectively with the mixture of F_1S variants, the above finding suggests that the affinity of ropivacaine is greater for the F_1 variant than for the S variant. Thus, changes in AGP concentration, rather than specific AGP variants, are of prime importance in local anaesthetic therapy with ropivacaine alone.

We examined the inhibitory effects of some drugs in combination with ropivacaine on the binding of ropivacaine. Examination of the binding disposition of ropivacaine to commercial human AGP (70 mg dL^{-1}) in the presence of dipyridamole, verapamil, lidocaine or disopyramide by using Lineweaver-Burk plots (data not shown) indicated that these drugs competitively inhibit the binding of ropivacaine to AGP. The inhibition constants (K_i) of dipyridamole, verapamil, lidocaine and disopyramide were 2.1, 5.2, 6.0 and 11.0 μM, respectively. The extent of the inhibitory effects against ropivacaine (F_1S type) presumably reflect the characteristics of selective binding of the drugs to the AGP variants.

Thus, we examined the inhibitory effect of dipyridamole, lidocaine or disopyramide on the binding disposition of ropivacaine in blood sampled from the 10 healthy volunteers. There was no clear difference in the inhibitory effect of lidocaine or disopyramide between the group with F_1S and the group without S variant (Figure 7). In contrast, the f_u values of ropivacaine in the F_1S group were clearly greater than those in the group without S variant in the case of dipyridamole, and the level of f_u in the group without the S variant tended to be greater in the cases of disopyramide and lidocaine in proportion to the K_i values. These results suggest that the inhibitory effect of dipyridamole may be stronger in the group with F_1S variants. Therefore, variations of f_u of ropivacaine in combination therapy may be caused by inter-individual variations in the ratio of AGP variants. Further, in the clinically relevant range, the f_u value of ropivacaine is increased about 2-fold by combination with dipyridamole (3.3 μg mL^{-1} vs 1.5 μg mL^{-1}).

Among the five patients, patients A, B, C and E lacked the S variant, and only patient D showed F_1S variants (Figure 8). Thus, 20% of the volunteers and 80% of the patients lacked the S variant. This result in patients with angina pectoris and myocardial infarction seems consistent with reports that the expression ratio of the AGP variants changes in various disease states (Mittermuller & Weidinger 1992; Duche et al 2000). The five patients received ropivacaine in combination with basic drugs such as lidocaine, diltiazem, nicorandil, dopamine and methoxamine at the time of the operation; only patient D additionally received landiolol and verapamil. It remains to be clarified where landiolol and verapamil bind selectively to some AGP variant types. Although patient B lacked the S variant, the f_u value was the highest among the five patients. The reason for this may be that the AGP concentration tended to be lower immediately after the operation. On the other hand, the f_u value of ropivacaine in patient D with F_1S variant tended to be greater than those of other patients without the S variant, except for patient B (Figure 3). We thought that in patient D, with the S variant, the AGP binding would have been strongly inhibited by verapamil, as seen in the case of dipyridamole in the healthy volunteers, although this remains to be confirmed.

Conclusions

Our results indicate that variations in the side-effects of ropivacaine administered alone to patients are caused by changes in the unbound concentration of ropivacaine in response to increased AGP concentration. However, in the case of combination therapy with other drugs, it is necessary to consider the inhibitory effect of co-administered drugs arising from their selective binding to AGP variants and inter-individual differences in AGP variants in patients.

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