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Letter

Effect of blood collection tubes containing separation gels on the measurement of drug concentrations in clinical toxicology

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ABSTRACT — Serum separation gels are problematic in therapeutic drug monitoring because they adsorb some of the target drugs; however, the adsorptive properties of drugs that cause clinical intoxication remain unelucidated. Drug adsorption to separators results in a decrease in the observed blood levels, which may lead to uncertain assessments of clinical toxicology. Therefore, this study aimed to clarify the effects of four brands of blood collection tubes with serum separation gels that are used in Japan on the blood concentrations of central nervous system-acting drugs. Amitriptyline-, amoxapine-, mirtazapine-, chlorpromazine-, and flunitrazepam-spiked plasma at respective intoxicated concentrations were incubated in blood collecting tubes with serum separators, namely Vacutainer, Neotube, Insepack, and Venoject, at 4°C or 25°C for up to 72 or 168 hr and compared with control tubes without a serum separator. The amitriptyline, chlorpromazine, and flunitrazepam concentrations significantly decreased, even in control tubes. All the tubes containing serum separators significantly reduced the observed drug concentration when incubated at 25°C, which was estimated using a power function. Except amoxapine, rest all drugs when incubated at 4°C showed a decrease in concentration, albeit to a lesser degree than at 25°C. The blood collection tube with the greatest decrease in concentration was the Vacutainer. In conclusion, although the possibility of drug degradation in plasma must be considered, control tubes are strongly recommended for clinical toxicology, in addition to therapeutic drug monitoring, because drug adsorption is less likely to occur in these tubes.

Key words: Blood collection tubes, Drug concentrations, Drug degradation

INTRODUCTION

Blood collection tubes containing separation gels facilitate serum separation, because the serum separator forms a barrier between the serum and blood clots during blood centrifugation after collection. Therefore, they are widely used for collecting blood during testing. Currently, the main serum separators in circulation are polyolefin-, polyester-, and acrylic-based materials. Some medicines have been reported to be adsorbed onto these separators, resulting in reduced blood concentrations. For example, the acrylic serum separators adsorb hydrophobic drugs such as phenytoin, phenobarbital, carbamazepine, quinidine, and lidocaine and decrease the observed serum

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drug concentrations by 20 to 50% after 24 hr of storage at 4°C (Bergqvist *et al.*, 1984; Koch and Platoff, 1990). In Japan, when therapeutic drug monitoring (TDM) is performed to measure the blood levels of certain drugs for a defined disease, insurance may claim specific drug treatment management fees. When performing TDM, collection of blood in control tubes without a serum separator is recommended, and the manufacturers of blood collection tubes and contract laboratories are advised to take precautions.

When treating acute drug poisoning, blood levels of intoxicated drugs must be accurately measured, to make adequate clinical decisions and/or academic validation. The most common substances responsible for drug poisoning in Japan are central nervous system (CNS)-acting drugs, such as benzodiazepines, antipsychotics, antidepressants, and antipyretic/analgesic drugs (Japan Poison Information Center, 2020; Kudo *et al.*, 2010). However, most CNS-acting drugs have not been subjected to TDM, and the effects of serum separators on the observed blood concentrations are unclear. Currently, there is no well-established systematization for measuring blood drug concentrations during drug intoxication (Otani *et al.*, 2020), and in some cases, measurement of blood drug concentrations must be performed using samples collected in blood collection tubes with serum separators. However, when the drug is adsorbed onto a serum separator, it decreases its apparent blood concentration, making it difficult to properly assess the relationship between clinical symptoms and blood drug concentrations (Bowen and Remaley, 2014). In fact, we encountered cases in which drug adsorption to the serum separator was highly probable during the course of measuring blood levels over time in patients with drug-intoxication. Therefore, this study aimed to clarify the effects of various serum separation gels on the observed concentrations of CNS drugs using a plain tube as a control, which did not use a serum separator, and four types of blood collection tubes containing a serum separator commercially available in Japan.

MATERIALS AND METHODS

Materials

Amitriptyline, amoxapine, mirtazapine, and doxepin were purchased from the Tokyo Chemical Industry (Tokyo, Japan). Chlorpromazine, flunitrazepam, and diazepam were purchased from Fujifilm Wako Chemicals (Osaka, Japan). Bond Elut QuEChERS was purchased from Agilent Technologies (CA, USA). Freshly frozen human plasma was purchased from the Japanese Red Cross Society (Tokyo, Japan) and Innovative Research Inc. (MI, USA). Venoject® II tubes (VP-P075K, plain tubes) and Venoject® II tubes with a separator gel (VP-AS076K60, Venoject) were purchased from Terumo (Tokyo, Japan). Neo-Tube® tubes (NP-SP0735, Neotube) were purchased from Nipro (Osaka, Japan). BD Vacutainer® SST™ II tube (368640, Vacutainer) was purchased from Becton Dickinson Japan (Tokyo, Japan). Insepack® II-D tubes (511462, Insepack) were purchased from Sekisui Medical (Tokyo, Japan). All other reagents used were of the highest commercially available grade.

Sample preparation

Amitriptyline, amoxapine, mirtazapine, chlorpromazine, or flunitrazepam was added to human plasma and adjusted to the concentration (μg/mL) at each drug intoxication, i.e., 0.5, 3.0, 1.0, 1.0, or 0.1, respectively (Schulz *et al.*, 2012). After shaking at 37°C for 1 hr, each drug-containing plasma was dispensed into control tubes, Venoject (polyolefin-based serum separator gel), Neotube (polyester-based serum separator gel), Vacutainer (acrylic-based serum separator gel), and Insepack (polyolefin-based serum separator gel). The mixtures were stirred for 1 min and centrifuged at 1300 × g for 10 min. Amitriptyline-spiked plasma in the blood collection tubes were incubated at room temperature (RT; 25°C) or 4°C for 6, 24, or 168 hr with vertical rotation (15 rpm). Amoxapine-, mirtazapine-, chlorpromazine-, or flunitrazepam-spiked plasma in the blood collection tubes were incubated at RT or 4°C for 6, 24, or 72 hr. In addition, the samples that were drug-extracted immediately after centrifugation, were used as the respective 0 hr samples.

Sample extraction

Amitriptyline or mirtazapine was extracted from the plasma samples by adding 80 μL of 20% Na_2CO_3, 1.2 mL of *n*-hexane/3-methyl-1-butanol (98.5:1.5), and 200 ng of doxepin as an internal standard (IS) to 200 μL of plasma and stirred vigorously. The organic phase was collected after centrifugation at 15000 × g for 10 min. After the solvent evaporated under a nitrogen stream, the residue was dissolved in 200 μL of the initial mobile phase and subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Amoxapine was extracted from the plasma samples by adding 200 mg of QuEChERS, 300 μL of acetonitrile, 250 μL of distilled water, and 20 ng of diazepam as an IS to 50 μL of plasma and stirred vigorously. The organic phase was collected after centrifugation at 15000 × g for 10 min and subjected to LC-MS/MS analysis. Flunitrazepam was extracted from the plasma samples by adding 60 μL of 20% Na_2CO_3,

1 mL of ethyl acetate, and 10 ng of diazepam as an IS to 200 µL of plasma and stirred vigorously. The organic phase was collected after centrifugation at 15000 × g for 10 min. After the solvent evaporated under a nitrogen stream, the residue was dissolved in 200 µL of the initial mobile phase and subjected to LC-MS/MS analysis. Chlorpromazine was extracted from the plasma samples by adding 200 mg of QuEChERS, 700 µL of acetonitrile, and 10 ng of diazepam to 200 µL of plasma and stirred vigorously. The organic phase was collected after centrifugation at 15000 × g for 10 min. After the solvent evaporated under a nitrogen stream, the residue was dissolved in 200 µL of the initial mobile phase and subjected to LC-MS/MS analysis.

Liquid Chromatography Tandem-Mass Spectrometry (LC-MS/MS) analysis

An LC-MS/MS system comprising Alliance 2695 and Masslynx (Waters, MA, USA) was used to measure amitriptyline, mirtazapine, and flunitrazepam concentrations. Chromatographic separation was achieved using an X-Bridge BEH C18 Column (2.1 mm x 150 mm, 3.5 µm; Waters). The column temperature was set at 40°C. The mobile phases used for measuring amitriptyline and mirtazapine were (A) 0.1% formic acid and (B) acetonitrile, respectively. The gradient started at 10% B, rose to 50% within 3.5 min, increased to 90% within next 5 min, remained constant for 2 min, returned to 10% in 0.5 min, and remained constant for 5 min. The flow rate was set at 0.2 mL/min and the injection volume was 10 µL. The mobile phases used for measuring flunitrazepam were (A) 0.1% formic acid and (B) acetonitrile containing 0.1% formic acid. The gradient started at 10% B, rose to 95% within 5 min, remained constant for 15 min, returned to 10% after 1 min, and stayed constant for 5 min. The flow rate was set at 0.2 mL/min and the injection volume was 10 µL. After electrospray ionization (ESI) in positive mode, the samples were analyzed in multiple reaction monitoring (MRM) mode. The flow rates of the drying and cone gases were set to 10 L/min, and 0.83 L/min, respectively. The temperatures of the source and drying gases were set to 120°C and 350°C, respectively. The instrumental conditions for each compound are listed in Table 1.

An LC-MS/MS system comprising an LC-40ADXR and LCMS-8045 (Shimadzu, Kyoto, Japan) was used for amoxapine and chlorpromazine measurements. Chromatographic separation was achieved on a Phenomenex Kinetex XB-C18 column (2.1 mm ID x 100 mm, 2.6 µm; Shimadzu) with an equivalent Phenomenex Security Ultra C18 guard column (2.1 mm ID; Shimadzu). The column temperature was set at 40°C. The mobile phase consisted of (A) 10 mM ammonium formate with 0.1% formic acid and (B) methanol containing 10 mM ammonium formate with 0.1% formic acid. The gradient started at 5% (B), rose to 95% within 7.5 min, remained constant for 2.5 min, immediately returned to 5%, and stayed constant for 5 min. The flow rate was set at 0.3 mL/min and the injection volume was 1 µL. After ESI in positive mode, the samples were analyzed in MRM mode. The flow rates of the nebulizer, drying, and heating gases were set to 3, 10, and 10 L/min, respectively. The temperatures of the interface, desolvation line, and heat block were set to 300°C, 250°C, and 400°C, respectively. The instrumental conditions for each compound are listed in Table 1.

Statistical Analysis

The difference in observed plasma drug concentrations between samples incubated at 4°C and RT at each time point (6, 24, and 168 hr for amitriptyline and 6, 24, and 72 hr for the other drugs) was analyzed using Student's *t*-test. Differences in observed plasma drug concentrations between the 0 hr sample and each time point for amitriptyline and other drugs were analyzed using Dunnett's test. The relationship between the incubation time of the blood collection tubes and the measured plasma drug concentrations was analyzed using simple regression analysis. The relationship between the logP (octanol/water partition coefficient) of the drug and percentage decrease in the observed drug concentration was analyzed using Pearson's product-moment correlation coefficient. All statisti-

Table 1. Liquid Chromatography - Mass Spectrometry (LC-MS/MS) conditions.

Compound	MRM transitions		Collision energy (V)	Retention Time (min)
	Precursor ion (*m/z*)	Product ion (*m/z*)		
Amitriptyline	278.2	104.9	20	9.40
Amoxapine	314.1	271	27	6.01
Mirtazapine	266.1	195.1	20	7.43
Chlorpromazine	319.1	86.1	22	6.58
Flunitrazepam	314.1	268.1	25	9.49
Diazepam (IS)	285.1	193.1	29	9.96* 6.80**
Doxepin (IS)	280.1	106.9	20	8.96

MRM: multiple reaction monitoring.
*LC conditions for measurement of flunitrazepam (Masslynx).
** LC conditions for measurement of amoxapine and chlorpromazine (LCMS-8045).

Table 2. Relative drug concentrations in plasma incubated in various blood collection tubes at 4°C.

Drugs	Blood collection tubes	4°C				
		0 hr	6 hr	24 hr	72 hr	168 hr
Amitriptyline	Control tube	100	99.3 ± 0.12	98.7 ± 4.97	-	101.2 ± 0.43
	Vacutainer	100	42.3 ± 0.80**	32.1 ± 0.38**	-	14.0 ± 0.51**
	Neotube	100	76.7 ± 2.63**	64.6 ± 1.39**	-	51.2 ± 1.78**
	Insepack	100	87.7 ± 1.85**	68.1 ± 4.93**	-	42.6 ± 0.96**
	Venoject	100	79.0 ± 0.48**	76.1 ± 1.53**	-	69.5 ± 0.67**
Amoxapine	Control tube	100	100.4 ± 9.39	92.6 ± 4.00	99.0 ± 0.12	-
	Vacutainer	100	77.2 ± 2.31**	55.2 ± 1.33**	55.8 ± 4.19**	-
	Neotube	100	92.6 ± 7.43	88.2 ± 5.24	80.2 ± 2.05	-
	Insepack	100	93.3 ± 8.17	92.2 ± 0.86	89.0 ± 3.37	-
	Venoject	100	100.0 ± 3.72	86.6 ± 1.32	83.0 ± 6.46	-
Mirtazapine	Control tube	100	104.0 ± 4.93	98.0 ± 6.27	103.6 ± 5.52	-
	Vacutainer	100	65.8 ± 1.27**	51.1 ± 3.11**	39.8 ± 1.75**	-
	Neotube	100	80.0 ± 6.26*	69.9 ± 2.26**	64.9 ± 4.01**	-
	Insepack	100	95.7 ± 4.68	82.8 ± 1.52*	81.9 ± 3.94*	-
	Venoject	100	91.0 ± 1.76	87.6 ± 3.19*	86.9 ± 0.57*	-
Chlorpromazine	Control tube	100	93.8 ± 3.51	90.4 ± 9.25	79.1 ± 8.97	-
	Vacutainer	100	46.0 ± 5.20**	32.0 ± 3.40**	19.4 ± 0.60**	-
	Neotube	100	66.7 ± 2.92	61.3 ± 3.77*	38.0 ± 1.03**	-
	Insepack	100	62.1 ± 5.66*	59.7 ± 12.26*	44.8 ± 1.78**	-
	Venoject	100	62.8 ± 3.01*	56.7 ± 2.46*	56.8 ± 4.65*	-
Flunitrazepam	Control tube	100	93.7 ± 5.11	80.8 ± 1.75*	84.2 ± 1.61**	-
	Vacutainer	100	82.0 ± 5.19**	68.2 ± 1.94**	73.1 ± 1.49**	-
	Neotube	100	72.2 ± 1.76**	73.5 ± 1.40**	72.0 ± 0.97**	-
	Insepack	100	98.2 ± 3.06	84.7 ± 1.37**	80.8 ± 1.39**	-
	Venoject	100	77.6 ± 8.14*	74.8 ± 1.48*	80.4 ± 2.51**	-

Data are expressed as % of relative concentration ± S.E. (n = 3). *$p < 0.05$, **$p < 0.01$ different from the 0 hr-control. –, not determined.

cal analyses were performed using JMP Pro 16.0.

RESULTS AND DISCUSSION

Because amitriptyline gets adsorbed onto the separator gel at RT (Orsulak *et al*., 1984; Schrapp *et al*., 2019), its stability and adsorption under our experimental conditions were examined for comparison with a previous report (Table 2 and 3). Apparent amitriptyline concentrations in plasma incubated at 4°C in control tubes did not change after 168 hr (Table 2). However, when incubated at RT, the observed concentration decreased to 75.2% of the initial concentration (0.5 μg/mL) at 168 hr (Table 3), indicating that amitriptyline could degrade under prolonged incubation in plasma at RT. When using either brand of blood collection tubes with serum separation gels, significant decreases in the amitriptyline concentration were observed as early as 6 hr at 4°C (Table 2) and RT (Table 3). The rate of decrease in observed amitriptyline concentrations after 24 hr-incubation in the blood collection tubes at 4°C and RT was in the following order: Vacutainer (acrylic-based serum separator gel) > Neotube (polyester-based serum separator gel) > Insepack (polyolefin-based serum separator gel) > Venoject (polyolefin-based serum separator gel). When using the blood collection tubes with serum separator gels, the observed concentrations obtained at RT were significantly lower than those at 4°C. These results

Table 3. Relative drug concentrations in plasma incubated in various blood collection tubes at room temperature.

Drugs	Blood collection tubes	RT				
		0 hr	6 hr	24 hr	72 hr	168 hr
Amitriptyline	Control tube	100	95.0 ± 1.81	93.5 ± 2.52	-	75.2 ± 0.28$^{**\#\#}$
	Vacutainer	100	26.9 ± 0.62$^{**\#\#}$	14.3 ± 0.20$^{**\#\#}$	-	2.5 ± 0.04$^{**\#\#}$
	Neotube	100	47.3 ± 2.46$^{**\#\#}$	32.9 ± 0.34$^{**\#\#}$	-	13.1 ± 0.21$^{**\#\#}$
	Insepack	100	47.2 ± 1.25$^{**\#\#}$	36.6 ± 0.65$^{**\#\#}$	-	10.0 ± 0.34$^{**\#\#}$
	Venoject	100	60.0 ± 1.12$^{**\#\#}$	49.5 ± 2.75$^{**\#\#}$	-	24.7 ± 1.55$^{**\#\#}$
Amoxapine	Control tube	100	104.1 ± 2.60	88.9 ± 3.26	92.1 ± 4.91	-
	Vacutainer	100	66.5 ± 2.62$^{**\#}$	31.5 ± 1.91$^{**\#\#}$	20.0 ± 0.92$^{**\#\#}$	-
	Neotube	100	78.5 ± 2.41$^{**\#}$	55.1 ± 2.67$^{**\#\#}$	46.2 ± 2.15$^{**\#\#}$	-
	Insepack	100	97.1 ± 3.77	72.0 ± 3.15$^{**\#\#}$	62.9 ± 3.63$^{**\#\#}$	-
	Venoject	100	63.4 ± 1.53$^{**\#\#}$	46.7 ± 2.44$^{**\#\#}$	39.0 ± 1.58$^{**\#\#}$	-
Mirtazapine	Control tube	100	108.1 ± 2.55	99.4 ± 1.42	115.3 ± 1.24	-
	Vacutainer	100	48.1 ± 2.85$^{**\#\#}$	35.7 ± 0.65$^{**\#\#}$	12.8 ± 1.95$^{**\#\#}$	-
	Neotube	100	57.9 ± 1.37$^{**\#\#}$	48.4 ± 0.75$^{**\#\#}$	34.3 ± 0.32$^{**\#\#}$	-
	Insepack	100	71.1 ± 5.97$^{**\#\#}$	65.2 ± 4.25$^{**\#}$	60.7 ± 1.72$^{**\#\#}$	-
	Venoject	100	87.3 ± 5.25	69.5 ± 1.02$^{*\#}$	63.4 ± 11.4$^{**\#\#}$	-
Chlorpromazine	Control tube	100	71.5 ± 2.43*	76.0 ± 4.27*	63.6 ± 9.24*	-
	Vacutainer	100	20.9 ± 4.66$^{**\#}$	8.8 ± 0.71**	6.9 ± 0.16**	-
	Neotube	100	29.8 ± 2.06$^{**\#}$	20.1 ± 1.45$^{**\#\#}$	17.6 ± 0.55**	-
	Insepack	100	41.7 ± 3.00**	26.0 ± 1.46$^{**\#\#}$	6.0 ± 0.01$^{**\#\#}$	-
	Venoject	100	40.9 ± 1.37**	39.2 ± 1.20**	37.9 ± 1.49**	-
Flunitrazepam	Control tube	100	82.6 ± 12.5	85.3 ± 4.56	80.0 ± 1.44	-
	Vacutainer	100	72.5 ± 1.12$^{**\#}$	48.5 ± 0.45$^{**\#\#}$	37.8 ± 2.89$^{**\#\#}$	-
	Neotube	100	70.8 ± 1.27**	44.4 ± 1.85$^{**\#\#}$	24.9 ± 0.69$^{**\#\#}$	-
	Insepack	100	92.6 ± 0.59	76.5 ± 8.52*	64.3 ± 0.82$^{**\#\#}$	-
	Venoject	100	80.1 ± 2.73*	64.4 ± 5.32**	49.3 ± 4.98$^{**\#\#}$	-

Data are expressed as % of relative concentration ± S.E. (n = 3). *p < 0.05, **p < 0.01 different from the 0 hr-control. #p < 0.05, ##p < 0.01 different from the respective incubation at 4℃. –, not determined.

indicated that amitriptyline was strongly adsorbed onto the separator, including Vacutainer as previously reported (Schrapp *et al*., 2019), and that adsorption could be evaluated under the experimental conditions of this study. Furthermore, the results indicated that the adsorption of drugs varied depending on the separating material or brand. Therefore, other CNS-acting drugs frequently encountered in clinical settings as intoxication cases, such as the antidepressants amoxapine and mirtazapine, antipsychotic chlorpromazine, and benzodiazepine flunitrazepam, were investigated in subsequent experiments. Moreover, in all blood collection tubes with serum separation gels, amitriptyline concentrations declined rapidly during the first 6 hr, followed by a gradual decrease. Therefore, for subsequent experiments, the maximum incubation time was 72 hr.

At 4°C and RT, plasma concentrations of amoxapine (initial concentration of 3.0 μg/mL) and mirtazapine (initial concentration of 1.0 μg/mL) incubated in control tubes did not change during 72 hr-incubation (Table 2 and 3), indicating that these drugs were stable under the current experimental conditions. Meanwhile, the plasma concentrations of chlorpromazine (initial concentration of 1.0 μg/mL) incubated in control tubes decreased significantly as early as 6 hr at RT (Table 3), indicating that the drug was unstable in plasma at RT. No change in the apparent concentration was observed when aqueous solutions of chlorpromazine were stored at RT, indicating

that plasma components were involved in the stability of this drug. Plasma concentrations of flunitrazepam (initial concentration of 0.1 μg/mL) decreased significantly after incubation for > 24 hr at 4°C in control tubes (Table 2). Although not significant, the flunitrazepam concentration also decreased during incubation at RT.

All drugs used in this study showed a significant decrease in concentration after incubation for more than 24 hr at RT in blood collection tubes containing either brand of serum separation gel (Table 3). Even at 4°C, mirtazapine, chlorpromazine, and flunitrazepam showed a significant decrease in concentration after incubation for 24 hr in all brands of blood collection tubes containing the separation gels. Amoxapine adsorbed successfully on Vacutainer at 4°C but showed resistance for adsorption on other serum separation gels (Table 2). Plasma containing either drug showed the lowest concentrations when incubated in Vacutainer at 4°C and RT (Table 2 and 3). The only exception was the flunitrazepam-containing plasma, which showed the lowest concentration when incubated at RT in the Neotube (Table 3). Observed concentrations of amoxapine, mirtazapine, chlorpromazine, and flunitrazepam in plasma incubated at RT in control tubes were equivalent to those incubated at 4°C. In contrast, concentrations of these drugs incubated in blood collection tubes containing a serum separation gel at RT were lower than those incubated at 4°C (Table 2 and 3). These results indicated that drug adsorption onto the separating gels occurred in a temperature-dependent manner.

Vacutainer showed the most significant decrease in concentration at 4°C, which appeared to be drug adsorption (Table 2). As Vacutainer was the only brand of acrylic-based material used in this study, it is not known whether the material could likely to adsorb drugs. Meanwhile, caution should be taken because Vacutainer adsorbs drugs easily at 4°C. This could be due to direct storage in the refrigerator and utilization in drug measurement after centrifugation in a test tube with a separating gel. Insepack and Venoject, which are polyolefin-based materials, had a relatively small effect on the concentrations of drugs such as mirtazapine and flunitrazepam. However, among the collecting tubes used, Insepack showed the greatest decrease in chlorpromazine concentration after 72 hr at RT. Therefore, depending on the storage conditions, any isolate could have a significant impact on drug concentrations.

When drug-containing plasma was stored in blood collection tubes containing the separation gel, the concentrations decreased more rapidly between 0 and 6 hr than at 24 hr or later. Therefore, a single regression analysis was performed to examine the effects of the physicochemical properties of the drugs on the measured drug concentrations (Fig. 1). The relationship between the drug concentration and incubation time was found to be roughly equivalent to the following equation in all cases: log(drug concentration) = a × log(time) + b (a: slope, b: intercept). The main causes of the decrease in the plasma concentration of drugs over time could be attributed to drug degradation and adsorption onto the separating materials. Separation materials are not expected to play a major role in drug degradation, whereas adsorption onto the separation gel depends on the material, drug, and temperature. The Vacutainer showed a somewhat different slope of decreasing concentration than that of the other brands (Fig. 1), which was consistent with the discussion above. For the other brands, the slope of the decrease in concentration was similar, except for the combination of Insepack and chlorpromazine.

The logP of drugs affects their adsorption onto the separators. In a study using Vacutainer, drugs with logP > 3 or 3.3 caused a significant decrease in plasma concentration (Steuer *et al*., 2016; Schrapp *et al*., 2019); therefore, the authors recommended using blood collection tubes without separators. However, this study demonstrated that amoxapine, mirtazapine, and flunitrazepam, with logP < 3, could also be adsorbed onto the separation gel. In this study, the blood collection tubes were rotated to reduce the variation in measurements between samples, whereas in a previous report, the blood collection tubes were stored in a static position. The procedure employed in this study increased the contact area between the plasma and separation gel more than previously reported; therefore, it is suggested that even drugs with a relatively small logP are adsorbed on the separator. A significant correlation was reported between the rate of decrease in the observed drug concentration and logP (Schrapp *et al*., 2019). Therefore, Pearson's product-moment correlation analysis was performed at logP of the drugs and the rate of decrease in drug concentrations, using drug concentrations after 6 hr at RT (Fig. 2) that were similar to the conditions described in a previous report (Schrapp *et al*., 2019). The results showed a positive correlation between logP and the rate of decrease in drug concentration for Vacutainer ($r = 0.96$, $p = 0.01$) and Insepack ($r = 0.94$, $p = 0.02$). In contrast, no correlation was observed for Neotube ($r = 0.88$, $p = 0.052$) or Venoject ($r = 0.80$, $p = 0.10$). Vacutainer has been reported to show a positive correlation between logP and adsorption (Steuer *et al*., 2016; Schrapp *et al*., 2019), which was also confirmed in this study. This showed that highly fat-soluble drugs were adsorbed onto the acrylic separator in a short period of time, causing a decrease in drug concen-

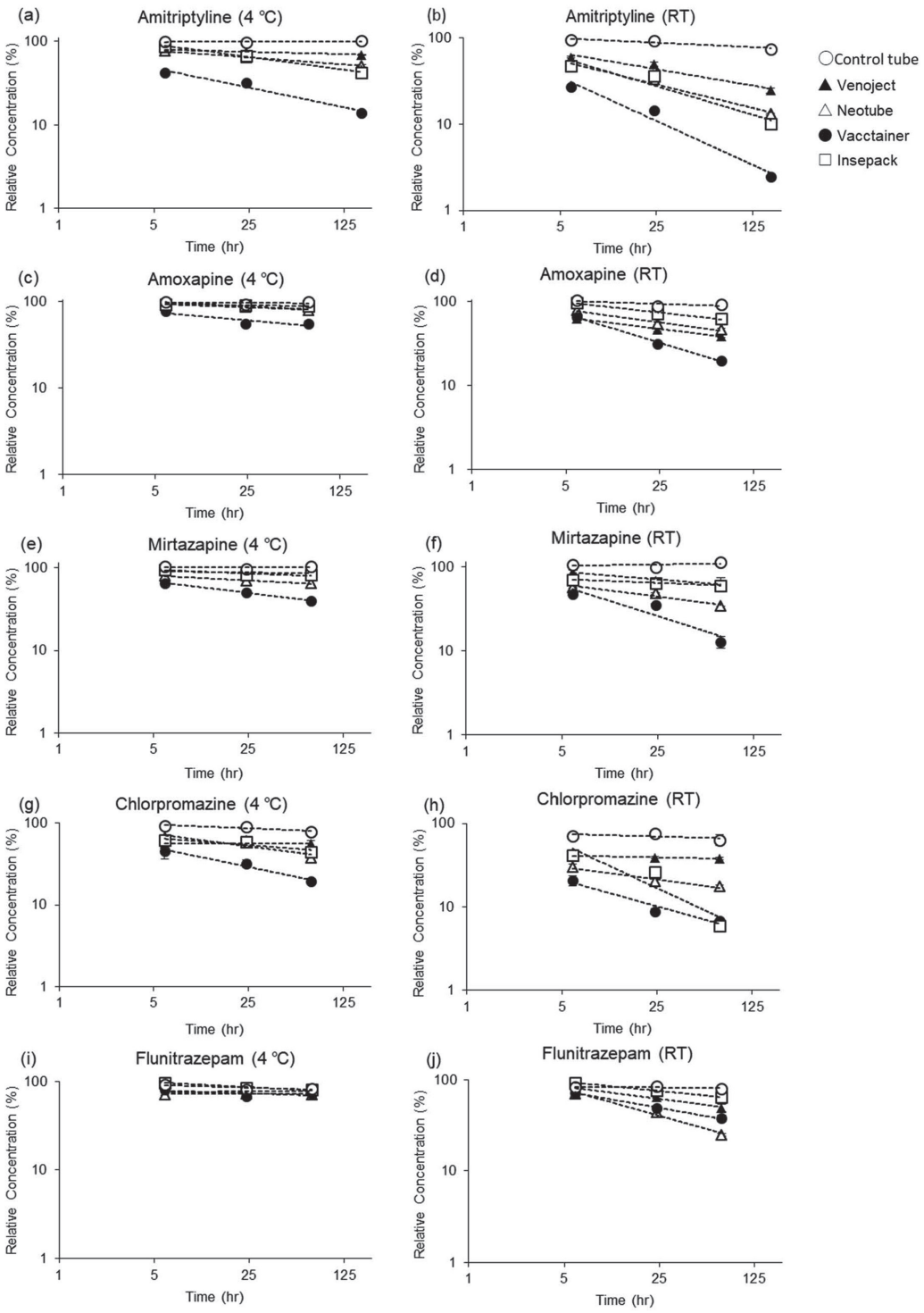

Fig. 1. Changes in drug concentrations in plasma. Concentration changes of amitriptyline (a, b), amoxapine (c, d), mirtazapine (e, f), chlorpromazine (g, h), and flunitrazepam (i, j). Plasma drug concentrations when stored at 4°C (a, c, e, g, i) and at room temperature (RT) (b, d, f, h, j) are represented as % of 0 hr-control. The approximation lines of power function are indicated by a dashed line. Data are expressed as relative concentrations to the 0 hr-control. Data are shown as mean ± S.E. (n = 3).

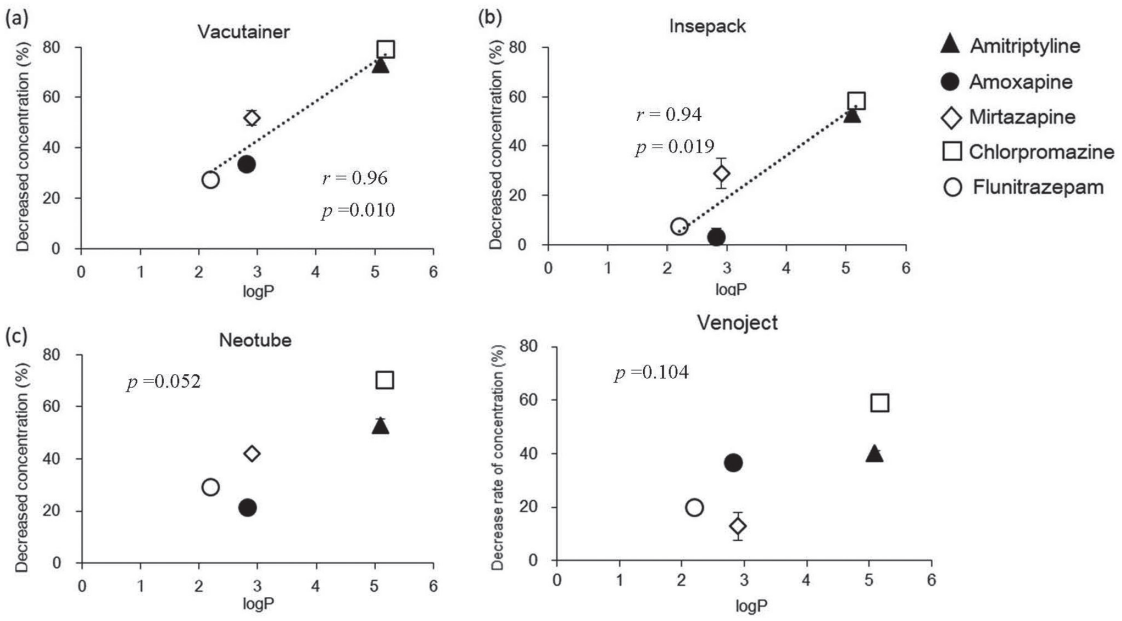

Fig. 2. Relationship between logP values and percent decreases in observed concentrations of drugs. Vertical axis indicates percentage decreases in drug concentrations when drug-spiked plasma was stored for 6 hr at RT in (a) Venoject, (b) Neotube, (c) Vacutainer, and (d) Insepack and horizontal axis indicates the logP of the drug. The regression lines are indicated by a dashed line, when a correlation was found between logP and the percentage decrease in drug concentration. Data are shown as mean ± S.E. (n = 3).

tration. Both Insepack and Venoject are blood collection tubes containing a polyolefin-based separator; however, a correlation was observed for Insepack but not for Venoject. This could be due to differences in gel properties, such as density and crystallinity, between different manufacturers, even for the same polyolefin-based product. The accuracy of the correlation analysis was insufficient because the number of drugs studied was small and drugs with similar logP values were used in the current study. Further studies with a larger number of drugs will make it possible to clarify the relationship between drug adsorption on various separation gels and logP.

In conclusion, control tubes are strongly recommended for TDM and clinical toxicology because drug adsorption is less likely to occur in control tubes and adequate blood levels can be measured. In addition, the amitriptyline, chlorpromazine, and flunitrazepam concentrations significantly decreased, even in control tubes. Moreover, unlike in TDM, immediate measurement of blood concentrations in cases of drug intoxication is often not possible; thus, it is expected that the samples will be stored for a longer period. Therefore, it was necessary to store frozen samples, even in control tubes.

Conflict of interest---- The authors declare that there is no conflict of interest.

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